IMPROVING CALVING RATES IN AFRIKANER CATTLE

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CONTENTS

LIST OF PUBLICATIONS

1. LISHMAN, A.W. & HUNTER, G.L., 1966.
   Sexual activity in the ewe following isolation from the ram during the breeding seasons. S. Afr. J. Agric. Sci.9, 993-1004.
2. LISHMAN, A.W. & HUNTER, G.L., 1966.
   Effect of progesterone treatment prior to joining breeding rams, on the incidence of oestrus and rate of lambing in a Merino flock. Proc. S. Afr. Anim. Prod. 5, 137-139.
3. HUNTER, G.L. & LISHMAN, A.W., 1967.
   Post-partum ovulation and oestrus in spring-lambing ewes. J. Reprod. Fert. 14, 473-475.
4. LISHMAN, A.W. & DE LANGE, G.M., 1967.
   The incidence of oestrus in Merino ewes following introduction of breeding rams in summer and spring. Proc. S. Afr. Soc. Anim. Prod. 6, 196-196.
5. HUNTER, G.L. & LISHMAN, A.W., 1967.
   the effect of the ram early in the breeding season on the incidence of ovulation and oestrus in sheep.
   Proc. S. Afr. Soc. Anim. Prod.,6, 199-201.
6. LISHMAN, A.W. & HUNTER, G.L., 1967.
   Oestrous activity in the ewe following isolation from the ram during anoestrus.
   S. Afr. J. Agric. Sci. 10, 747-752
7. LISHMAN, A.W., 1969.
   The seasonal pattern of oestrus amongst ewes as affected by isolation from, and joining with rams. Agroanimalia 1, 95-102.
8. LISHMAN, A.W., DE LANGE, G.M. & VILJOEN, J.T., 1969.
   Ability of masculinized ewes to stimulate onset of the breeding season in maiden Merino ewes. Proc. S. Afr. Soc. Anim. Prod. 8, 141-142.
9. LISHMAN, A.W., BOTHA, W.A. & LOUW, B.P., 1974.
   Release of LH in ewes treated with progestogen and oestrogen during the anoestrous season. S. Afr. Anim. Sci., 4, 143-145.

10. LISHMAN, A.W., STIELAU, W.J., DREOSTI, I.E., STEWART, A.M. & BOTHA, W.A., 1974. Plasma luteinizing hormone levels in ewes failing to exhibit oestrus during lactation and in ewes isolated from rams. S. Afr. J. Anim. Sci. 4, 45-49.
11. LISHMAN, A.W., STIELAU, W.J. & BOTHA, W.A., 1974. Reproduction in the ewe in relation to plane of nutrition, bodymass and change of bodymass. I. The incidence of oestrus between lambing and reconception. Agroanimalia 6, 25-34.
12. LISHMAN, A.W., STIELAU, W.J. & BOTHA, W.A., 1974. Reproduction in the ewe in relation to plane of nutrition, bodymass and change of bodymass. II. Lambing performance. Agroanimalia 6, 75-80.
13. LISHMAN, A.W., STIELAU, W.J., SWART, C.E. & BOTHA, W.A., 1974. Nutrition of the ewe and the ovarian sensitivity to gonadotrophin. Agroanimalia 6, 7-12.
14. LISHMAN, A.W., STIELAU, W.J., DREOSTI, I.E., BOTHA, W.A., STEWART, A.M. & SWART, C.E., 1974. The release of LH at oestrus in ewes on two planes of nutrition during lactation. J. Reprod. Fert., 41, 227-230.
15. LISHMAN, A.W., 1975. Reduced sensitivity to oestrogen in ewes continuously associated with rams. S. Afr. J. Anim. Sci., 5, 235-238.
16. LISHMAN, A.W. & DE LANGE, G.M., 1975. Oestrous activity and lambing rates in ewes following varying periods of association with vasectomized rams prior to the commencement of the mating period. Agroanimalia 7, 93-100.
17. POULTNEY, B.G., LISHMAN, A.W., LOUW, B.P., BOTHA, W.A. & ARANGIE, P.A.R., 1977. The effects of feed restriction, oestrogen priming and stage of the oestrous cycle on Gn-RH-induced release of LH in ewes. S. Afr. J. Anim.Sci. 7, 141-145.
18. POULTNEY, B.G., BOTHA, W.A. & LISHMAN, A.W., 1977. Undernutrition during the lactation and/or post-weaning periods and the reproductive performance of merino ewes. S. Afr. J. Anim. Sci. 7, 137-139.

19. Inskeep, E.K. & Lishman, A.W. 1978. Factors affecting post partum anoestrus in beef cattle. Beltsville Symp. Agric. Res., (3) Anim. Reprod. 277-289.

20. HAMILTON, C.D. & LISHMAN, A.W., 1979.
    Reducing the partum-to-mating interval in autumn lactating ewes through the use of exogenous hormones. S. Afr. J. Anim. Sci. 9, 59-64.

21. HAMILTON, C.D., LISHMAN, A.W. & LAMB, P.A., 1979.
    The effect in ewes of oestrogen priming and GnRH on LH release and luteal function during early lactation in spring. S. Afr. J. Anim. Sci. 9, 197-203.

22. LISHMAN, A.W., ALLISON, S.M.J., FOGWELL, R.L., BUTCHER, R.L. & INSKEEP, E.K., 1979.
    Follicular development and function of induced corpora lutea in underfed postpartum anoestrous beef cows. J. Anim. Sci. 48, 867-875.

23. MEAKER, H.J., COETSEE, T.P.I. & LISHMAN, A.W., 1979.
    Protein and/or energy supplementation of lactating beef cows during the early summer period in Northern Natal. S. Afr. J. Anim. Sci. 9, 231-236.

24. FLETCHER, I.C., LISHMAN, A.W., THRING, B. & HOLMES, J.A., 1980.
    Plasma progesterone levels in lactating ewes after hormone induced ovulation during the non breeding season. S. Afr. J. Anim. Sci. 10, 151-158.

25. MEAKER, H.J., COETSEE, P.P.N., SMITH, M. & LISHMAN, A.W., 1980.
    The relationship between bodymass and fertility of beef cows of different ages. S. Afr. J. Anim. Sci. 10, 83-89.

26. LEWIS, G.S., LISHMAN, A.W., BUTCHER, R.L. DAILEY, R.A. & INSKEEP, E.K., 1981.
    Factors affecting function of induced corpora lutea in postpartum anoestrous ewes. J. Anim. Sci. 52, 1122-1129.

27. SMITH, M.F., LISHMAN, A.W., LEWIS, G.S., HARMS, P.G., ELLERSIECK, M.R., INSKEEP, E.K., WILTBANK, J.N. & AMOSS, M.S., 1983.
    Pituitary and ovarian responses to gonadotropin releasing hormone, calf removal, and Synchro-Mate B in anoestrous beef cows. J. Anim. Sci. 57, 418-424.

28. LOUW, B.P., LISHMAN, A.W., VAN NIEKERK, A. & LESCH, S.F., 1983. Influence of season on tonic luteinizing hormone (LH) and progesterone levels in cattle in a sub-tropical environment. S. Afr. J. Anim. Sci. 13, 253-256.
29. LISHMAN, A.W., LYLE, A.D., SMITH, V.W. & BOTHA, W.A., 1984. Conception rate of beef cows and growth of suckling calves as influenced by date of calving and supplementary feeding. S. Afr. J. Anim. Sci. 14, 10-19.
30. LISHMAN, A.W., SNYMAN, J.W. & MOOLMAN, J.Z., 1984. Reconception and bodymass changes of energy supplemented first calver beef cows and growth of their creepfed calves. S.Afr. J. Anim. Sci. 14, 19-25.
31. LOUW, B.P., LISHMAN, A.W., VAN NIEKERK, A., GROBBELAAR, J., LESCH, S.F., 1984. Effect of restricted suckling on LH and ovarian steroids after induced ovulation in *Bos taurus* and *Bos indicus* cows. S. Afr. J. Anim. Sci. 14, 91-96.
32. GROBBELAAR, J., BOTHA, W.A. & LISHMAN, A.W., 1984. The effect of progestogen and oestradiol priming on luteal function in seasonally anoestrus GnRH-treated ewes. S. Afr. J. Anim. Sci. 14, 143-145.
33. CRICHTON, J.S. & LISHMAN, A.W., 1984. Identification of sexually low-response rams using a libido test and their performance when allowed to mate under field conditions. S. Afr. J. Anim. Sci. 14, 177-178.
34. LISHMAN, A.W. & HARWIN, G.A., 1985. Failure to induce ovulation by short-term calf removal in lactating beef cows on dry-lot. S. Afr. J. Anim. Sci. 15, 21-22.
35. CRICHTON, J.S. & LISHMAN, A.W., 1985. Libido evaluation of virgin beef bulls. S. Afr. J. Anim. Sci. 15, 22-24.
36. WELLS, P.L., HOLNESS, D.H., FREYMARK, P.J., McCABE, C.T. & LISHMAN, A.W., 1985. Fertility in the Afrikaner cow. 2. Ovarian recovery and conception in suckled and non-suckled cows post partum. Anim. Reprod. Sci. 8, 315-326.

37. VAN NIEKERK, A., LISHMAN, A.W. & LESCH, S.F., 1986.
The reproductive responses of two breeds of beef cows and the performance of their progeny in two contrasting environments. S. Afr. J. Anim. Sci. 16, 209-214.

38. WELLS, P.L., HOLNESS, D.H., McCABE, C.T. & LISHMAN, A.W., 1986.
Fertility in the Afrikaner cow. 3. Once a day suckling and its effect on the pattern of resumption of ovarian activity and conception rate in early lactation. Anim. Reprod. Sci. 12, 1-12.

39. CRICHTON, J.S., LISHMAN, A.W. & LESCH, S.F., 1987.
Failure to demonstrate a relationship between beef bull libido and conception rate. S. Afr. J. Anim. Sci. 17, 27-30.

40. LOUW, B.P., LISHMAN, A.W. & THOMAS, C.R., 1988.
The influence of loss and gain of bodymass on ovarian activity in beef cows. S. Afr. J. Anim. Sci. 18, 1-7.

41. BUTTERFIELD, W.A. & LISHMAN, A.W., 1988.
Embryo mortality and early post-oestrous cycle embryonic death estimated from oestrous cycle lengths and milk progesterone analysis. S. Afr. J. Anim. Sci. 18, 79-82.

42. CRICHTON, J.S. & LISHMAN, A.W., 1988.
Factors influencing sexual behaviour of young Bos indicus bulls under pen and pasture mating conditions. Appl. Anim. Behav. Sci. 21(4), 281-292.

43. GROBBELAAR, J., LISHMAN, A.W., BOTHA, W.A., ELS, D.L., LOUW, B.P. and VAN NIEKERK A., 1989.
Attempts to improve the function of GnRH-induced corpora lutea in early post-partum ewes through oestradiol and/or PMSG priming or exogenous luteotrophin. Anim. Reprod. Sci. 19, 245-263.

44. PEARSON, H.G. & LISHMAN, A.W., 1991
Augmentation of luteal function in the lactating ewe after induction of ovulation with a gonadotropin releasing hormone. S. Afr. J. Anim. Sci. 21, 94-90.

45. CRICHTON, J.S., LISHMAN, A.W. & HUNDLEY, M., 1991.
Role of dihydrotestosterone in the control of sexual behaviour in castrated male sheep. J. Reprod. Fert. 3, 9-17.

46. LISHMAN, A.W. & INSKEEP, E.K., 1991
Deficiencies in luteal function during re-initiation of cyclic breeding activity in beef cows and in ewes. S.Afr.J.Anim.Sci. 21, 59 - 76.

47. BUTCHER, R.L., REBER, J.E., LISHMAN, A.W., BREUEL, K.F., SCHRICK, F.N., SPITZER, J.C. & INSKEEP, E.K., 1992.
Maintenance of pregnancy in postpartum beef cows that have short-lived corpora lutea.
J. Anim. Sci. 70, 3831 - 3837.

48. BREUEL, K.F., LEWIS, P.E., SCHRICK, F.N., LISHMAN, A.W., INSKEEP, E.K. & BUTCHER, R.L., 1992.
Factors affecting fertility in the postpartum cow: Role of the oocyte and follicle in conception rates. Biol. Reprod. 48, 655 - 661.

49. STEWART, I.B., LOUW, B.P. & LISHMAN, A.W., 1993. Suckling behaviour and fertility in beef cows on pasture. I. Suckling behaviour. S. Afr. J. Anim. Sci. 23, 176 - 179.

50. STEWART, I.B., LOUW, B.P. & LISHMAN, A.W., 1993. Suckling behaviour and fertility in beef cows on pasture. II. Influence of twelve-hour calf separation on interval to first oestrus after onset of mating period. S. Afr. J. Anim. Sci. 23, 180 - 181.

51. STEWART, I.B., LOUW, B.P., LISHMAN, A.W. & STEWART P.G., 1995.
Late-night suckling inhibits onset of postpartum oestrous activity in beef cows. S. Afr. J. Anim. Sci. 25, 26 -29.

52. STEWART, I.B., LOUW, B.P., LISHMAN, A.W., MORGENTHAL. J.C. & LEVIN, J., 1997.
Interaction between melatonin and suckling in prolonging post partum oestrous activity in suckled cows.
(Unpublished).

53. WELLS, P.L., HOLNESS, D.H. & LISHMAN, A.W., 1979.
The resumption of ovarian activity and conception in Afrikaner and Mashona cows after partial weaning either before or during the breeding period.
(Unpublished).

54. WELLS, P.L., HOLNESS, D.H. & LISHMAN, A.W., 1979.
The pattern of behaviour prior to and during oestrus in Afrikaner and Mashona cows and the effect of calf removal and absence of bulls.
(Unpublished).

55. WELLS, P.L., HOLNESS, D.H. & LISHMAN, A.W., 1979.
Suckling behaviour during daylight in Afrikaner, Mashona and Hereford cows.
(Unpublished).

PREFACE

The research outlined in this dissertation has been aimed at eliminating the problem of low calving rates in beef cattle and in particular in the Afrikaner breed. This breed is considered to be highly adapted to tropical and sub-tropical areas. At the time these studies were initiated some 60% of the National Beef Herd of South Africa consisted of Afrikaner-type cattle.

For many years it had been known that calving rates in beef breeding herds were in the region of 50%. At this level it is impossible to maintain herd size, if all non-productive females are culled annually. Consequently, cows that tended to calve only in alternate years were being retained in the breeding herds. Such a policy was excused on the basis that beef farming was practised mainly in the extensive farming areas of the country and that periodic droughts were part of the environment. Thus, it was argued that, where restricted calving seasons were applied, lactating cows could not rear a calf and reconceive in the same year. The Afrikaner and allied breeds were chosen for use in such areas because they survived and sometimes reproduced. The question that needed answering is to what extent the adaptability depended on lowered productivity and whether simple means could be devised to overcome what appeared to be genetically depressed fertility.

Due to the high cost of procuring sufficient suitable cows for research much of the early work was conducted using sheep as experimental material. In this way it was hoped that effective use could be made of the limited resources. At the same time that answers were being obtained to questions posed, experience in conducting research and application of new techniques, could be acquired. A concerted effort was, however, made not to lose sight of the major objective of the research viz., to account for, and to improve, low calving rates in Afrikaner cows. The team effort necessitated by the of the research had as its natural outcome papers under multiple-authorship. The collaboration took several forms and included research :-

1. Conducted at my initiative during my employment as researcher in the Department of Agriculture and while stationed at Cedara Research Institute.
2. Initiated by others and for which I took over responsibility while at Cedara.
3. Performed by MSc and PhD students who worked under my supervision.

4. Carried out during two sabbatical at West Virginia University where I had the privilege of collaborating with Keith Inskeep and his graduate students.

This dissertation centres on calving rates in Afrikaner cows. Because of the multi-faceted approach that developed, the thesis is divided into eight interlinked parts which are introduced and explained in greater detail at the start of each section. Essentially, the work was conducted along seven main lines :-

1. The extent to which manipulation of environmental factors such as, season, exposure to introduction of males and/or modification of the nutritional level would modify reproductive patterns in females.
2. Understanding the mechanisms through which under nutrition reduced reproductive rates.
3. Attempts to overcome the inhibitory effects of lactation on re-breeding by the use of exogenous hormones and the problems that arise from such practises.
4. Reduction in the suckling-induced inhibition of postpartum rebreeding by manipulation of suckling by the calf.
5. Investigations regarding the suckling behaviour in Afrikaners in an attempt to explain the prolonged lactation in this breed. This work was extended to *Bos taurus* breeds under intensive conditions.
6. Oestrous behaviour in the Afrikaner cow and its role in reducing the chances of successful mating occurring.
7. The role of the male in reducing conception rates.

The research will be presented in the approximate chronological order in which it was conducted. However, this was not always feasible if some adherence to the aspects listed above, is applied.

PART A

EFFECT OF EXPOSURE TO MALES ON SEXUAL ACTIVITY IN FEMALES

INTRODUCTION

In the mid 1960's research in the Department of Animal Science at the University of Natal was centred on the influence of rams on initiation of the spring breeding season in ewes and on the effect of presence of rams on the duration of oestrus and time of ovulation. Some twenty years earlier E.J. Underwood and co-workers and twenty years later P.G. Schinckel had shown that at the transition from anoestrus to the breeding season the introduction of rams to ewes that had previously been isolated from contact with rams, stimulated the ewes to exhibit oestrous one cycle (17 days) later.
This phenomenon had also been seen in horses and cattle. It explained why in sheep farming practice a marked increase in the number of ewes lambing in autumn occurred some three weeks after the lambing season first commenced.

Studies of the breeding season in sheep had been customarily conducted by associating vasectomized males with a group of ewes and noting of the ewes exhibiting oestrus each day. Under such circumstances no stimulation (by rams) at the onset of the spring breeding season occurred. P.G. Schinckel had proposed that the continuous association of ewes with vasectomized rams led to a deepening or lengthening of the seasonal anoestrus.

Paper 1

The objective of the research reported in Paper 1 of this thesis was an investigation of the aforementioned hypothesis. Earlier observations at the Ukulinga Research Station of the University of Natal had shown that the spring breeding season usually commenced in November and continued (in the absence of pregnancy) until June or July. The experiment reported in Paper 1 compared seasonal changes in the occurrence of oestrus in ewes either continuously with sterile rams or isolated from rams for a period during anoestrus.

Findings :-

1. Contrary to expectation, it was demonstrated that isolation of ewes from rams, particularly during the winter anoestrus, led to a cessation of sexual activity. This activity could restored by re-association with rams, but the "ram stimulus" became a significant factor only about the time that the seasonal breeding stimuli could be expected to be increasing (late October).

Implications/Significance :-

Two important concepts were established in this study viz.,

1. Isolation from rams leads to a cessation of sexual activity. This was the first time that this phenomenon had been demonstrated for any species.
2. The practice of continuously associating ewes with rams for many months in order to define the limits of the natural breeding season may not provide a realistic picture of what happens in farming practice. In the latter situation, rams and ewes are in association for only some six weeks each year.

Paper 2

The next step in this theme was to investigate whether 30-day periods of association and isolation from rams during the breeding season would allow the "ram effect" to be manifested. This should then have prolonged the breeding period. A secondary objective was to determine whether a 30-day period of isolation from rams during the breeding season was long enough to allow the ram effect to be re-initiated and whether the isolation period needed to be extended to 60 days.

Findings :-

1. The breeding season was not prolonged by intermittent association. Isolation for 30 days did reduce sexual activity significantly provided it occurred during May. However, isolation during March or April (height of breeding season) was not effective.

2. When the period of isolation was extended to 60 days this always reduced oestrous activity compared to ewes continuously associated with rams .

Implications/Significance :-

1. As the season advances towards seasonal anoestrus, oestrous activity in ewes is to some extent maintained by continuous association with rams. This reaffirmed the findings outlined in Paper 1.
2. At the peak of the breeding season (March/April) the seasonal stimuli can support continued oestrous activity during a 30-day period of isolation from rams, provided this did not occur during the declining stages of the breeding season.
3. When isolation continues for 60 days ewes may show oestrus only after stimulation by rams.

Paper 3

The objective in Paper 3 was to confirm the findings outlined in Papers 1 and 2. In particular, the aim was to define the breeding season in ewes in the absence of a continued ram stimulus (reflecting seasonal stimuli only). Ewes were kept in isolation from rams and new groups were joined at monthly intervals for 12 months.

Findings :-

1. With only minor exceptions, the level of oestrous activity was lower amongst ewes isolated from rams than in those that had been stimulated by rams.
2. Introduction of rams during the anoestrous season did not stimulate oestrous activity to the same degree as seen during the breeding season.
3. Introduction of rams was a stronger stimulus than continuous presence of rams.

Implications/Significance :-

1. Fresh joining of ewes with rams could virtually eliminate seasonal anoestrus, even though the ram stimulus was low during the accepted non-breeding period.

2. A ram stimulus could be obtained throughout the year.
3. Association of sexes for long periods of time does NOT lead to a deepening of anoestrous, but eliminates the stimulus due to fresh joining.

Paper 4

The research described thus far has outlined findings obtained with ewes that were not exposed to an annual mating-lactating-weaning regime. Evidence had been accumulated suggesting that experimental procedures utilising ewes mated only to sterile rams did not duplicate the on-farm situation. Thus, experimental findings might not be very useful in explaining why lambing rates may vary, depending on the month of matings relative to the presumed natural breeding season.

In a small experiment, a Merino breeding flock was mated either during December or January. According to the accepted findings most of the ewes should have been cycling when first exposed to breeding rams, particularly with the January mating.

Findings :-

1. Virtually identical mating patterns were seen at the two times, with a low evidence of oestrous for the first 17 days after joining. The peaks that could be expected from ram stimulation then occurred on the 18th and 24th days after joining.

Implications/Significance :-

1. Many ewes do not enter the breeding season even when the seasonal stimuli are expected to be high, unless these ewes were stimulated by joining with rams.

Paper 5

Paper 5 investigated the suggestion (P.G. Schinckel) that the first ovulation of the spring breeding season is initiated within a few days of joining with rams.

Findings :-

1. Within 6 to 8 days of joining, rams stimulated ovulation in 75% of the ewes exposed.

Paper 6

For oestrus to occur prior to ovulation the brain centres responsible for overt oestrous behaviour require to be primed with progesterone. This can come either from a waning corpus luteum or from an exogenous source.

P.G. Schinckel had shown that when rams were introduced into a ewe flock, at the transition from anoestrus to the breeding season ovulation without oestrus occurred in a high proportion of the flock. Such ewes then show oestrus one cycle later. Paper 6 describes the attempt to convert these silent ovulations into ones accompanied by oestrus.

Findings :-

1. Progesterone priming (prior to joining with rams) for nine days increased the percentage of ewes mated within five days after ram introduction by nearly 40%.
2. Priming for only three days was of little value except that it shifted the subsequent oestrus from a peak at 24 days post joining to four days earlier. The significance of this finding became evident nearly 25 years later.

Paper 7

When ewes are joined with rams in spring a large proportion of the flock (up to 70%) may be mated after having undergone only one oestrous cycle. In this situation the likelihood of pregnancy failure could be higher than in ewes in which the reproductive tract has been "conditioned" during the course of several oestrous cycles.

Paper 7 describes an attempt to ascertain whether conditioning is important or not. The investigation was repeated over three years.

Findings :-

1. Contrary to expectation, a lengthening of the breeding season by continuously exposing ewes to sterile rams increased the % of barren ewes and the % conceiving **AFTER** the first oestrus.

Implications/Significance :-

1. Perhaps selection should be aimed at retaining animals that respond easily to stimuli rather than to those with a short anoestrous period and a long breeding season.
2. The negative association between % ewes conceiving to first oestrus and the % not lambing implies a lowered fertility in repeat breeders. Limiting the mating period to not more than two oestrous cycles can assist in culling of poor breeders.

Paper 8

Overt oestrus in the ewe is detected only in response to mating attempts by rams. It is thus virtually impossible to study oestrous activity in ewes without the confounding influence of exposure to rams.

Following suggestions by the author of this thesis, S.D. Parsons demonstrated that masculinized ewes could reduce the duration of oestrus, just as rams did. The purpose of Paper 7 was to determine whether masculinized ewes could be utilized to detect oestrus or whether the customary ram-effect would result.

Findings :-

1. After exposure of anoestrus ewes to either vasectomized rams or masculinized ewes for 15 days, the mating pattern were similar to that seen in ewes exposed to entire rams.
2. A delay of a few days in the response to masculinized ewes was seen.

Implications/Significance :-

1. Masculinized ewes can stimulate onset of the breeding season in anoestrous ewes.
2. Male-mating behaviour should perhaps be directed at the recipient animals rather than be incidental. Strong confirmation of the latter suggestion was obtained in later observations (See Paper 8 for details).

Paper 9

T.J. Robinson and associates had detected individual differences in the sensitivity of ewes to ovarian hormones. The possibility exists that continuous presence of rams with ewes might reduce the sensitivity of their nervous centres controlling oestrous behaviour, to oestrous inducing hormones. Paper 9 investigated this possibility. An earlier experiment (not presented here) had resulted in nearly 80% more ewes that had previously been isolated from rams, exhibiting oestrus in response to progesterone-oestrogen treatment, when compared to ewes continuously with rams. The latency to heat was also significantly shorter in isolated ewes. In an expanded experiment (Paper 9) both entire and ovariectomized ewes were injected with progesterone and oestrogen to induce oestrus.

Findings :-

1. Female sheep that were continuously exposed to the mating stimulus (vasectomized rams) for several months showed adaptation to the stimulus and were less sensitive to oestrus-inducing hormones.
2. The ovary is not required for this phenomenon to occur.

Implications/Significance :-

1. It would appear from these findings, that in order to induce a high incidence of oestrus at the onset of the spring breeding season ewes should be isolated from rams during the anoestrous period.

Paper 10

Before the advent of radioimmuniossays little was known concerning the hormonal mechanisms controlling anoestrus. R.H. Dutt (1960) and R.H. Robertson & J.S.M. Hutchinson (1962) had proposed that the rate of synthesis and/or release of luteinizing hormone (LH) from the anterior pituitary might be involved. This paper considered circulating levels of LH in anoestrus lactating ewes.

Findings :-

1. Based on samples collected at infrequent intervals (daily or once only) lactation anoestrus was not due to insufficient average levels of LH in the circulation. However, in ewes isolated from rams the plasma LH levels were lower than in ewes continuously with rams.

Implications/Significance :-

1. Early evidence of minor surges in the tonic release of LH was obtained. This phenomenon has of course been thoroughly investigated in later studies where blood samples have been obtained at intervals of minutes rather than days.

The importance of this section in relation to Afrikaner fertility will become evident in Part F.

PAPER 1

S. Afr. J. Agric. Sci. (1967), 10, 747–752

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OESTROUS ACTIVITY IN THE EWE FOLLOWING ISOLATION FROM THE RAM DURING ANOESTRUS

(Met opsomming in Afrikaans)
(Avec résumé en français)

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A. W. LISHMAN(¹) and G. L. HUNTER, Faculty of Agriculture, University of Natal, Pietermaritzburg

ABSTRACT

From July 3, 1964, 27 Merino and 17 Döhne Merino ewes were run with vasectomized rams (Group 1). From September 1, five groups (2a to 2e) each consisting of 12 Merino and 10 Döhne Merino ewes which had been isolated from rams from the end of May, were joined with vasectomized rams in turn at 17-day intervals.

Following joining with rams, the incidence of oestrus in Group 1 was significantly greater among the Döhne Merinos. In the smaller groups (2a to 2e) this breed difference was also observed during the first 17 days after joining, but was generally not significant. Compared to Group 1, the incidence of oestrus tended to be lower in the isolated groups during the first 17 days after joining in each case, but after oestrus had apparently been stimulated by the presence of rams, its incidence was greater in the isolated groups. Compared with freshly joined ewes, a significant ram stimulus during the second 17-day period following joining was not achieved until late in October. The incidence of oestrus in ewes may thus depend on their association with rams and it may be erroneous to assume that the sexual activity of ewes separated from rams is the same as that of ewes in contact with rams.

INTRODUCTION

Underwood, Shier & Davenport (1944) observed that at the commencement of the breeding season the introduction of rams into the ewe flock resulted in increased occurrence of oestrus in ewes previously isolated from rams. Schinckel (1954) reported that ewes which had been continuously associated with sterile rams during anoestrus resumed oestrous activity at a later date than ewes isolated from rams during this time. He therefore suggested that continuous association of the two sexes led to deepening or lengthening of anoestrus. However, the deepened anoestrus may merely be the result of the lack of the stimulus produced by fresh association of the sexes at the start of the breeding season. In the experiment to be described the incidence of oestrus was compared in ewes which during anoestrus were either run continuously with rams or were isolated from rams for varying periods.

PROCEDURE

The experimental animals consisted of 87 Merino and 67 Döhne Merino-type ewes. The Merinos were obtained from farmers in the Natal Region during the period May to September 1963, brought to the Ukulinga Experiment Station, Pietermaritzburg, and used in other experiments until May 21, 1964. During the period March to May 1964, the Döhne Merino ewes were transferred to Ukulinga from the Eastern Cape and Orange Free State Regions. No information was available regarding their association with rams prior to their arrival at Ukulinga. Thus from the latter part of May, all ewes were kept separate from rams until the start of the present experiment. The Döhne Merino ewes consisted of ewes that lambed during the autumn of 1964, as well as dry ewes and maiden two-tooth ewes. All the Merinos were dry.

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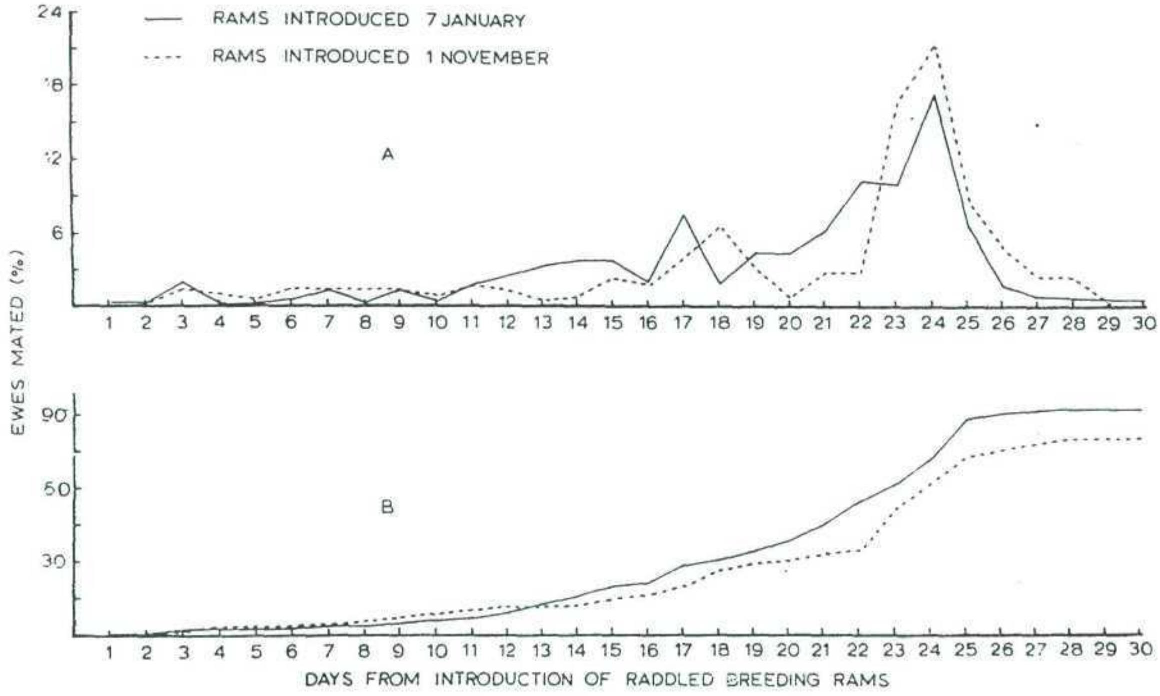

Fig. 1.—*The percentage of ewes mated daily (A) and the daily cumulative total number of ewes served during the 30-day observation period (B) after introduction of rams in summer and spring.*

oestrus commences and thus when a ram stimulus can be obtained. The findings reported here do not support this contention, since the ewes were obtained from flocks in which mating normally occurred during October and according to Lyle & Hunter's hypothesis breeding should have been at a high level during January, if not during November.

It would thus appear that, within the period covered in this study, many ewes do not commence breeding until stimulated by joining with rams. However, in the absence of suitable controls other factors, which may have influenced the present results, cannot be excluded.

Conclusion

When Merino ewes are isolated from rams until the mating period, whether this mating occurs at the commencement or later stages of the accepted breeding season, a high incidence of oestrus is obtained only after the ewes have been stimulated by the rams.

Opsomming

DIE VOORKOMS VAN ESTRUS BY MERINO-OOIE NA BYVOEGING VAN RAMME GEDURENDE SOMER EN LENTE

Die aantal ooie wat daagliks gedek is, is bepaal nadat teelramme gedurende Januarie en weer gedurende November, 1966, by Merino-ooie, wat voor paring van ramme afgesonder was, gevoeg is.

Gedurende hierdie twee paringstye het die voorkoms van estrus feitlik identiese patrone gevolg, met 'n lae voorkoms gedurende die eerste 16 of 17 dae gevolg deur hoogtepunte op die 17de of 18de dag en 24ste dag. Die gevolgtrekking word gemaak dat wanneer ooie tot die aanvang van die paringstyd van ramme afgesonder is, of hierdie paring teen die aanvang of latere stadiums van die erkende teelseisoen plaasvind, 'n hoë peil van geslagsaktiwiteit verkry word eers nadat die ooie deur die ramme gestimuleer is.

References

CLEGG, M. T., COLE, H. H. & GANONG, W. F., 1965. Proc. Conf. Estrous Cycle Control in Domestic Animals. Misc. Publs. U.S. Dep. Agric. No 1005, 96–103. (Anim. Breed. Abstr. 34, No 1303).

DUTT, R. H. & BUSH, L. F., 1955. J. Anim. Sci. 14, 885–896.

HART, D. S., 1950. J. agric. Sci., Camb. 40, 143–149.

HAFEZ, E. S. E., 1952. J. agric. Sci., Camb. 42, 189–265.

HUNTER, G. L., 1959. J.agric. Sci., Camb. 52, 282–295.

HUNTER, G. L., 1962. Proc. S. Afr. Soc. Anim. Prod. 1, 67–69.

HUNTER, G. L., 1964. Proc. S.Afr. Soc. Anim. Prod. 3, 196–206.

LISHMAN, A. W. & HUNTER, G. L., 1967. S.Afr. J. agric. Sci. 10, 747–752.

LYLE, A. D. & HUNTER, G. L., 1965. Proc. S.Afr. Soc. Anim. Prod. 4, 140–142.

RADFORD, H. M., 1961a. Aust. J. agric. Res. 12, 139–146.

RADFORD, H. M., 1961b. Aust. J. agric. Res. 12, 147–153.

RADFORD, H. M. & WATSON, R. H., 1957. Aust. J. agric. Res. 8, 460–470.

RADFORD, H. M., WATSON, R. H. & WOOD, G. F., 1960. Aust. vet. J. 36, 57–66.

SMITH, I. D., 1965. Wrld. Rev. Anim. Prod. 1965, No. 4 95–102.

YEATES, N. T. M., 1949. J. agric. Sci., Camb. 39, 1–42.

YEATES, N. T. M., 1955. Aust. J. agric. Res. 7, 440–446.

PAPER 5

PROC. S. AFR. SOC. ANIM. PROD. (1967) HAND. S. AFR. VER. DIEREPROD.

EFFECT OF THE RAM EARLY IN THE BREEDING SEASON ON THE INCIDENCE OF OVULATION AND OESTRUS IN SHEEP

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The stimulation of oestrous activity in ewes which results from the joining of the sexes early in the breeding season is a phenomenon of some importance particularly in the numerous South African flocks which are mated in spring or early summer (Parsons & Hunter, 1965). In practice, a marked peak in the incidence of oestrus occurs in flocks about 17–24 days after the introduction of the rams. A second, smaller peak of activity often occurs one cycle later, i.e. about 34 days after joining. Lyle & Hunter (1965) have briefly reviewed the earlier work on the topic and have shown that the presence of teaser rams for only six days during October is sufficient to induce a greater incidence of oestrus in the ewes 14–31 days after teasing started, compared with ewes which remained isolated from rams until day 14. These results indirectly support the suggestion of Schinckel (1954a), viz. that the first ovulation of the season occurs in many of the ewes within a few days of the introduction of the ram and that, in the absence of a waning corpus luteum from a previous ovulation, coincident oestrus is not displayed at this time, but occurs one cycle later. Lishman & Hunter (1966) provided further indirect evidence in support of Schinckel's suggestion by showing that injections of progesterone before the introduction of the rams in spring can cause a marked increase in the incidence of oestrus within 4 days of joining. To substantiate his own suggestion with direct evidence, early in the breeding season Schinckel (1954b) slaughtered two groups each of 36 ewes, in order to determine the incidence of ovulation. The ewes in one of these groups were run with a ram for five or six days immediately prior to slaughter, while the second (control) group of ewes remained isolated from rams before slaughter. Unfortunately, all but ten of the control ewes were slaughtered 1–4 weeks before any of the mated group and, furthermore, twenty of these control ewes, which had not ovulated at slaughter, were judged from the size of their follicles to be "approaching the breeding season". Many of these may well have ovulated within a few days had they been laparotomized instead of slaughtered. In addition, Schinckel assumed that the initiation of the breeding season in all ewes in the mated group which exhibited coincident oestrus and ovulation during the 5-6 day mating period (thirteen of the thirty-six ewes) was not stimulated by the ram. This presumption was apparently based on the grounds that oestrus would not have been displayed unless a waning corpus luteum from a previous ovulation was in the ovaries. Schinckel's own evidence showed that this is not invariably the case.

In view of the importance of the "ram effect" phenomenon in this country, there is a need for more substantial direct evidence to confirm Schinckel's (1954a) suggestion that the first ovulation of the season in ewes can be initiated by the introduction of rams and also for some measure of the variation in the interval between joining and ovulation. The small experiment to be reported was designed to meet these needs and also to examine the effects on the incidence of ovulation and oestrus of feeding a concentrate ration at this time of year.

Procedure

Sixteen non-pregnant, mature German Merino ewes were available. These had been maintained for some months in pens and group-fed a ration consisting of unrestricted quantities of *Eragrostis curvula* hay, maize silage, salt and bone meal. The experiment commenced during the spring of 1966, which is the time of year when such ewes are about to start their new breeding season (Hunter, 1964). The ewes were randomly allocated in four replications to the four treatment groups of a 2^2-factorial experiment.

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The replications and treatments were planned to correspond with those of another similar experiment (see Hunter & Lishman, 1967). For this reason, the replications entered the experiment at weekly intervals starting on October 28. The date of entry into the experiment in each case was designated day 0 and the commencement and cessation of treatments, as well as (for statistical analysis) the occurrence of ovulation and oestrus in the ewes were measured in days from the appropriate day 0.

The two factors employed in the experiment were (1) feeding: on days 3–28 the ewes either received a supplementary ration of 1 lb uncrushed maize grain, or received no supplementary ration; and (2) teasing: the ewes were teased with vasectomized rams either from day 3 or from day 14 until the first oestrus of the season had been recorded for each ewe. From the appropriate day (3 or 14) the ewes were removed from the pens where they had been isolated from rams and placed with active teaser rams. In addition, at 8 hr intervals, fresh vasectomized rams were introduced to these pens for periods of 15 min to record the incidence of oestrus. By means of laparotomies performed on day 20, the ovaries of all ewes were examined for corpora lutea, using local anaesthesia and a laparotomy cradle (Lamond & Urquhart, 1961). The date of the first ovulation of the breeding season was estimated for each ewe with the aid of the illustrations of Quinlan & Maré (1931), showing the appearance of corpora lutea at different stages of the ewe's reproductive cycle. It was necessary to repeat the laparotomies on two ewes in which ovulation had not occurred by day 20.

Results and Discussion

Following the introduction of teaser rams on day 3, ovulation occurred on day 6.2 ± 2.64 while following joining on day 14 ovulation occurred on day 18.5 ± 2.64. The mean difference (12.3 ± 4.36 days) is significant ($P<0.05$). In twelve of the sixteen ewes (75%) ovulation was apparently stimulated by joining the rams—in these ewes the first ovulation of the breeding season occurred 0–8 days after joining. Two of the remaining four ewes ovulated 6 days prior to joining and two were apparently not stimulated by the ram, since these first ovulated 15 and 18 days after joining. Of the fourteen ewes which ovulated in the presence of rams, two, seven, four and one had 0, 1, 2 and 5 silent ovulations respectively before showing their first oestrus of the breeding season. These observations may be compared with data from twenty-four similar ewes (Hunter & Lishman, 1967) among which, following lambing in spring, three, fifteen and six ewes had 0, 1 or more than 2 silent ovulations respectively before showing oestrus.

As a result of the dispersion of first oestrus by the variation in numbers of silent ovulations, the difference in interval to first heat, following joining on day 3 or on day 14, was not significant. However, half the ewes showed first oestrus 16–23 days after joining and three ewes were first on heat 33–35 days after joining, having first undergone two silent ovulations. These data are in accordance with the observations made under practical conditions referred to earlier.

Even though the number of animals in the experiment was small, the results support Schinckel's (1954a) suggestion that in many spring-mated ewes the first ovulation of the breeding season occurs within a few days of their joining the rams. The variation in interval between joining and what has been presumed to be a stimulated ovulation, appears to be rather substantial—a range of 9 days in this experiment. This requires confirmation in an experiment with larger numbers, which should also include an unteased group to determine the "natural" variation of ovulation dates at this time of year in ewes not stimulated by the presence of rams. It will also be of value to determine whether, in successive years, the same ewes tend to respond to the ram stimulus.

Although the supplementary maize ration significantly affected the ewes' liveweight changes during the period in which it was fed, viz. days 3–28 ($P<0.001$), it had no effect on the intervals from day 0 to ovulation or to oestrus. However, if fed earlier in relation to the time of introduction of the rams, a supplementary ration may influence the commencement of ovarian cycles, since Smith (1966) has shown that at this time of year the spontaneous occurrence of oestrus in ewes can be influenced by the current level of nutrition. There was no relationship between either the ewes' liveweight on day 3, or liveweight change between days 3 and 28, and the interval to first ovulation, whether this interval was measured from day 0 or from the day the ewes joined the rams.

Acknowledgements

The animals and facilities for this experiment were provided by the Department of Agricultural Technical Services. The authors' best thanks are due to the staff of the Ukulinga Experiment Station for care of the experimental animals, to Miss I. M. Gravett, of the Department of Biometry, for advice, and to Mr K. Webb, of the university's Computor Centre, for assistance with part of the analysis.

Opsomming

INVLOED VAN DIE RAM VROEG IN DIE TEELSEISOEN OP DIE VOORKOMS VAN OVULASIE EN BRONSTIGHEID BY SKAPE

Deur ondersoek van die eierstokke en waarneming met koggelramme is die voorkoms van eerste ovulasie en estrus met die aanvang van die teelseisoen in die lente by sestien volwasse Duitse Merino-ooie bepaal. In 'n 2^2-faktoriale eksperiment is gevind dat daaglikse byvoeding met 1 lb mielies per kop nie die aanvang van geslagsaktiwiteit beïnvloed het nie. Koggelramme is van of 3 of 14 dae na aanvang van die proef (Dag 0) by die ooie gebring. As gevolg van hierdie verskil in prikkeling, was die gemiddelde verskil in datum van eerste ovulasie (12.3 ± 4.36 dae) betekenisvol ($P < 0.05$). Klaarblyklik is 75% van die ooie deur bybringing van ramme gestimuleer en ovulasie in hierdie ooie het 0–8 dae na bybringing plaasgevind. Twee ooie het vóór byvoeging van ramme ge-ovuleer. Twee, sewe, vier en een van die veertien ooie wat in die teenwoordigheid van ramme ge-ovuleer het, het onderskeidelik 0, 1, 2 en 5 ovulasies sonder estrus voor die eerste estrus van die teelseisoen getoon. Weens hierdie variasie was daar, as gevolg van die verskil in prikkeling, geen betekenisvolle verskil in die periode vanaf Dag 0 tot eerste estrus nie. By 50% van die ooie is eerste estrus 16–23 dae na byvoeging van ramme waargeneem; by nog 19% het die seisoen se eerste estrus nie tot een siklus later voorgekom nie.

References

HUNTER, G. L., 1964. Proc. S. Afr. Soc. Anim. Prod. 3, 196–206.

HUNTER, G. L. & LISHMAN, A. W., 1967. J. Reprod. Fert. 14, 473–476.

LAMOND, D. R. & URQUHART, E. J., 1961. Aust. vet. J. 37, 430–431.

LISHMAN, A. W. & HUNTER, G. L., 1966. Proc. S. Afr. Soc. Anim. Prod. 5, 137–139.

LYLE, A. D. & HUNTER, G. L., 1965. Proc. S. Afr. Soc. Anim. Prod. 4. 140–142.

PARSONS, S. D. & HUNTER, G. L., 1965. Proc. S. Afr. Soc. Anim. Prod. 4, 136–139.

QUINLAN, J. & MARÉ, G. S., 1931. 17th Rep. Div. Vet. Serv. Anim. Ind., Onderstepoort, p. 663.

SCHINCKEL, P. G., 1954(a). Aust. vet. J. 30, 189–195.

SCHINCKEL, P. G., 1954(b). Aust. J. agric. Res. 5, 465–469.

SMITH, I. D., 1966. Proc. Aust. Soc. Anim. Prod. 6, 69–79.

PAPER 6

PROC. S. AFR. SOC. ANIM. PROD. (1966) HAND. S. AFR. VER. DIEREPROD.

EFFECT OF PROGESTERONE TREATMENT PRIOR TO JOINING BREEDING RAMS, ON THE INCIDENCE OF OESTRUS AND RATE OF LAMBING IN A MERINO FLOCK

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During anoestrus, the ovary of the ewe contains no corpus luteum and the first ovulation of the new breeding season is generally not accompanied by heat (Grant, 1934; Cole & Miller, 1935; Robinson, 1950). Prior priming with progesterone, either from a waning corpus luteum produced following a recent ovulation or from exogenous sources, is necessary for oestrogen to cause overt oestrus (Cole & Miller, 1933; Hammond, Hammond & Parkes, 1942; Robinson, 1954 a, b; 1955 a, b). Sexual activity at the transition from anoestrus to the breeding season can be induced by introducing rams into ewe flocks previously isolated from rams (Underwood, Shier & Davenport, 1944; Lyle & Hunter, 1965). Within a few days of joining, ovulation without heat apparently occurs in a high proportion of the flock (Schinckel, 1954 a, b) and, with the resultant corpora lutea regressing in their ovaries, these ewes show overt oestrus one cycle later (Parsons & Hunter, 1965).

The experiment to be described was conducted to determine whether progesterone therapy prior to introduction of the breeding rams in spring, influences the subsequent rates of mating and lambing.

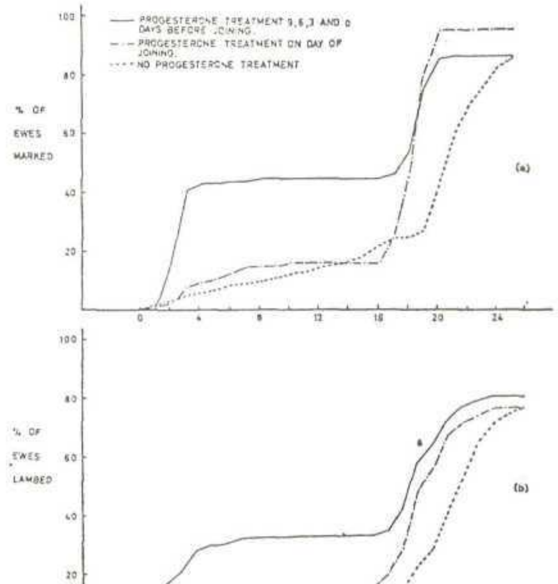

Fig. 1.—*Daily cumulative totals of ewes marked and ewes lambing in three treatment groups.*

Procedure

The trial was conducted in part of a Merion flock which regularly spent from September to early March on a farm in the Impendhle district of Natal and the remainder of the year in the Greytown district. The introduction of breeding rams to this flock in mid-October had previously produced a peak of sexual activity approximately one oestrous cycle later (Parsons & Hunter, 1965). The majority of the ewes had lambed the previous autumn and all ewes had been separated from rams since December, 1963. The portion of the flock available for the experiment consisted of 612 four-tooth ewes. Early in October, 1964, these were randomly allocated to three treatment groups and tagged for identification.

According to Robinson & Moore (1956), a progesterone sensitization period of only three days, followed by treatment with oestrogen, is sufficient to induce overt oestrus in ovariectomized ewes. However, the proportion of ewes responding is higher when a longer sensitization period is employed. Instead of the usual level of 10 mg progesterone per ewe daily, to simplify the management of the flock the progesterone treatment due over a three-day period was given as a single injection. Progesterone injections applied less frequently than once every 24 hours have given satisfactory results in the past (Dutt, 1953; Hunter, Bishop & Brown, 1958; Lishman & Hunter, 1961). The ewes in group 1 were given 30 mg progesterone in oil 9, 6, 3 and 0 days prior to the introduction of the rams. Group 2 ewes received a single injection of 30 mg progesterone on the day of joining. Group 3 was not treated with progesterone.

On October 16, 18 breeding rams fitted with coloured raddle-blocks (Radford, Watson & Wood, 1960) were joined with the ewe flock and the raddle colour was changed 14 days later. Newly marked ewes were identified and counted daily for 26 days i.e. until November 11, and the breeding rams were removed on December 2. During treatment and mating and throughout the summer the groups were grazed together on spring-burned sourveld. Two weeks prior to lambing in March the flock was trekked 90 miles by road to the sweetveld for winter grazing. Here, records were kept of the numbers of ewes in each group which lambed daily.

Results

The lambing records indicated that several ewes had been served, although they had not been visibly raddle-marked during mating. The detection of oestrus in this experiment, utilising raddle crayons, was thus unreliable, probably due to excessive rain during the mating period and to temperatures being too low for clear marking with the available raddle-blocks. During the five-day period following the introduction of the rams, the daily counts indicated that a minimum of 43.6, 9.3 and 5.8% of the ewes had been marked in Groups 1, 2 and 3 respectively. Using the Chi-square test, the proportion marked was significantly higher in Group 1 than in groups 2 and 3 ($P < .001$), while Groups 2 and 3 did not differ significantly. The daily incidence of oestrus as denoted by raddle marks is shown for the 26-day observation period in Fig. 1 a.

Approximately 20 per cent of the ewes in each group was eliminated from the lambing data, due to lost tags and occasional deaths. Fig. 1*b* shows the daily incidence of lambing in the three treatment groups up to 176 days from the introduction of the rams. By 155 days after joining significantly more of the identifiable ewes had lambed in Group 1 ($P < .01$), while Groups 2 and 3 did not differ significantly. There was a suggestion that progesterone treatment may have depressed conception rates during the first five days following joining. However, at the end of lambing, the percentages of wet ewes in the three groups were 86,87 and 85; these do not differ significantly.

Discussion

These observations confirm the need for priming with progesterone if oestrus is to be displayed in conjunction with ovulation and, in particular, provide some evidence that ovulation induced by associating the sexes at the start of the breeding season is "silent" due to a lack of progesterone sensitization. However, in interpreting the results it must be borne in mind that during anoestrus progesterone alone can cause ovulation and oestrus (Dutt, 1952; 1953; Robinson, 1954a; 1955b). It is possible that if the preliminary progesterone treatment is administered to ewes in more "physiological" doses, for example by injecting it once or even twice a day, higher proportion of ewes may display oestrus shortly after introducing the rams. Furthermore, a proportion of the sheep used in this experiment were maiden ewes being mated for the first time. The reaction to the introduction of the ram might be greater in parous ewes, which would imply that the reaction is not altogether inate, but is to some extent a conditioned response.

In the Natal Region (Adler, 1964), and probably throughout the country, Merino sheep are most commonly mated in spring, and early summer for autumn lambs. The stimulatory effect of the rams causing initiation of ovarian cycles when the sexes are joined for mating at this time, is therefore likely to be an important influence on fertility in these flocks. It has been suggested (Schinckel, 1954b) that the pathway of the ram stimulus involves the hypothalamus and gonadotrophin output by the pituitary. An understanding of this mechanism and especially of the factors which may modify the ram effect, is of some practical importance in the quest to improve the fertility of spring-mated Merinos.

Acknowledgements

Thanks are due to Mr. P. J. Nel who provided the facilities for this experiment and whose unlimited co-operation ensured the success of the investigation. Messrs. Ciba Ltd. of Switzerland were responsible for the generous donation of crystalline progesterone.

Opsomming

INVLOED VAN PROGESTEROON-BEHANDELING VOOR BYVOEGING VAN TEELRAMME, OP DIE VOORKOMS VAN ESTRUS EN TEMPO VAN LAMMING IN 'N MERINO KUDDE

'n Kudde van 612 viertand Merino-ooie wat vanaf Desember 1963 van ramme geskei was, is ewekansig verdeel in drie behandelingsgroepe. Die ooie in Groep 1 is behandel met 30 mg progesteroon 9, 6 en 3 en 0 dae voor teelramme met merkers tot die hele kudde op 16 Oktober 1964 toegevoeg is. Op hierdie dag is Groep 2 behandel met 30 mg progesteroon terwyl Groep 3 geen progesteroon ontvang het nie. Ooie wat gemerk is, is daagliks vir 26 dae getel en die ramme is op 2 Desember verwyder. Gedurende die lamseisoen is die lampersentasies van die groepe bestudeer.

Betekenisvol meer ooie in Groep 1 is gemerk binne vyf dae en het gelam binne 155 dae na toevoeging van ramme (kyk Fig. 1). Die finale lampersentasies van die drie groepe het nie betekenisvol verskil nie. Hierdie resultate dui aan dat die ovulasie wat volg op die bybring van ramme teen die aanvang van die teelseisoen, as gevolg van 'n gebrek aan progesteroon nie gepaard gaan met estrus nie.

References

ADLER, E. D., 1964. Proc. S. Afr. Soc. Anim. Prod. 3, 13.
COLE, H. H. & MILLER, R. F., 1933. Amer. J. Physiol. 104, 165.
COLE, H. H. & MILLER, R. F., 1935. Amer. J. Anat. 57, 39.
DUTT, R. H., 1952. J. Anim. Sci. 11, 792.
DUTT, R. H., 1953. Iowa State Coll. J. Sci. 28, 55.
GRANT, R., 1944. Trans. Roy. Soc. Edin. 58, 16.
HAMMOND, J. (Jr.), HAMMOND, J. & PARKES, A. S., 1942. J. Agric. Sci. 32, 308.
HUNTER, G. L., BISHOP, G. P. & BROWN, D. L., 1958. J. Agric. Sci. 51, 129.
LISHMAN, A. W. & HUNTER, G. L., 1961. S. Afr. J. Agric. Sci. 4, 35.
LYLE, A. D. & HUNTER, G. L., 1965. Proc. S. Afr. Soc. Anim. Prod. 4, 140.
PARSONS, S. D. & HUNTER, G. L., 1965. Proc. S. Afr. Soc. Anim. Prod. 4, 136.
RADFORD, H. M., WATSON, R. H. & WOOD, G. F., 1960. Aust. Vet. J. 36, 57.
ROBINSON, T. J., 1950. J. Agric. Sci. 40, 275.
ROBINSON, T. J., 1954a. J. Endocrin. 10, 117.
ROBINSON, T. J., 1954b. Endocrinology 55, 403.
ROBINSON, T. J., 1955a. J. Endocrin. 12, 163.
ROBINSON, T. J., 1955b. J. Agric. Sci. 46, 37.
ROBINSON, T. J. & MOORE, N. W., 1956. J. Endocrin, 14, 97.
SCHINCKEL, P. G. 1954a. Aust. Vet. J. 30, 189.
SCHINCKEL, P. G. (1954b. Aust. J. Agric. Res. 5, 465.
UNDERWOOD, E. J., SHIER, F. L. & DAVENPORT, N., 1944. J. Dept. Agric. W. Austr. 21, 135.

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PAPER 7

OESTROUS ACTIVITY AND LAMBING RATES IN EWES FOLLOWING VARYING PERIODS OF ASSOCIATION WITH VASECTOMIZED RAMS PRIOR TO THE COMMENCEMENT OF THE MATING PERIOD

(*Met opsomming in Afrikaans*)
(*Avec résumé en français*)

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ABSTRACT

Prior to the commencement of the annual breeding period in November, groups of ewes which had lambed in autumn were either (a) continuously associated with rams, (b) associated with and isolated from rams for alternate periods of 17 days, (c) joined with rams for one or two oestrous cycles or (d) isolated from rams.

With one exception, both continuous and alternate association with vasectomized rams maintained the incidence of oestrus above 40% throughout the period prior to joining with entire breeding rams. Isolation from rams tended to reduce the incidence of oestrus during late September.

The experimental treatments had no consistent effect on the pattern of mating with breeding rams or on the conception rate. However, association of the ewes with vasectomized rams for 17 or 34 days prior to exposure to breeding rams significantly shortened the interval to first mating.

On the average, of the ewes that were in anoestrus prior to joining with breeding rams, 7,2% conceived after the first oestrus while 8,4% did not lamb. Amongst the ewes that were cycling, the corresponding percentages of 15,7 and 16,2 proved to be significantly greater than for the anoestrous ewes.

Opsomming

VOORKOMS VAN ESTRUS BY OOIE EN LAMPRESTASIES NA VARIËRENDE PERIODES VAN ASSOSIASIE MET KOGGELRAMME VOOR AANVANG VAN DIE DEKPERIODE

Die duur van die anestrusperiode en lamprestasies is bestudeer by ooie wat in die herfs gelam het en voor aanvang van die jaarlikse dekperiode in November met ramme òf (a) voortdurend geassosieer is, (b) vir alternatiewe periodes van 17 dae geassosieer en afgesonder is, (c) vir een of twee estruskringlope geassosieer is òf (d) van ramme afgesonder is.

Die voorkoms van bronstigheid gedurende die tydperk voor byvoeging van teelramme was, op een uitsondering na, deurgaans bokant 40% vir beide voortdurende en afwisselende assosiasie met koggelramme. Daar was 'n neiging tot 'n verlaagde voorkoms van bronstigheid gedurende laat September, as gevolg van afsondering van ramme.

Die proefbehandelings het geen konsekwente invloed op die patroon van paring met teelramme of op die besettingsyfer gehad nie. Die interval tot eerste paring is betekenisvol verkort by ooie wat vir 17 of 34 dae met koggelramme geassosieer was.

Van die ooie wat in anestrum voor byvoeging van teelramme was het gemiddeld 7,2% na die eerste estrus beset geraak, terwyl 8,4% gladnie gelam het nie. Die ooreenkomstige persentasies van 15,7 en 16,2 by geslagsaktiewe ooie, was beduidend hoër.

Résumé

L'ACTIVITÉ DES CHALEURS ET DES DEGRÉS D'AGNELAGE DES BREBIS À LA SUITE DE PÉRIODE VARIÉÈS D'ASSOCIATIONS AVEC DES BÉLIERS VASECTOMISÉS PRÉCÉDANT LE COMMENCEMENT DE LA PÉRIODE D'ACCOUPLEMENT

Préalablement au commencement de la période annuelle d'accouplement de novembre, des groupes de brebis qui avaient mis bas en automne furent soit, (a) continuellement mises en présence de béliers, soit (b) mises en présence de béliers et isolées d'eux pour des périodes alternant tous les 17 jours, soit (c) mises avec les béliers pour un ou deux cycles de chaleurs ou encore (d) isolées totalement des béliers.

A l'exception d'un seul cas, l'association continuelle et l'association alternée avec des béliers vasectomisés maintint l'incidence des chaleurs au delà de 40% tout au long de la période précédant la saison d'accouplement complet avec les béliers. L'isolement des béliers tendit à réduire l'incidence des chaleurs durant les derniers jours de septembre.

Les traitements expérimentaux n'eurent aucun effet consistant sur le mode d'accouplement avec les béliers de reproduction ou sur le degré conceptionnel. Cependant l'association des brebis avec des béliers vasectomisés pour des périodes de 17 ou de 34 jours précédant la mise en présence de béliers de reproduction diminua de manière significative l'interval jusqu'au premier accouplement.

En moyenne, parmi les brebis qui n'étaient pas en chaleur antérieurement à leur association avec les béliers de reproduction, 7,2% conçurent après les premières chaleurs, tandis que 8,4% ne mirent pas bas. Parmi les brebis qui furent sous cycle les pourcentages correspondants de 15,7 et de 16,2 s'avérérent être significativement plus grands que chez les brebis qui n'étaient pas en chaleur.

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INTRODUCTION

It is common practice in commercial sheep production to join breeding ewes with entire breeding rams for a period of some six weeks each year. Ewes which fail to conceive, or those that experience early embryonic mortality are likely to return to oestrus only after the breeding rams have been removed from the flock. Chopping & Lindsay (1970) concluded that ewes which mated, but did not lamb, contributed greatly to the wastage of potential lambs. Mullaney (1966) and Lishman, Stielau & Botha (1974b) have shown that wastage due to this cause can exceed 30%.

The spring mating season is characterized by a 17-day period when few ewes exhibit oestrus, followed by a period of approximately 10 days during which nearly 70% of the ewes are served (Parsons & Hunter, 1965; Lishman & Hunter, 1966; Lishman & De Lange, 1967; Lyle & Hunter, 1967a). During the peak mating season the limited number of rams (usually 3%) is expected to serve a large number of ewes each day. Not only will each ewe be mated less often, (Hulet, Ercanbrack, Price, Blackwell & Wilson, 1962; Mattner, Braden & Turnbull, 1967) but the quality of the semen may also be reduced. Consequently, fertility and embryo survival may be detrimentally affected.

When the ewes are joined with rams in spring and early summer many of the females are in a state of sexual rest. However, introduction of rams stimulates a large proportion of the ewes to ovulate and exhibit oestrus one cycle later (Schinckel, 1954). Such ewes will therefore probably be mated after having undergone only one oestrous cycle. In such cases the possibility exists of an increased rate of foetal atrophy compared to ewes in which the reproductive tract has been "conditioned" during the course of several oestrous cycles (Dutt, 1954; Hulet, Voigtlander, Pope & Casida, 1956). This conclusion is supported by the finding that the number of services per conception is reduced when oestrous activity is initiated early in the post-partum period. (Thatcher & Wilcox, 1973; Britt, Morrow, Kittok & Seguin, 1974).

Ewes can be prevented from becoming anoestrous by changing their association with males. Thus, Lamond, Wells & Miller (1963) induced a high incidence of oestrus during the anoestrous season by isolating the ewes from rams for a short period and then rejoining them with rams. A similar effect has been produced by continuously associating ewes with rams (Lishman & Hunter, 1967; Lishman, 1969).

The onset of the new breeding season in spring can also be hastened by introducing vasectomized teaser rams into the ewe flock (Lyle & Hunter, 1965; Lyle & Hunter, 1967b) and breeding can then be effected after the ewes have undergone several oestrous cycles.

The object of this study was to determine whether the duration of the non-breeding season could be altered by manipulation of the extent to which ewes are associated with rams and whether the lambing rate would be influenced.

PROCEDURE

Immediately after parturition (March 1967) Merino and Dormer ewes were randomly allocated to four groups and until introduction of entire breeding rams on 8 November the ewes were treated as follows:

Group 1—continuously associated with vasectomized teaser rams—continuous group.

Group 2—associated with and isolated from teaser rams for alternate periods of 17 days—alternate group.

Group 3—teaser rams joined with the ewes for 34 days prior to 8 November—teased group.

Group 4—isolated from rams—isolated group.

During 1968 and 1969 the experiment was repeated with the following modifications:

(i) All ewes were isolated from rams until conclusion of the lactation period.

(ii) Mating with entire breeding rams commenced on 1 November.

(iii) Only Merino ewes were utilised.

A second flock (Flock 2) was also included during 1968 and here all the ewes were associated with rams during the 84-day lactation period. In this flock, Group 3 joined teaser rams 17 days prior to 1 November.

Observations for oestrus were made twice daily using vasectomized rams. After initiation of the annual breeding period the entire rams were fitted with raddle crayons (Radford, Watson & Wood, 1960) and the ewes that had been mated were recorded daily for the 42-day mating period.

After parturition the ewes were fed a ration of milled legume hay, maize silage and concentrate meal. The quantity of feed was regulated so as to maintain body condition. Summer grazing consisted of kikuyu (*Pennisetum clandestinum*) pasture and grazing was initiated when the ewes joined the breeding rams.

RESULTS

Seasonal changes in the incidence of oestrus

The incidence of oestrus was maintained above 40% throughout the anoestrous season where the ewes were continuously or alternately associated with teaser rams (Fig. 1). The only exception was that observed during the period commencing 28 September, 1969 and in this instance the difference between the aforementioned treatments was significant ($P<0,01$). Although isolation from rams tended to reduce the subsequent incidence of mating (Fig. 1) the number of ewes exhibiting oestrus spontaneously was lower in Group 3 (previously isolated) than in Group 1 (continuously with rams) only during the periods commencing 28 September 1968 (Flock 1, $P<0,05$) and 1969 ($P<0,01$).

Mating following introduction of entire rams

The majority of the ewes were mated within 25 days after commencement of the annual breeding period and the pattern of mating is depicted in Fig. 2. The mating pattern differed between treatments from year to year and even within the same year.

Association of the ewes with vasectomized rams for 17 or 34 days prior to the introduction of entire rams resulted in a shortening of the interval from introduction of breeding rams to first mating. (Table 1).

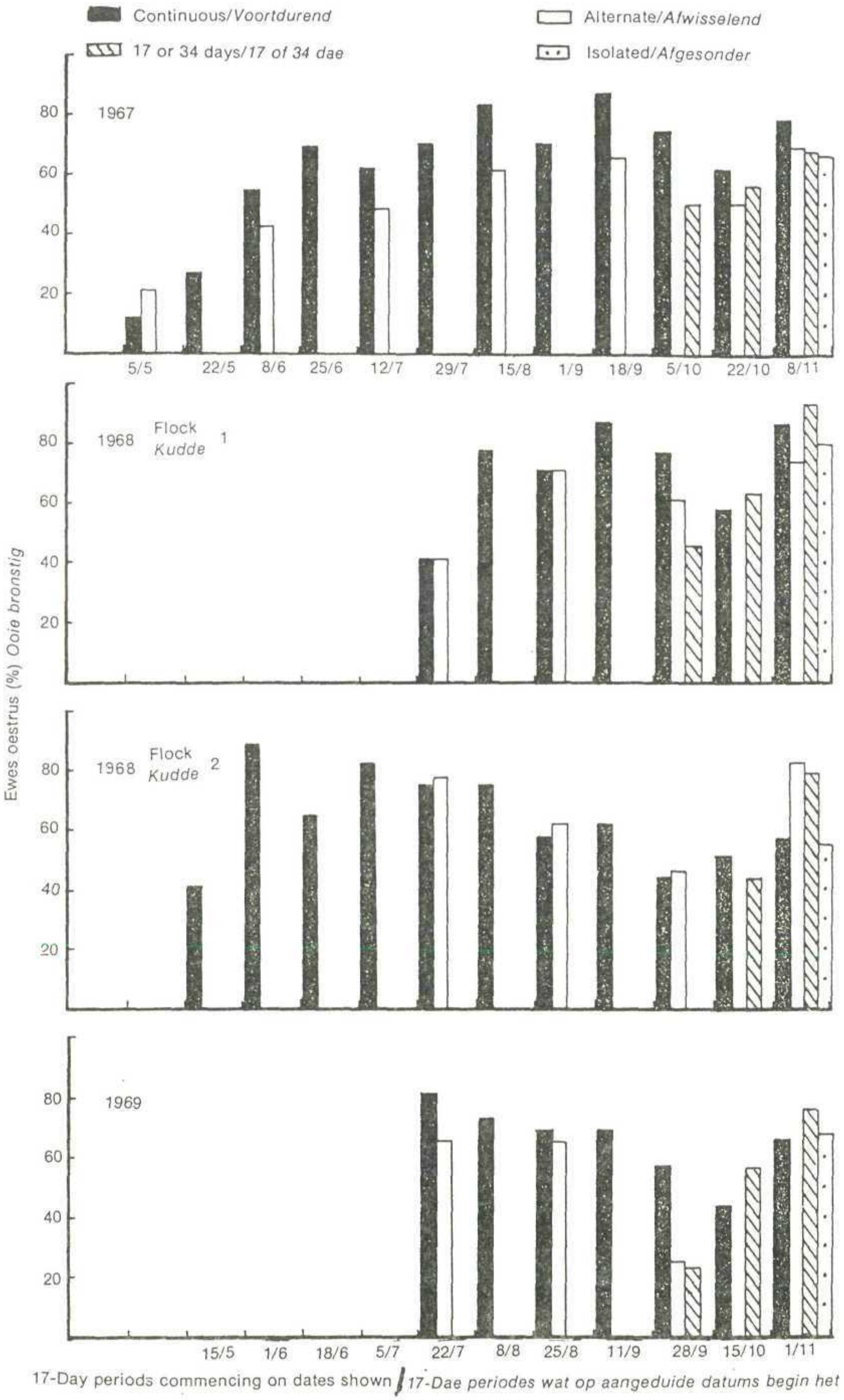

FIG. 1 The proportion of ewes oestrus following various periods of association with rams
FIG. 1 *Die verhouding ooie bronstig na verskillende periodes van assosiasie met ramme*

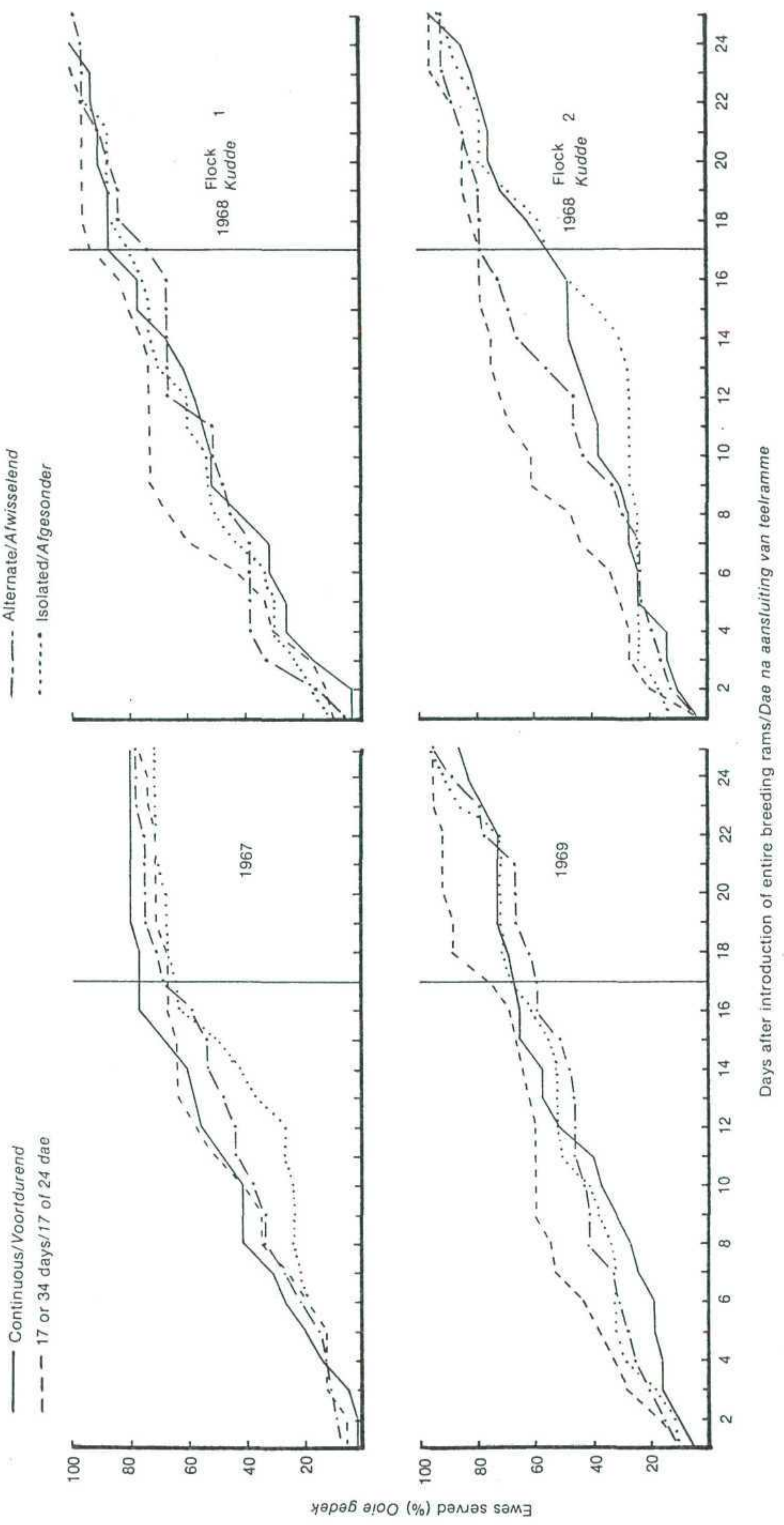

FIG. 2 Daily cumulative total numbers of ewes served by entire rams following varying periods of association with vasectomized rams
FIG. 2 *Daaglikse kumulatiewe getal ooie gedek deur teelramme na variërende periodes van assosiasie met koggelramme*

TABLE 1 The interval between introduction of entire breeding rams and the first subsequent mating. Figures which do not differ significantly are joined by an unbroken vertical line to the right, while the broken line to the left joins figures which differ significantly. *Indicates P=0,05

TABEL 1 *Die interval tussen byvoeging van vrugbare teelramme en die eerste daaropvolgende dekking. Syfers wat nie betekenisvol verskil nie word verbind deur 'n ononderbroke vertikale lyn aan die regterkant, terwyl die gebroke lyn aan die linkerkant syfers wat betekenisvol verskil, verbind. *Dui aan P=0,05*

Association with rams *Assosiasie met ramme*	Group *Groep*	Interval (days) to mating / *Interval (dae) tot dekking*							
		1967		1968				1969	
				Flock 1 *Kudde 1*		Flock 2 *Kudde 2*			
		Mean *Gemiddeld*	S E *S F*	Mean *Gemiddeld*	S E *S F*	Mean *Gemiddeld*	S E *S F*	Mean *Gemiddeld*	S E *S F*
Continuous/Voortdurend	1	6,6	0,90	10,9	1,18	13,8	1,48	10,9	1,11
Alternate/*Afwisselend*	2	8,9	1,13	* 10,4	1,36	12,1	1,36	* 15,4	0,84
Teased/*Gekoggel*	3	7,4	1,25	8,4	1,08	9,9	1,36	* 8,1	1,12
Isolated/*Afgesonder*	4	* 12,6	0,68	10,3	1,33	* 15,1	1,65	10,6	1,45

TABLE 2 Reproductive performance of ewes following varying periods of association with rams

TABEL 2 *Reproduksie by ooie na variërende periodes van assosiasie met ramme*

Year/*Jaar*	Association with rams *Assosiasie met ramme*	N	Reproductive performance/*Reproduksie*									% not mated *% nie gedek*	% not lambing* *% nie gelam**
			First oestrus *Eerste estrus*			Second oestrus *Tweede estrus*			Third oestrus *Derde estrus*				
			No. mated *Getal gedek*	Lambed/*Lam* No. *Getal*	Lambed/*Lam* %	No. mated *Getal gedek*	Lambed/*Lam* No. *Getal*	Lambed/*Lam* %	No. mated *Getal gedek*	Lambed/*Lam* No. *Getal*	Lambed/*Lam* %		
1967†....	Continuous/*Voortdurend.*	24	22	13	59,1	6	3	50,0	0	—	—	8,3	27,3
	Alternate/*Afwisselend....*	26	26	16	61,5	6	3	50,0	0	—	—	0	26,9
	Teased/*Gekoggel........*	16	16	7	43,7	7	6	85,7	0	—	—	0	18,7
	Isolated/*Afgesonder.....*	23	23	13	56,5	8	5	62,5	1	0	0	0	21,7
Flock 1/ *Kudde* 1	Continuous/*Voortdurend.*	31	31	25	80,6	5	2	40,0	1	0	0	0	12,9
	Alternate/*Afwisselend....*	31	31	24	77,4	3	3	100,0	0	—	—	0	12,9
	Teased/*Gekoggel........*	30	30	26	86,7	3	3	100,0	0	—	—	0	3,3
	Isolated/*Afgesonder......*	30	30	24	80,0	6	4	66,7	0	1	50,0	0	0
1968..... Flock 2/ *Kudde* 2	Continuous/*Voortdurend.*	29	29	20	68,9	5	2	40,0	1	0	0	0	24,1
	Alternate/*Afwisselend....*	30	30	23	76,7	4	2	50,0	1	1	100,0	0	13,2
	Teased/*Gekoggel........*	29	29	24	82,7	5	3	60,0	0	—	—	0	6,8
	Isolated/*Afgesonder.....*	28	28	19	67,8	5	4	80,0	0	—	—	0	20,4
1969.....	Continuous/*Voortdurend.*	38	33	27	89,8	4	2	50,0	1	1	100,0	13,1	9,1
	Alternate/*Afwisselend....*	39	39	31	79,5	5	4	80,0	0	—	—	0	10,2
	Teased/*Gekoggel........*	41	39	31	79,5	5	4	80,0	0	—	—	4,9	10,2
	Isolated/*Afgesonder.....*	43	42	40	95,2	2	2	100,0	0	—	—	2,3	0

† The Dormer ewes were disposed of before lambing/*Die Dormer-ooie is voor lamtyd verplaas*
* Expressed as a % of the number served/*Uitgedruk as 'n % van die getal gedek*

Conception and lambing rates

During any one year the conception rate at the first oestrus was not significantly influenced by the experimental treatments (Table 2). There was also no consistent indication that ewes which returned to service conceived less easily than ewes which were settled at the first oestrus following joining with breeding rams.

In general, continuous association of the ewes with vasectomized rams resulted in a greater number of barren ewes (ewes not served + ewes not lambing) than any other treatment.

Lambing rates as influenced by reproductive patterns prior to fertile mating

Frequent or continuous association of the ewes with vasectomized rams (Groups 1 and 2) did not prevent the occurrence of a seasonal anoestrus in all ewes. Furthermore, when the ewes were isolated from rams a variable proportion apparently continued to exhibit oestrous cycles.

In order to examine the effect of the reproductive state prior to mating with entire rams, each ewe was classified as "anoestrus" or "sexually active" when joined with entire rams. Ewes that had been isolated from rams for some time were classified as sexually active if they exhibited oestrus within 17 days (one oestrous cycle) after being re-associated with rams (either vasectomized or entire).

A pooling of the results (Table 3) showed that both the percentage of ewes not lambing ($P<0,05$) and those conceiving after the first oestrus ($P<0,01$) were lower amongst the anoestrous than amongst the sexually active ewes.

DISCUSSION

In spite of the considerable amount of research on reproduction in sheep the factors which determine the limits of the breeding season in the Merino ewe remain poorly understood. The present findings are in accordance with the hypothesis, suggested by Lyle & Hunter (1967a) and subsequently supported by Lishman, Stielau & Botha (1974a), that when mating takes place at a relatively fixed time each year the ewes tend to adapt to this procedure. The breeding season then apparently commences spontaneously at about this time each year. This point is illustrated by the high percentage of ewes mated within 17 days of being joined to entire breeding rams after a period of isolation from rams (Fig. 1, Fig. 2). In contrast, the incidence of oestrus a month earlier was appreciably lower in similarly treated ewes. It has been suggested that in view of the "seasonal" pattern of oestrus, a varying proportion of the flock is unlikely to mate in spring and early summer and that the lambing percentage is accordingly reduced (Adler, 1964). Our results do not support this contention and it is evident (Table 3) that only a very minor percentage of the ewes were not mated. This again suggests that conclusions based on the usual method of determining the limits of the breeding season viz., observing oestrus over a period of 12 months or more in a group of ewes continuously associated with sterile rams, may not be ideal. The Merino and allied breeds apparently can be bred successfully at virtually any time of the year provided this is preceded by a number of years during which a relatively fixed breeding time is adhered to.

TABLE 3 Reproductive performance of ewes which were either anoestrous or sexually active prior to joining with entire breeding rams
TABEL 3 *Reproduksie by ooie òf anestrus òf geslagsaktief voor byvoeging van teelramme*

Year *Jaar*	Association with rams *Assosiasie met ramme*	Anoestrous ewes/*Anestrus ooie*				Sexually active ewes/*Geslagsaktiewe ooie*			
		Not mated *Nie gedek*	Not Lambing *Nie gelam*	Conceived at/*Beset by*		Not mated *Nie gedek*	Not lambing *Nie gelam*	Conceived at/*Beset by*	
				First oestrus *Eerste estrus*	Subsequent oestrus *Daaropvolgende estrus*			First oestrus *Eerste estrus*	Subsequent oestrus *Daaropvolgende estrus*
1967.....	Continuous/*Voortdurend*....	0	0	1	0	2	6	11	4
	Alternate/*Afwisselend*......	0	0	1	0	0	7	15	3
	Teased/*Gekoggel*..........	0	0	6	2	0	3	2	3
	Isolated/*Afgesonder*.......	0	0	3	1	0	5	10	4
	Sub Total/*Subtotaal*.......	0	0	11	3	2	21	38	14
1968–Flock 1/*Kudde* 1	Continuous/*Voortdurend*....	0	1	4	0	0	3	21	2
	Alternate/*Afwisselend*......	0	1	5	2	0	3	19	1
	Teased/*Gekoggel*..........	0	0	13	2	0	1	13	1
	Isolated/*Afgesonder*.......	0	0	5	1	0	1	19	4
	Sub Total/*Subtotaal*.......	0	2	27	5	0	8	72	8
1968–Flock 2/*Kudde* 2	Continuous/*Voortdurend*....	0	3	9	0	0	4	11	2
	Alternate/*Afwisselend*......	0	1	15	1	0	3	8	1
	Teased/*Gekoggel*..........	0	0	15	2	0	2	9	1
	Isolated/*Afgesonder*.......	0	2	10	0	0	3	9	5
	Sub Total/*Subtotaal*.......	0	6	49	3	0	12	37	9
1969.....	Continuous/*Voortdurend*....	4	1	11	0	1	2	16	3
	Alternate/*Afwisselend*......	0	1	15	0	0	3	16	4
	Teased/*Gekoggel*..........	1	3	26	3	1	1	5	1
	Isolated/*Afgesonder*.......	1	0	13	0	0	0	27	2
	Sub Total/*Subtotaal*.......	6	5	65	3	2	6	64	10
	Total/*Totaal*.............	6	13	152	14	4	47	211	41
	%.....................	—	7,2(1)	—	8,4(2)	—	15,7(1)	—	16,2(2)

(1) Expressed as a % of the ewes served/*Uitgedruk as 'n % van die ooie gedek*

(2) Expressed as a % of the ewes lambing/*Uitgedruk as 'n % van die ooie wat gelam het*

In view of the evidence suggesting that fertility is improved when fertile mating is preceded by one or more oestrous cycles (Thatcher & Wilcox, 1973; Britt, *et al.*, 1974) it would appear advisable to select ewes that are able to continue cycling throughout the anoestrous season. However, this procedure may in fact reduce the overall fertility level of the flock (Table 3). It is perhaps more appropriate to favour females which respond readily to the stimulus of joining with rams, but which enter a state of sexual rest when not associated with males. The explanation for the increased fertility of the ewe which does not cycle continuously may perhaps be associated with the phenomenon of so-called pituitary fatigue as observed in the repeat-breeder dairy cow. The finding that non-parous ewes produce fewer lambs than parous ewes (Quinlivan, Martin, Taylor & Cairney, 1966) is in accordance with this proposal.

The results in Table 2 show an association between the % ewes conceiving at the first oestrus and the % not lambing. This implies that ewes which do not conceive at the first oestrus are less fertile than those which do. In order to reduce the chance of ewes with inherent low fertility conceiving and transmitting this characteristic to the progeny, the annual breeding period should be limited to not more than two oestrous cycles.

REFERENCES

ADLER, E. D., 1964. Breeding practices, and the fertility of cattle, pigs and sheep in the Natal Region. *Proc. S. Afr. Soc. Anim. Prod.*, 3, 13–24.

BRITT, J. H., MORROW, D. A., KITTOK, R. J. & SEGUIN, B. E., 1974. Uterine involution, ovarian activity and fertility after melengestrol acetate and oestradiol in early post-partum cows. *J. Dairy Sci.* 57, 89–92.

CHOPPING, M. H. & LINDSAY, D. R., 1970. An approach to the study of reproductive wastage in sheep. *Proc. Aust. Soc. Anim. Prod.* 8, 312–316.

DUTT, R. H., 1954. Fertility rate and embryonic death loss in ewes early in the breeding season. *J. Anim Sci.* 13, 464–473.

HULET, C. V., VOIGTLANDER, H., POPE, A. L. & CASIDA, L. E., 1956. The nature of early-season infertility in sheep. *J. Anim. Sci.* 15, 607–616.

HULET, C. V., ERCANBRACK, S. K. PRICE, D. A., BLACKWELL, R. L. & WILSON, L. O., 1962. Mating behaviour of the ram in the one-sire pen. *J. Anim. Sci.* 21, 857–864.

LAMOND, D. R., WELLS, K. E. & MILLER, S. J., 1963. Study of a breeding problem in Merino ewes in central Queensland. *Aust. vet. J.* 39, 295–298.

LISHMAN, A. W., 1969. The seasonal pattern of oestrus amongst ewes as affected by isolation from and joining with rams. *Agroanimalia* 1, 95–102.

LISHMAN, A. W. & De LANGE, G. M., 1967. The incidence of oestrus in Merino ewes following introduction of breeding rams in summer and spring. *Proc. S. Afr. Soc. Anim. Prod.* 6, 196–198.

LISHMAN, A. W. & HUNTER, G. L., 1966. Sexual activity in the ewe following isolation from the ram during the breeding season. *S. Afr. J. agric. Sci.* 9, 993–1004.

LISHMAN, A. W. & HUNTER, G. L., 1967. Oestrous activity in the ewe following isolation from the ram during anoestrus. *S. Afr. J. agric. Sci.* 10, 747–752.

LISHMAN, A. W., STIELAU, W. J. & BOTHA, W. A., 1974a. Reproduction in the ewe in relation to plane of nutrition, body mass and change of body mass. I. Incidence of oestrus between lambing and reconception. *Agroanimalia* 6, 25–34.

LISHMAN, A. W., STIELAU, W. J. & BOTHA, W. A., 1974b. Reproduction in the ewe in relation to plane of nutrition, body mass and change of body mass. II. Lambing performance. *Agroanimalia* 6, 75–80.

LYLE, A. D. & HUNTER, G. L., 1965. The effect on the occurrence of oestrus in the ewe during spring of six and 14 day teasing periods, prior to mating. *Proc. S. Afr. Soc. Anim. Prod.* 4, 140–142.

LYLE, A. D. & HUNTER, G. L., 1967a. The effects of ram introduction during spring, summer and autumn on the incidence of oestrus in three Merino breeding flocks in East Griqualand. *Proc. S. Afr. Soc. Anim. Prod.* 6, 202–207.

LYLE, A. D. & HUNTER, G. L., 1967b. Teasing groups of ewes at staggered intervals as a means of levelling the ram mating load and flock lambing rate. *S. Afr. J. agric. Sci.* 10, 597–608.

MATTNER, P. E., BRADEN, A. W. H. & TURNBULL, K. E., 1967. Studies in flock mating of sheep. I. Mating behaviour. *Aust. J. exp. Agric. Anim. Husb.* 7, 103–109.

MULLANEY, P. D., 1966. Pre-natal losses in sheep in western Victoria. *Proc. Aust. Soc. Anim. Prod.* 6, 56–59.

PARSONS, S. D. & HUNTER, G. L., 1965. Practices controlling fertility and flock improvement in sheep farming. *Proc. S. Afr. Soc. Anim. Prod.* 4, 136–139.

QUINLIVAN, T. D., MARTIN, C. A., TAYLOR, W. B. & CAIRNEY, I. M., 1966. Estimates of pre- and perinatal mortality in the New Zealand Romney Marsh ewe. II. Pre- and perinatal loss in those ewes that conceived to second service and those that returned to second service and were mated a third time. *J. Reprod. Fert.* 11, 391–398.

RADFORD, H. M., WATSON, R. H. & WOOD, G. F., 1960. A crayon and associated harness for the detection of mating under field conditions. *Aust. vet. J.* 36, 57–66.

SCHINCKEL, P. G., 1954. The effect of the ram on the incidence and occurrence of oestrus in ewes. *Aust. vet. J.* 30, 189–195.

THATCHER, W. W. & WILCOX, C. J., 1973. Postpartum oestrus as an indicator of reproductive status in the dairy cow. *J. Dairy Sci.* 56, 608–610.

ABILITY OF MASCULINISED EWES TO STIMULATE ONSET OF THE BREEDING SEASON IN MAIDEN MERINO EWES

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Lishman and Hunter (1965) described circumstances under which the use of rams to detect oestrus in ewes could result in undesired influences. These workers suggested that the problem might be solved by the use of masculinised teaser ewes and described techniques which resulted in male mating behaviour in female sheep. Parsons and Hunter (1968) demonstrated that when masculinised ewes were continuously associated with normal oestrual ewes, a significant reduction in the duration of oestrus resulted. However, the influence of teaser ewes on the onset of the breeding season has not previously been reported and the experiment to be described was designed to investigate this aspect.

During October 1967, 84 two-tooth, maiden Merino ewes were randomly divided into three euqally sized groups. Mating with fertile rams was planned to commence on November 1 and during the preceding 15 days the ewes received the following treatments:

Group 1 – Isolated from contact with other sheep,
Group 2 – Joined with vasectomized rams,
Group 3 – Joined with masculinised (teaser) ewes.

Four ewes were masculinised by daily intramuscular injections of 1 mg stilboestrol dipropionate and treatment was commenced seven days prior to their joining the ewes in Group 3. Injections were continued for a further 15 days.

During the 15 day period prior to November 1 three vasectomized rams and three teaser ewes were associated with the appropriate groups of ewes. Each day one ram and one masculinised ewe were replaced by others which had been rested for a day.

On November 1 the three groups of maiden ewes were combined into one flock and joined with three fertile rams fitted with "Sire-sine" harnesses and marking crayons (Radford, Watson & Wood, 1960). The ewes that had been served were identified daily and during the course of the experiment the animals were grazed on kikuyu pasture.

The number of ewes in each group which mated during the 24 days after joining with raddled fertile rams is presented in Fig. 1.

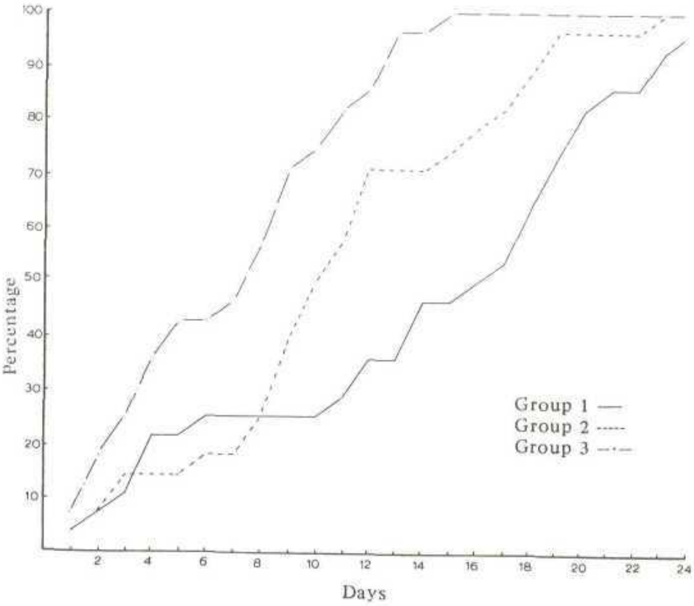

Fig. 1. – *Daily cumulative totals of ewes marked in three treatment groups*

From Fig. 1 it can be seen that the incidence of oestrus in the ewes previously associated with vasectomized rams (Group 2) or masculinized ewes (Group 3) followed a similar pattern. However, amongst the ewes in Group 1 which were isolated until November 1, only 53.6 % of the females were served during the first 17 days (one oestrous cycle) after joining with fertile rams. According to the Chi-square test, significantly more ewes had mated in Group 3 by days 12 ($P<.01$), 13 ($P<.01$), 17 ($P<.05$) and 18 ($P<.05$) of the fertile mating period than in Group 1. The highest number of ewes marked on any one day was 14.3 % which occurred on day 9 in Groups 2 and 3 and day 19 in Group 1. In Group 2 this represented day 24 after joining vasectomized rams and agrees with previous findings on this station (Lishman & de Lange, 1967).

The difference in response between ewes associated with vasectomized rams and those joined with masculinised ewes is possibly due to two factors. Firstly, the ewes in Group 3 averaged 87.0 lb at mating, while those in Groups 1 and 2 were on the average 5 lb lighter. In ewes of this type sexual activity can be expected to be correlated with maturity, as indicated by liveweight. Secondly, the masculinised ewes had been grazed together during the seven days prior to being joined with the maiden ewes in Group 3. The former tended to remain together after joining the maiden ewes and were inclined to limit their attention to each other. It is interesting to note that the masculine behaviour persisted for at least five months in one ewe, although no further hormone treatment was applied.

In view of the present finding viz., that masculinised ewes can stimulate onset of the breeding season and also reduce the duration of oestrus, as reported by Parsons and Hunter (1967), it would appear that the use of such animals to detect oestrus is associaed with stimuli similar to that produced by vasectomized or fertile males. However, masculinized females possess one advantage in that they can be used without the danger of spreading diseases normally transmitted during copulation.

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References

LISHMAN, A.W. & HUNTER, G.L., 1965. Proc. S. Afr. Soc. Anim. Prod. 5, 142.

LISHMAN, A.W. & DE LANGE, G.M., 1967. Proc. S. Afr. Soc. Anim. Prod. 7, 196.

PARSONS, S.D. & HUNTER, G.L., 1967. J. Reprod. Fert. 14, 61.

RADFORD, H.M., WATSON, R.H. & WOOD, G.F., 1960. Aust. vet. J. 36, 57.

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REDUCED SENSITIVITY TO OESTROGEN IN EWES CONTINUOUSLY ASSOCIATED WITH RAMS

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OPSOMMING: VERLAAGDE SENSITIWITEIT VIR ESTROGEEN BY OOIE VOORTDUREND IN KONTAK MET RAMME

Die voorkoms van bronstigheid was beduidend hoër (P<0,001) na oestradiol bensoaat (ODB) behandeling van ooie wat gedurende die anestrus seisoen vir drie maande van ramme afgesonder was, as by ooie wat nie van ramme afgesonder was nie. Bronstigheid is ook na 'n korter latente periode by die afgesonderde ooie waargeneem as by die ooie wat voortdurend by ramme was. In 'n tweede proef is die eierstokke van 44 van die 84 ooie verwyder voor afsondering van, of assosiasie met ramme. Vyf maande later het die ooie progesteroon vir drie, ses of 12 dae ontvang voor behandeling met ODB. Beide die totale aantal ooie bronstig en die wat binne 40 uur na ODB inspuiting estrus getoon het, was beduidend hoër by die ooie wat afgesonder was as by die wat voortdurend by ramme was. Beduidend meer ooie was binne 40 uur bronstig na ses of 12 dae van progesteroonbehandeling as na drie dae. Alhoewel die latente periode tot bronstigheid korter was by die afgesonderde ooie as dié wat nie afgesonder was nie, was die verskil net by die intakte ooie betekenisvol. Die verwydering van die eierstokke het nie die resultate beduidend beïnvloed nie.

SUMMARY:

Oestradiol benzoate (ODB) injection of ewes isolated from rams for three months during the anoestrous season resulted in a significantly greater (P<0,001) incidence of oestrus than in anoestrous ewes not isolated from rams. Mating was initiated after a shorter latent period in the isolated ewes than in those continuously with rams. In a second experiment 44 of the 84 ewes were ovariectomized one month prior to the commencement of either isolation from or continuous association with rams. Five months after the ovariectomies, all ewes received progesterone for three, six or twelve days prior to ODB injection. The total number of ewes exhibiting oestrus and the number which mated within 40 h after ODB injection were significantly higher among the isolated ewes than amongst those continuously with rams. Significantly more ewes exhibited oestrus after 6 or 12 d of progesterone than where progesterone was administered for 3 d. Although the latency to oestrus was shorter in the isolated ewes than in those not isolated, the difference was significant only in the ewes not ovariectomized. The presence or absence of ovaries did not influence the results significantly.

Schinckel (1954) observed that ewes which had been continuously associated with sterile rams during the non-breeding season resumed sexual activity at a later date than ewes isolated from rams during anoestrus. He therefore suggested that continuous association of the two sexes led to a deepening or lengthening anoestrus. Individual differences in the sensitivity of ewes to oestrogen have been demonstrated (Robinson, 1955a; Robinson & Moore, 1956) and it is possible that the continuous presence of the ram during the anoestrous season might modify the sensitivity of the nervous mechanisms mediating oestrous behaviour in the ewe. This experiment was conducted to examine the effect of association with rams on the response of ewes to stimuli which evoke oestrus.

Procedure

Experiment 1

On July 3, a flock of Merino ewes (two-tooth and mature) was randomly divided into two groups and one group transferred to partly-roofed pens where they were joined with vasectomized teaser rams (continuous group). The second group remained in similar pens situated approximately 1 km from the nearest ram (isolated group).

Observations for oestrus (permitted service) amongst the ewes continuously associated with rams were made by teasing at 8 h intervals and of the ewes which had not exhibited oestrus for at least 40 d prior to the commencement of progesterone injection on September 15, 24 animals were randomly selected for inclusion in the experiment. The same number of ewes was randomly selected from the flock that had been isolated from rams.

Treatment of each ewe consisted of daily intramuscular injections of 10 mg progesterone in 1 ml arachis oil, administered for 3 d at 08h00, followed 24 h later by injection (i.m.) of 20 μg ODB. The length of the preliminary progesterone sensitization period was selected for a response of approximately 50% in terms of ewes exhibiting behavioural oestrus (Robinson, 1955b; Robinson, Moore & Binet, 1956) so that differences in sensitivity to oestrogen would not be obliterated. The ODB dosage of 20 μg is equivalent to a physiological dose (Robinson, 1955b; Robinson *et al.*, 1956). The ewes that had been isolated from rams were joined with vasectomized rams 24 h after ODB injection. When observations for oestrus had been terminated the ovaries of selected ewes were examined for recent ovulations.

Experiment 2

One June 22, a group of 40 mature, entire Merino ewes and a similar group of 44 ewes which had been ovariectomized one month earlier were randomly subdivided into two groups. One group of ovariectomized and one of entire ewes were isolated from rams, and the

Table 1

Incidence of, latency to, and duration of oestrus following progesterone-oestrogen treatment and the occurrence of ovulation in ewes either isolated from, or continuously associated with rams

			Latency to oestrus (h)		Duration of oestrus (h)		Occurrence of ovulation:			
							Ewes oestrus		Ewes not oestrus	
Association with rams	n	No. of ewes oestrus	Mean	S E	Mean	S E	No. examined	No. ovulating	No. examined	No ovulating
Continuous	24	3	45,3	±7,0	21,3	±12,2	2	1	10	9
Isolated	24	22	34,5	±1,6	26,9	± 2,4	12	6	2	2

Table 2

Incidence of oestrus in ovariectomized and entire ewes, either continuously associated with or isolated from rams and treated for varying periods with progesterone prior to oestrogen injection

		Percentage ewes oestrus following progesterone for:										
		3 days			6 days			12 days			All progesterone treatments	
Association with rams	Type of ewe	n	Oestrus within 40h	Oestrus within 80h	n	Oestrus within 40h	Oestrus within 80h	n	Oestrus within 40h	Oestrus within 80h	Oestrus within 40h	Oestrus within 80h
Continuous	Entire	7	14,3	42,8	6	33,3	33,3	7	42,8	85,7	30,0	55,5
	Spayed	7	0	28,6	7	50,1	50,0	6	16,7	16,7	20,0	30,0
Isolated	Entire	7	42,8	42,8	7	85,7	100,0	6	83,3	100,0	70,0	80,0
	Spayed	8	50,0	62,5	7	71,4	71,4	7	57,1	57,1	59,1	63,6

Table 3

Latency to, and duration of oestrus in ovariectomized and entire ewes treated with ODB following isolation or continuous association with rams

		Latency to oestrus (h)		Duration of oestrus (h)	
Association with rams	Type of ewe	Mean	S E	Mean	S E
Continuous	Entire	37,6	±1,36	24,7	±10,58
	Spayed	35,3	±6,14	22,1	±12,16
Isolated	Entire	29,8	±2,46	23,9	± 9,36
	Spayed	26,6	±2,25	16,4	± 7,55

Table 4

Incidence of, latency to, and duration of oestrus following ODB treatment of ewes four weeks after being re-associated with rams and in ewes continuously associated with rams

				Latency to oestrus (h)		Duration of oestrus (h)	
Association with rams	Type of ewe	n	Ewes oestrus	Mean	S E	Mean	S E
Continuous	Entire	20	5	42,58	±4,84	24,38	±8,60
	Spayed	20	9	32,10	±11,04	18,78	±10,44
Isolated	Entire	20	3	36,07	±18,54	28,33	±10,60
	Spayed	21*	8	28,99	±8,26	23,88	±4,29

* One ewe died

remaining groups joined with vasectomized rams. Amongst the latter ewes observations for oestrus were made twice daily using a fresh group of vasectomized rams.

On October 22, each sub-group was again subdivided into three groups and progesterone administered daily at 08h00 for 12, 6 or 3 d prior to ODB injection. Twenty-four hours after the last progesterone injection each ewe received 20 μg ODB and 24 h later the isolated ewes were joined with vasectomized rams. Continuous observations for oestrus were initiated 12 h later and ceased after 80 h. During this period fresh rams were introduced at intervals of 4 h. Commencing on November 26 the ewes were subjected to a uniform treatment consisting of progesterone for 6 d followed by 20 μg ODB. Observations for oestrus were made as before.

General management of ewes

The ewes were fed a daily ration of 1 kg milled lucerne hay, 110 g maize and unmilled grass hay *ad lib.* A mineral lick was available at all times. The ewes were weighed at regular intervals as a check on adequate nutrition.

Results

Experiment 1

The proportion of isolated ewes which exhibited oestrus was significantly greater ($P < 0,001$) than in the continuous group (Table 1). Furthermore, among the isolated ewes the interval between ODB injection and the commencement of oestrus was significantly shorter than for the continuous group. The duration of oestrus showed marked variation and the differences observed (Table 1) were not significant.

An examination of the ovaries failed to account for the low incidence of oestrus amongst the ewes not isolated from rams (Table 1).

Experiment 2

A significantly greater proportion of the ewes which had been isolated from rams exhibited oestrus both within 40 h ($P < 0,001$) and within 80 h ($P < 0,01$) after ODB injection than amongst the ewes continuously associated with rams (Table 2). There was no significant difference between the entire and ovariectomized ewes. Compared to pre-treatment for three days, the progesterone sensitization periods of six and 12 days significantly increased the response observed within 40 h after ODB injection.

Due to the great variation between individuals, most differences in the latency to and duration of oestrus were not significant. Continuous presence of the ram significantly increased the latent period amongst the entire ewes ($P = 0,05$).

When re-tested four weeks later the increased sensitivity of the isolated ewes to ODB was no longer significant (Table 4), although the results obtained for the entire ewes were remarkably similar to those recorded in Experiment 1.

Discussion

It has been clearly demonstrated that the presence of the ram, especially during the anoestrous season, is necessary to maintain oestrous activity in ewes (Lishman & Hunter, 1966; 1967; Lishman, 1969). This contradicts the hypothesis of a deepened anoestrus when ewes are continuously associated with rams (Schinckel, 1954). In contrast, the present findings support Schinckel, since they suggest that when ewes are subjected to the mating stimulus for several months, adaptation to this stimulus occurs. Such females apparently became less sensitive to stimuli which induce overt oestrus. The finding that 17% of the ewes continuously associated with rams exhibited oestrus later than the average onset of breeding (Radford & Watson, 1957) agrees with this conclusion. These findings support the contention that in order to induce a high incidence of oestrus, where the mating period is timed to commence at the onset of the breeding season (spring), the ewes should be isolated from the rams during the anoestrous period.

At this stage, only speculation is possible on the mechanisms whereby continuous association with rams reduces the sensitivity to oestrogen. The observation that the ovary is not required for the effect of continued presence of the male to be mediated, favours the conclusions that the anterior hypothalamic centres controlling oestrous behaviour are involved. In considering the components of the total "ram stimulus" it has generally been assumed that the factors of sight, sound and smell are of prime importance and that where ewes are to be isolated from the ram stimulus these factors should be precluded from influencing the ewes. However, the recent observation (Louw, unpublished) of almost complete anoestrus in a group of ewes separated from rams by a single pen (4 m wide) containing weaned lambs, suggests that none of the components listed is effective in producing the ram stimulus. It is suggested therefore that the mating behaviour of the ram must be directed toward the ewe in order to produce the stimulus. This agrees with the hypothesis suggested by Parsons & Hunter (1967) to account for the reduced duration of oestrus in ewes frequently exposed to the mating stimulus during oestrus.

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Reference

LISHMAN, A.W., 1969. The seasonal patterns of oestrus amongst ewes as affected by isolation from and joining with rams. *Agroanimalia,* 1, 95.

LISHMAN, A.W. & HUNTER, G.L., 1966. Sexual activity in the ewe following isolation from the ram during the breeding season. *S. Afr. J. agric. Sci.* 9, 993.

LISHMAN, A.W. & HUNTER, G.L., 1967. Oestrous activity in the ewe following isolation from the ram during anoestrus. *S. Afr. J. agric. Sci.* 10, 747.

PARSONS, S.D. & HUNTER, G.L., 1967. Effect of the ram on duration of oestrus in the ewe. *J. Reprod. Fert.* 14, 61.

RADFORD, H.M. & WATSON, R.H., 1957. Influence of rams on ovarian activity and oestrus in Merino ewes in the spring and early summer. *Aust. J. agric. Res.* 8, 460.

ROBINSON, T.J., 1955a. Quantitative studies on the hormonal induction of oestrus in spayed ewes. *J. Endocr.* 12, 163.

ROBINSON, T.J., 1955b. Endocrine relationships in the induction of oestrus and ovulation in the anoestrous ewe. *J. agric. Sci. Camb.* 46, 37.

ROBINSON, T.J. & MOORE, N.W., 1956. The interaction of oestrogen and progesterone on the vaginal cycle of the ewe. *J. Endocr.* 14, 97.

ROBINSON, T.J., MOORE, N.W. & BINET, F.E., 1956. The effect of the duration of progesterone pre-treatment on the response of the spayed ewe to oestrogen. *J. Endocr.* 14, 1.

SCHINCKEL, P.G., 1954. The effect of the ram on the incidence and occurrence of oestrus in ewes. *Aust. vet. J.* 30. 189.

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PLASMA LUTEINIZING HORMONE LEVELS IN EWES FAILING TO EXHIBIT OESTRUS DURING LACTATION AND IN EWES ISOLATED FROM RAMS

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OPSOMMING: LUTEINISERENDE HORMOON PEILE IN DIE PLASMA VAN OOIE ANESTRUS GEDURENDE LAKTASIE EN BY OOIE VAN RAMME AFGESONDER

Daar was geen betekenisvolle verskil in die peil van luteiniserende hormoon in die plasma van ooie wat geen oestrus gedurende laktasie getoon het nie en die tussen-estrus peil van ooie wat gereeld bronstig was. Die LH peil was beduidend laer by ooie wat van ramme afgesonder was as by soortgelyke, maar anestrus, ooie wat voortdurend by ramme geloop het. Die basale LH peil by ooie wat gereeld bronstig was, het relatief min gevarieer van dag tot dag, terwyl merkwaardigde skommelinge in die daaglikse monsters van ooie wat enstrus was, verkry is.

SUMMARY

The plasma LH level of ewes which did not exhibit oestrus during lactation was not significantly different from the inter-oestrus level of ewes showing regular oestrus. The LH level in the plasma of ewes kept isolated from rams was significantly lower than amongst similar, but anoestrous, ewes continuously associated with rams. In ewes which were cycling regularly the basal LH level remained relatively constant from day to day during inter-oestrus, whereas in anoestrous ewes the daily samples showed considerable fluctuation.

Cyclic oestrous activity in the ewe is not resumed immediately after parturition, and when breeding management dictates short intervals between successive lambings, the duration of the post-partum anoestrous period is important. The factors which affect the delay to first oestrus following parturition have been described by Hunter (1968, 1971), but little is known concerning the hormonal mechanisms controlling anoestrus.

It is probable that the breeding and non-breeding seasons in sheep are partly related to the rate of synthesis and/or release of luteinizing hormone (LH) from the adenohypophysis (Dutt, 1960; Robertson & Hutchinson, 1962). Accordingly, the level of LH in the circulation would differ between the anoestrous ewe and the ewe showing regular oestrus. Roche, Foster, Karsch, Cook & Dzuik (1970) drew no conclusions regarding the LH levels in such ewes, but noted that during the anoestrous period the LH levels were lowest during mid-anoestrus.

In this investigation of anoestrus, plasma levels of LH were measured in ewes which did not exhibit oestrus during lactation. Ewes which had been isolated from rams during anoestrus were also studied since such animals exhibit a lower incidence of oestrus (Lishman & Hunter, 1967) and ovulation (Hunter, 1969) than ewes which are associated with rams.

Procedure

Maiden and mature Merino ewes that had lambed in autumn were used in the experiment. The ewes were fed a ration of milled lucerne hay, maize silage and a concentrate mixture in such amounts that one group maintained body condition (high plane) while the other experienced an average loss of 20% in bodymass (low plane) during the 84-day lactation period. During the post-weaning period all ewes were fed the same amounts of feed. After lambing at the end of March 1970 twice-daily observations for oestrus were made using vasectomized rams.

At the conclusion of the lactation period blood samples were obtained from the ewes that had not exhibited oestrus during the suckling period. Samples obtained during the inter-oestrus period from ewes which were showing oestrus regularly at this time (late June), served as controls.

Just prior to the commencement of the new breeding season (mid-October) blood samples were taken from ewes which had been isolated from rams since weaning. Samples were drawn from at least five maiden and five mature ewes which had been randomly selected from amongst the animals on each of the two planes of nutrition. These were compared with ewes that had been continuously associated with rams, but which were anoestrus at the time of sampling. In all cases blood samples were collected by jugular venipuncture on five consecutive days and at the same time each day (14h00). Samples (10cm^3) were collected into heparinized test tubes, the plasma separated by centrifugation and the latter stored at -15°C until assayed.

LH was measured using the double-antibody radioimmunoassay developed by Niswender, Reichert, Midgley & Nalbandov (1969). Purified ovine LH (Papkoff preparation G3-206) was iodinated with ^{125}I (Radiochemical Centre, Amersham). NIH-LH-S16 was used as the standard hormone and all plasma values were expressed in terms of this preparation. The anti-ovine LH serum was used at an initial dilution of 1:100000 (Lishman, Stielau, Dreosti & Stewart, 1973). All determinations of plasma LH were performed in duplicate, using not more than 0,2cm^3 of plasma.

Results

Ewes anoestrus throughout lactation

The mean plasma LH levels during five consecutive days, in the various groups, is shown in Table 1. Although

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Table 1

Plasma LH levels in ewes not exhibiting oestrus during lactation (anoestrus) compared to inter-oestrous samples from cycling ewes (oestrus)

Plane of nutrition during lactation	Reproductive state during lactation	Age of ewe	Number of animals	Average plasma LH level for five daily samplings ng/cm^3 ± S.E.	No. of ewes in which a single daily sample exceeded 5,0 ngLH/cm^3
High	Oestrus	Maiden	5	2,95 ± 0,67	0
		Mature	6	3,75 ± 0,61	0
	Anoestrus	Maiden	3	4,32 ± 0,87	1
		Mature	6	4,19 ± 0,61	2
Low	Oestrus	Maiden	3	3,13 ± 0,87	0
		Mature	5	3,53 ± 0,67	0
	Anoestrus	Maiden	13	3,96 ± 0,42	5
		Mature	10	3,27 ± 0,48	1

Table 2

Plasma LH levels in anoestrous ewes continuously associated with rams and similar ewes isolated from rams

Plane of nutrition during lactation	Association with rams following weaning	Age of ewe	Number of animals	Average plasma LH level for five daily samplings ng/cm^3 ± S.E.	No. of ewes in which at least one daily sample exceeded 5,0 ng LH/cm^3
High	Continuous	Maiden	5	3,35 ± 0,49	1
		Mature	5	4,09 ± 0,49	2
	Isolated	Maiden	7	2,76 ± 0,42	1
		Mature	8	2,95 ± 0,39	2
Low	Continuous	Maiden	5	3,85 ± 0,49	1
		Mature	7	4,66 ± 0,42	4
	Isolated	Maiden	7	2,94 ± 0,42	1
		Mature	9	3,19 ± 0,39	1

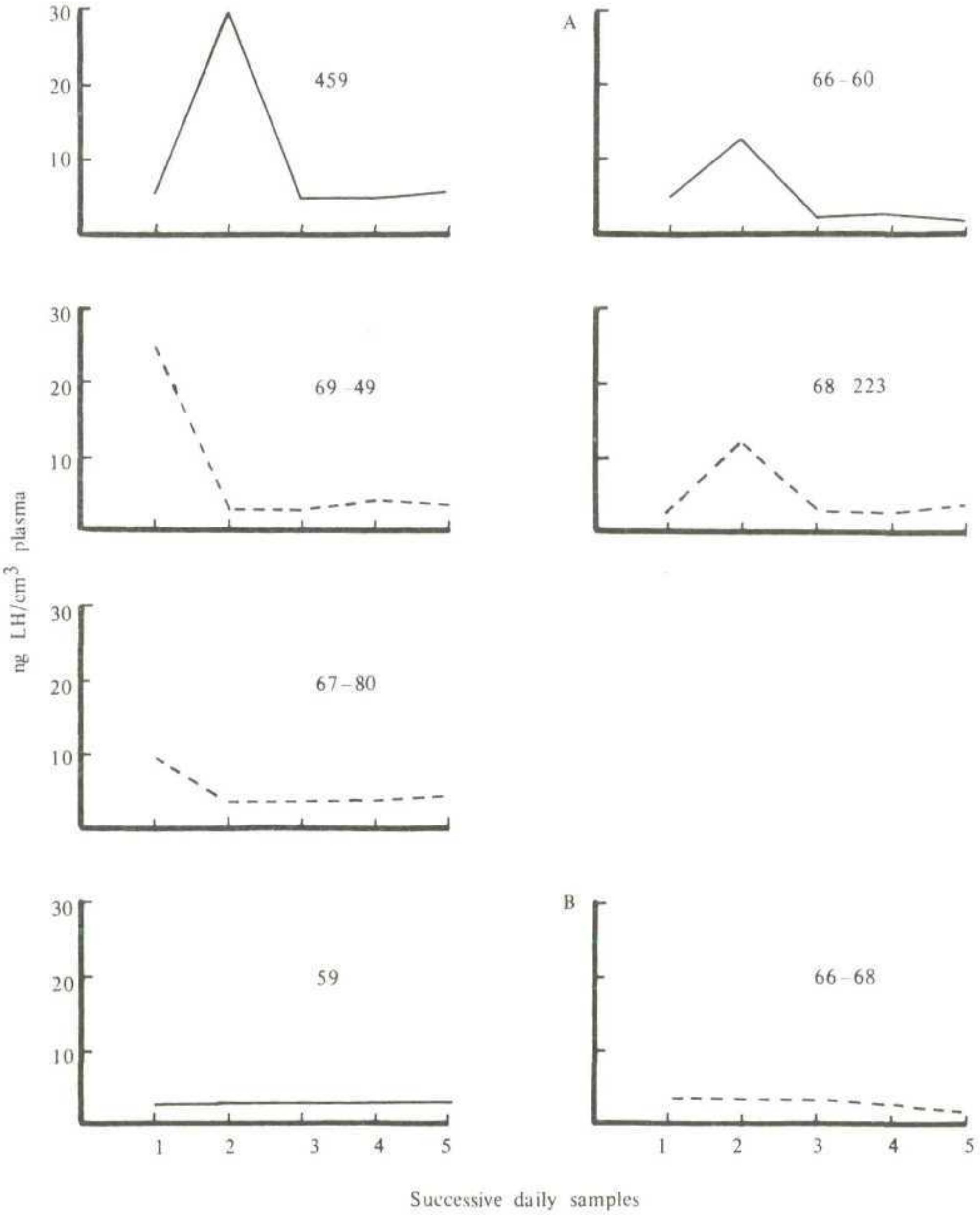

Fig. 1. *Plasma LH levels illustrating marked daily variation in ewes not exhibiting oestrus during lactation (A) compared to dioestrous levels of two randomly selected cycling ewes (B).*

—— *High plane of nutrition during lactation.*

- - - - *Low plane of nutrition during lactation.*

mean plasma LH levels in maiden ewes tended to differ from those in mature ewes the differences were not statistically significant (least squares analysis). Similarly, neither the reproductive state nor the plane of nutrition had a significant effect on the mean plasma LH level (Table 1). In certain individuals one of the daily samples showed a marked deviation from the remaining values (Table 1) and the more striking examples are illustrated in Fig. 1. This phenomenon was not observed amongst the ewes exhibiting regular oestrus.

Ewes isolated from rams

During late anoestrus (October, 1970) plasma LH levels were lower in ewes isolated from rams than in ewes in continuous contact with rams (least square analysis, $P = 0{,}01$; Table 2). The occurrence of elevated levels of LH ($>5{,}0$ ng/cm^3) in anoestrous ewes was again evident.

Discussion

The results presented here do not support the hypothesis that some ewes fail to ovulate and remain anoestrus throughout lactation because of an inadequate, tonic release of LH from the pituitary gland. This is in accordance with the finding that pituitary stores of gonadotrophin are not reduced during anoestrus (Roche *et al.*, 1970). However, the findings of Roche *et al.* (1970) and Pelletier & Thimonier (1973) suggest that the ability of the pituitary to rapidly synthesise and release gonadotrophin, in amounts sufficient to induce ovulation, is impaired during lactation.

It has recently been demonstrated that in sheep (Cumming, Brown, Blockey & Goding, 1971) and in man (Nankin & Troen, 1972; Root, De-Cherney, Russ, Duckett, Garcia & Wallach, 1972; Murray & Corker, 1973) there is a pulsatile release of LH from the pituitary gland. However, the suppressive action of gonadal steroids maintains the tonic release of LH at a relatively low level (Brown, Cumming, Goding & Hearnshaw, 1972).

The minor surges in plasma LH reported here and also observed in anoestrous (Butler, Bolt & Malvern, 1971) and ovariectomized ewes (Roche *et al.*, 1970; Reeves, O'Donell & Denorscia, 1972) probably reflects an interruption of the negative feed back system (Reeves *et al.*, 1972) Under such conditions there is a cyclic release of LH (Reeves *et al.*, 1972) rather than repetitive abrupt discharges.

Such fluctuations in hormone levels can have an important influence on the results obtained, depending on the sampling procedure followed. Findings based on samplings repeated several times each day and continued over a number of days would be preferable.

Evidence currently available suggests that various factors can influence the level of LH in the circulation. This is indicated by the finding that both in underfed (Howland, 1971a, 1972; Howland & Skinner, 1973) and young (Howland, 1971b) laboratory animals the serum LH level is reduced. Our results do not demonstrate a consistent effect of age or nutritional level, but suggest that a further factor viz., association with rams can influence the basal level of LH in the blood. The latter is in conformity with the results of Lishman & Hunter (1966, 1967), Hunter (1969) and Lishman (1969) where it was demonstrated that ewes which are isolated from rams tend to enter a state of anoestrus.

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References

BROWN, J.M., CUMMING, I.A., GODING, J.R. & HEARNSHAW, H., 1972. Control of baseline levels of luteinizing hormone in the ewe. *J. Reprod. Fert. 28,* 134.

BUTLER, W.R., BOLT, D.J. & MALVERN, P.V., 1971. Transient surges in LH release in ovariectomized sheep. *The Physiologist, 14,* 117.

CUMMING, I.A., BROWN, J.M., BLOCKEY, M.A. de B. & GODING, J.R., 1971. Regulation of the oestrous cycle in the ewe. *J. Reprod. Fert, 24,* 148.

DUTT, R.H., 1960. The effect of germ cell damage on animal reproduction. *J. Dairy Sci. 43,* 123 (Suppl.).

HOWLAND, B.E., 1971a Gonadotrophin levels in female rats subjected to restricted feed intake. *J. Reprod. Fert. 27,* 467.

HOWLAND, B.E., 1971b. Effect of restricted feed intake on ovarian compensatory hypertrophy in the rat. *J. Anim. Sci. 33,* 83.

HOWLAND, B.E., 1972. Ovarian weight and ovarian compensatory hypertrophy in the rat as affected by duration of underfeeding. *J. Reprod. Fert. 28,* 321.

HOWLAND, B.E. & SKINNER, K.R., 1973. Effect of starvation on LH levels in male and female hamsters. *J. Reprod. Fert. 32,* 505.

HUNTER, G.L., 1968. Increasing the frequency of pregnancy in sheep. I. Some factors affecting rebreeding during the post-partum period. *Anim. Breed. Abstr. 36,* 347.

HUNTER, G.L., 1969. The rôle of the ram when synchronising the mating of sheep with progestogens. *Proc. S. Afr. Soc. Anim. Prod. 8,* 143.

HUNTER, G.L., 1971 Is there a lactation anoestrus in the sheep? *S. Afr. J. Anim. Sci. 1,* 55.

LISHMAN, A.W., 1969. The seasonal pattern of oestrus amongst ewes as affected by isolation from and joining with rams. *Agroanimalia 1*, 95.

LISHMAN, A.W. & HUNTER, G.L., 1966. Sexual activity in the ewe following isolation from the ram during the breeding season. *S. Afr. J. agric. Sci. 9*, 993.

LISHMAN, A.W. & HUNTER, G.L., 1967. Oestrous activity in the ewe following isolation from the ram during anoestrus. *S. Afr. J. agric. Sci. 10*, 747.

LISHMAN, A.W., STIELAU, W.J., DREOSTI, I.E. & STEWART, A.M., 1973. Radio-immunological evaluation of antisera to ovine luteinizing hormone. *S. Afr. J. Anim. Sci. 3*, 67.

MURRAY, M.A.F. & CORKER, C.S., 1973. Levels of testosterone and luteinizing hormone in plasma samples taken at 10-minute intervals in normal men. *J. Endocr. 56*, 157.

NANKIN, H.R. & TROEN, P., 1972. Overnight patterns of serum luteinizing hormone in normal men. *J. clin. Endocr. Metab. 35*, 705.

NISWENDER, G.D., REICHERT, L.E., MIDGLEY, A.R. & NALBANDOV, A.V., 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology 84*, 1166.

PELLETIER, J. & THIMONIER, J., 1973. Comparison of the induced preovulatory LH discharge in lactating and dry sheep during seasonal anoestrus. *J. Reprod. Fert. 33*, 310.

REEVES, J.J., O'DONNELL, D.A. & DENORSCIA, F., 1972. Effect of ovariectomy on serum luteinizing hormone (LH) concentrations in the anoestrous ewe. *J. Anim. Sci. 35*, 73.

ROBERTSON, H.A. & HUTCHINSON, J.S.M., 1962. The levels of FSH and LH in the pituitary of the ewe in relation to follicular growth and ovulation. *J. Edocr. 24*, 143.

ROCHE, J.F., FOSTER, D.L', KARSCH, F.J., COOK, B. & DZIUK, P.J., 1970. Levels of luteinizing hormone in sera and pituitaries of ewes during the oestrous cycle and anoestrus. *Endocrinology 86*, 568.

ROOT, A., DE-CHERNEY, A., RUSS, D., DUCKETT, G., GARCIA, C.R. & WALLACH, E., 1972. Episodic secretion of luteinizing and follicle-stimulating hormones in agonadal and hypoganadal adolescents and adults. *J. clin. Endocr. Metab.*, *35*, 700.

Our experience, as well as that of Underwood *et al.* (1944) and others, has been that following the introduction of rams both at the commencement of the breeding season (Parsons & Hunter, 1965) and during the breeding season (Lishman & Hunter, 1966), an increase in the incidence of oestrus commonly occurs in ewes after the17th day after joining. Observations at this institution have indicated that when ewes were tested for oestrus throughout the year, using vasectomized rams, the incidence of oestrus dropped markedly during June or July and rose again during November (Hunter, 1962; 1964). The object of this experiment was to determine the pattern of oestrous activity during the transition from anoestrus to the breeding season, in ewes which had been isolated from rams but which were not stimulated by the introduction of rams. To achieve this, the occurrence of heat in groups of ewes joined by rams at 17-day intervals was determined for the first 17 days after joining in each case. Thus, on July 3, 1964, a group consisting of 27 Merino and 17 Döhne Merino ewes was randomly selected from the main flock and transferred to partly-roofed pens, where it was joined with vasectomized teaser rams (Group 1). From the remaining ewes which had been isolated from rams since the end of May, five groups each consisting of 12 Merino and 10 Döhne Merino ewes (Groups 2a to 2e) were selected and remained in a paddock about half a mile from the nearest ram. At 17-day intervals commencing on September 1, these groups in turn were joined with Group 1 in the pens, where they were run with vasectomized rams and regularly teased. Observations for oestrus continued in all groups until November 24. The period of the experiment, from September 1 to November 24, was divided into five equal periods of 17 days for analysis purposes. These periods coincided with the first 17 days of teasing of Groups 2a to 2e respectively, as follows:

Period 1: September 1–17
2: September 18–October 4
3: October 5–21
4: October 22–November 7
5: November 8–24.

Observations for oestrus were made among ewes in the pens by teasing at eight-hour intervals with fresh, vasectomized rams. A ewe was regarded as being on heat if she permitted service by these teaser rams. All ewes were fed in troughs at the approximate rate of 2 lb hammer-milled lucerne hay, ¼ lb yellow maize (uncrushed) and 1 lb veld (*Themeda triandra*) hay per day. A salt-bonemeal lick and water were freely available. The ewes were weighed at weekly intervals as a check on adequate nutrition—during the course of the experiment there was an increase of approximately five per cent in weight.

RESULTS

The incidence of oestrus during each 17 day period is presented in Table 1. The Chi-square tests indicated that in Group 1 the Döhne Merino ewes were sexually more active than the Merino ewes (Periods 2 and 4: $P<0\cdot001$; Periods 1 and 5: $P<0\cdot01$, Period 3: $P<0\cdot05$). In the isolated Groups 2a to 2e, where the numbers of ewes were small, except in Group 2b during Period 2 ($P<0\cdot05$), breed differences were not significant. The incidence of oestrus in each isolated group during the first 17 days after the introduction of teaser rams represents the sexual activity of isolated ewes not stimulated by rams. These data, together with those from the same periods, for the ewes continuously associated with rams (Group 1), are presented in Fig. 1. The lower incidence of oestrus in the Merino ewes and in the isolated ewes is demonstrated in this figure, although with the small numbers in the groups it was only in November that the chi-square value in the comparison between the two groups of Döhne Merinos approached significance ($\chi^2=3\cdot008$).

TABLE 1.—The dates on which teaser rams were joined with groups of Merino and Döhne Merino ewes and the incidence of oestrus during 17-day periods

TABEL 1.—*Die datums waarop koggelramme by groepe Merino- en Döhne Merino-ooie gevoeg is en die voorkoms van estrus gedurende 17-dae periodes*

Treatment *Behandeling*	Group *Groep*	Rams joined *Ramme bygevoeg*	Breed *Ras*	No. of ewes *Getal ooie*	No. of ewes showing oestrus during 17-day periods commencing: *Getal ooie bronstig gedurende 17-dae periodes wat begin het op:*				
					Sept. 1 *Sept. 1*	Sept. 18 *Sept. 18*	Oct. 5 *Okt. 5*	Oct. 22 *Okt. 22*	Nov. 8 *Nov. 8*
Continuously with rams *Voortdurend by ramme*	1	July 3 *Julie 3*	Merino	27	3	2	5	4	12
			Döhne Merino	17	10	13	9	12	15
Isolated from ram *Van ram afgesonder*	2a	Sept. 1 *Sept. 1*	Merino	12	0	2	5	8	8
			Döhne Merino	9*	3	5	2	7	8
	2b	Sept. 18 *Sept. 18*	Merino	12		0	3	7	8
			Döhne Merino	9*		4	5	7	7
	2c	Oct. 5 *Okt. 5*	Merino	12			0	9	9
			Döhne Merino	10			2	6	9
	2d	Oct. 22 *Okt. 22*	Merino	12				1	11
			Döhne Merino	10				4	6
	2e	Nov. 8 *Nov. 8*	Merino	12					4
			Döhne Merino	10					5

*One ewe found to be pregnant and was excluded/*Een ooi dragtig gevind en uitgeskakel*

The results in Table 1 may be summarized as follows:—Merino ewes continuously with rams showed a relatively low incidence of oestrus until November. While this was also true of isolated Merino ewes before they were stimulated by rams (i.e. for the first 17 days of teasing in each group), joining with rams stimulated increasing oestrous activity within two to three 17-day periods. Compared to the Merino ewes in Group 1, this activity reached significant levels in Groups 2a, 2b and 2c during Period 4 and in Group 2d during Period 5. Furthermore, compared with freshly joined ewes, a significant ram stimulus during the second 17-day period following joining was also not achieved until Period 4, i.e. in late October, after rams had been introduced into Group 2c on October 5. On the other hand, many of the Döhne Merinos continuously associated with rams tended to continue oestrous cycles, but, as in the case of the Merinos, in the absence of rams these ewes tended to stop oestrous activity until they were stimulated by joining with the rams.

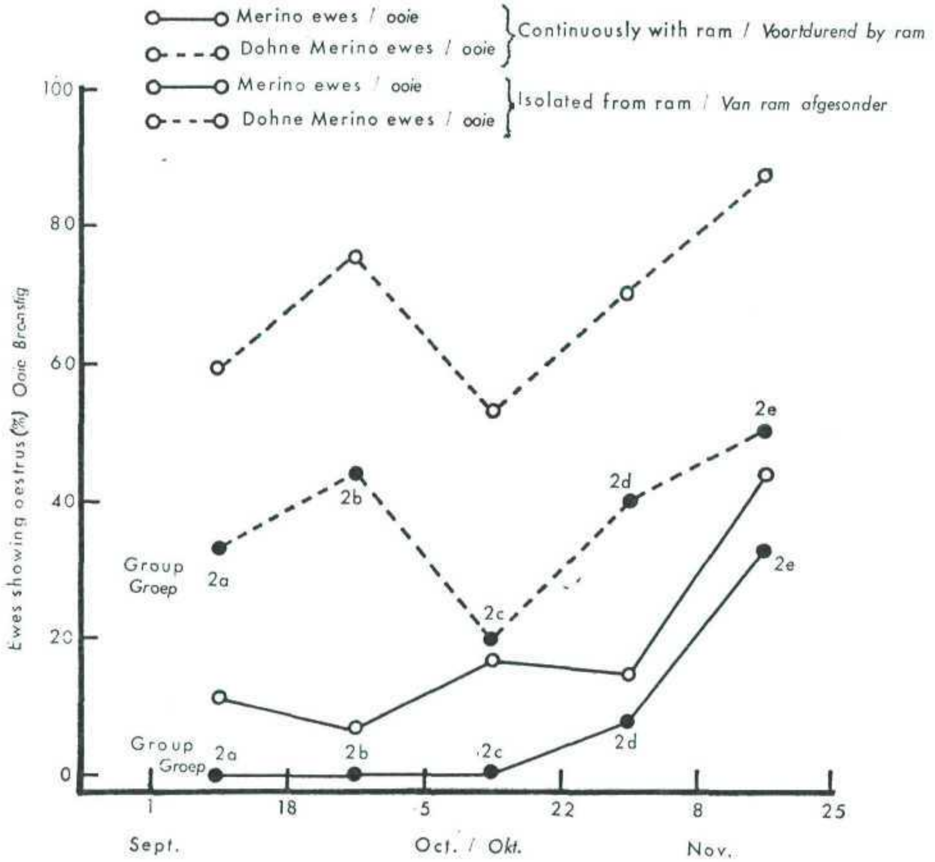

FIG. 1.—Oestrous activity in ewes either continuously associated with rams (Group 1) or isolated from rams prior to each 17-day teasing period (Groups 2a–e)

FIG. 1.—*Bronstigheid by ooie, òf voortdurend by ramme (Groep 1), òf van ramme afgesonder voor elke 17-dae periode (Groepe 2a–e)*

DISCUSSION

The differences in occurrence of oestrus recorded in this experiment for the Merino and Döhne Merino ewes may be due to genetic differences or to the geographical origin of the ewes or both. Differences in the incidence of oestrus apparently due to the previous geographical (nutritional?) environment (Hunter, 1962) and to environment and/or strain differences (Watson, 1962) have already been reported.

The relatively low incidence of heat in the unstimulated ewes (i.e. during the first 17 days following introduction of rams to Groups 2a to 2e), compared to those continuously associated with rams (Group 1), suggests that isolation from the ram during winter leads to a cessation of sexual activity. Isolation for periods of 60 days during the breeding season has also resulted in a lower incidence of oestrus (Lishman & Hunter, 1966). From these results, as well as from those of Riches & Watson (1954) and Watson (1962), it is becoming increasingly clear that the incidence of oestrus in ewes isolated from rams may differ considerably from its occurrence in ewes associated with rams and that the use of rams to determine the display of oestrus may stimulate the ewes under a variety of circumstances and thereby alter their pattern of sexual activity. The influence of the introduction and continued presence of rams on ovarian activity remains to be determined.

Opsomming

BRONSTIGHEID BY OOIE NA AFSONDERING VAN DIE RAM GEDURENDE ANESTRUS

Veranderinge in die voorkoms van estrus gedurende lente en vroeë somer is vergelyk tussen ooie wat gedurende anestrus, òf voortdurend by ramme geloop het, òf vir verskillende tydperke van ramme afgesonder is. Vanaf 3 Julie 1964, het 27 Merino- en 17 Döhne Merino-ooie by koggelramme geloop (Groep 1). Vanaf 1 September is vyf groepe (2a tot 2e), elk bestaande uit 12 Merino- en 10 Döhne Merino-ooie, wat vanaf die einde Mei van ramme afgesonder is, om die beurt met tussenpose van 17 dae by ramme gevoeg.

Die voorkoms van estrus by ooie in Groep 1 na byvoeging van ramme was betekenisvol hoër by die Döhne Merino's as by die Merino's. Alhoewel nie deurgaans betekenisvol nie, is hierdie rasverskil gedurende die eerste 17 dae na byvoeging van ramme ook waargeneem in die kleiner groepe (2a tot 2e.). In vergelyking met Groep 1 was daar gedurende die eerste 17 dae na byvoeging van ramme 'n neiging tot 'n laer voorkoms van bronstigheid in elk van die afgesonderde groepe. Hierna, toe bronstigheid klaarblyklik deur die teenwoordigheid van die ramme gestimuleer is, was die voorkoms van estrus hoër in die afgesonderde groepe. In vergelyking met ooie wat pas by die ram gevoeg is, is 'n betekenisvolle ramstimulus gedurende die tweede 17-dae periode na bybringing van ramme slegs in laat Oktober waargeneem. Die voorkoms van estrus by ooie mag dus afhang van hul assosiasie met ramme en dit mag foutief wees om aan te neem dat die geslagsaktiwiteit van ooie wat van ramme afgesonder is dieselfde is as dié van ooie wat by ramme loop.

Résumé

LE RUT DES BREBIS SEPAREES D'AVEC LES BELIERS DURANT L'ANOESTRUS

On compara les variations dans l'occurrence de l'oestrus de brebis qui, durant l'anoestrus, furent, soit gardées avec les béliers, soit en séparées pendant des périodes variables. Vingt-sept brebis mérinos et 17 brebis döhne-mérinos furent mises avec

les béliers à partir du 3 *juillet* 1964 (*groupe* 1). *A partir du* 1*er septembre, cinq groupes* (2*a*–2*e*) *de* 12 *brebis mérinos et de* 10 *brebis döhne-mérinos, séparés d'avec les béliers depuis la fin-mai, furent mis avec les béliers à des intervalles de* 17 *jours.*

L'occurrence de l'anoestrus des brebis döhne-mérinos du groupe 1 *fut significativement plus élevée que celle des brebis mérinos du même groupe, après qu'on les eût mises avec les béliers. Bien qu'en général cette différence entre les deux races ne fût pas significative, elle fut également observée chez les plus petits groupes* 2*a*–2*e*. *Comparés au groupe* 1, *les autres groupes firent preuve d'un rut diminué pendant les* 17 *premiers jours passés en compagnie des béliers. Après quoi, le rut ayant probablement été stimulé par la présence des béliers, l'occurrence de l'oestrus fut plus élevée chez les brebis des groupes séparés. Ce n'est que vers la fin-octobre qu'on put observer que les brebis furent stimulées significativement par les béliers au cours de la deuxième période de* 17 *jours, passée en compagnie des béliers. L'occurrence de l'oestrus des brebis peut donc dépendre de la présence des béliers et ce peut être une erreur que de croire que l'activité sexuelle des brebis séparées d'avec les béliers est la même que celle des brebis se trouvant avec les béliers.*

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REFERENCES

HUNTER, G. L., 1962. Observations on oestrus in Merinos. Proc. S. Afr. Soc. Anim. Prod. No. 1, 67–69.

HUNTER, G. L., 1964. The effect of season and mating on oestrus and fertility in the ewe. Proc. S. Afr. Soc. Anim. Prod. No. 3, 196–206.

LISHMAN, A. W. & HUNTER, G. L., 1966. Sexual activity in the ewe following isolation from the ram during the breeding season. S. Afr. J. Agric. Sci. 9, 993–1004.

PARSONS, S. D. & HUNTER, G. L., 1965. Practices controlling fertility and flock improvement in sheep farming. Proc. S. Afr. Soc. Anim. Prod. No. 4, 136–139.

RICHES, J. H. & WATSON, R. H., 1954. The influence of the introduction of rams on the incidence of oestrus in Merino ewes. Aust. J. Agric. Res. 5, 141–147.

SCHINCKEL, P. G., 1954. The effect of the ram on the incidence and occurrence of oestrus in ewes. Aust. Vet. J. 30, 189–195.

WATSON, R. H., 1962. Seasonal variation in occurrence of oestrus in Merino ewes in Southern Victoria. Aust. Vet. J. 38, 310–323.

UNDERWOOD, E. J., SHIER, F. L. & DAVENPORT, N., 1944. Studies in sheep husbandry in Western Australia. V. The breeding season of Merino, crossbred and British breed ewes in the Agricultural districts. J. Dep. Agric. W. Aust. 21, 135–143.

PAPER 2

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SEXUAL ACTIVITY IN THE EWE FOLLOWING ISOLATION FROM THE RAM DURING THE BREEDING SEASON

(*Met opsomming in Afrikaans*)
(*Avec résumé en français*)

◆

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ABSTRACT

Adult Merino-type ewes which had exhibited oestrus between November 1964 and the end of February 1965 were allocated into ten treatment groups in order to investigate the influence of—(*a*) intermittent periods and (*b*) 30-day or 60-day periods of isolation from rams, on the ensuing incidence of oestrus.

In ewes which were continuously associated with vasectomized rams the number of ewes exhibiting oestrus showed a decline during the 30-day period commencing July 29. The periods of intermittent isolation did not prolong the breeding season as measured by the occurrence of oestrus in ewes continuously associated with rams. Intermittent isolation also did not reduce the sexual activity as compared to ewes continuously associated with rams.

Isolation from rams for a single period of 30 days, commencing at the height of the breeding season, did not result in a significant reduction of sexual activity as compared to ewes continuously associated with rams. However, isolation during the 30-day period commencing May 30, was followed by less overt oestrus during September and October than isolation during the 30-day periods commencing March 31 and April 30, respectively. When isolation continued for 60 days the number of ewes exhibiting heat during the first 17 days following re-association with rams was significantly lower than for ewes continuously associated with rams.

The ewes exhibiting heat during each 30-day period were grouped into those which exhibited heat during the first 17 days of each 30-day period and those showing their first oestrus after the 17th day of each period. After grouping in this manner it was apparent that while the majority of ewes continuously present with rams had exhibited oestrus during the first 17 days, in the groups that had been isolated, a number of ewes exhibited first heat only after the 17th day. It thus appeared that joining with rams after isolation stimulated some ewes to exhibit oestrus after the 17th day.

INTRODUCTION

It is commonly believed that during the breeding season the ewe continues to exhibit cyclic oestrous activity, even in the absence of rams. Results obtained in an earlier experiment suggested that complete isolation from the male influence during the declining stages of the breeding season may have resulted in an unusually early cessation of oestrous activity. Lamond, Wells & Miller (1963) reported that periodic removal and reintroduction of rams at the approach of anoestrus, resulted in a prolongation of the breeding season.

Following a period of isolation from rams, adult ewes which are in anoestrus may be stimulated at the onset of the breeding season to exhibit heat approximately 18 to 24 days after joining with rams. The interval to induced oestrus may be longer than 24 days, but at least 17 days usually elapse from the date of ram introduction

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before the stimulated oestrus can be shown (Underwood, Shier & Davenport, 1944; Schinckel, 1954a; Eyal, 1958; Edgar, 1960; Lyle, 1965). The experiment to be described was planned to study the occurrence of oestrus following removal and subsequent reintroduction during the second half of the breeding season.

PROCEDURE

At the Ukulinga Experiment Station, Pietermaritzburg, 270 adult Merino-type ewes, all which had been regularly teased and had exhibited oestrus between November 1964 and the end of February 1965 were randomly allocated into ten treatment groups and maintained in pens throughout the experiment. The treatments (see Table 1) were designed to investigate (*a*) the effect on oestrous activity of intermittent periods of isolation from rams (Groups 1–3), and (*b*) the influence of 30-day or 60-day periods of isolation from rams on the ensuing incidence of oestrus (Groups 4–9). Since, by continuous teasing, the peak of the breeding season and the centre of the anoestrous period had previously been found to occur about April and August respectively (Hunter, 1962), isolation treatments in (*b*) commenced during April. Sexual activity in this experiment continued for longer than anticipated and observations were consequently extended to October 27. While being run with rams, the occurrence of oestrus was determined by teasing at eight-hour intervals using vasectomized rams other than those continuously with the ewes.

During isolation from rams, the groups were housed in pens approximately three-quarters of a mile from the nearest ram. The rams running with the ewes were changed weekly and the individual groups of ewes were kept separate at all times. All ewes were fed in troughs at the approximate rate of 2 lb hammer-milled lucerne hay, ¼ lb maize meal and 1 lb veld (*Themeda triandra*) hay per day. The feeding of maize meal had to be discontinued during June due to restrictions imposed following a country-wide drought. A salt-bone meal lick and water were available at all times.

RESULTS

Ewes which died or did not exhibit oestrus during the course of the experiment were eliminated from the treatment groups. The number of ewes finally included in each group is presented in Table 1. A 2 × 2 Chi-square test utilizing Yates' correction was used throughout to test for treatment differences.

Total number of ewes exhibiting oestrus

Continuous association with rams: Until the end of July (i.e. through Period 5) there was no seasonal variation in the number of ewes in group 10 which exhibited oestrus at least once during each 30-day period (Table 1). However, during the 30-day period commencing August 28 significantly fewer ($P < 0{\cdot}001$) ewes in this group exhibited heat than during the 30-day period commencing June 29. Similarly, the number of ewes on heat during the period commencing 28 September was significantly lower than during the 30-day period commencing July 29 ($P < 0{\cdot}01$).

Intermittent isolation: The total number of ewes on heat in Group 10 during the 30-day periods was never observed to be significantly different from those in Groups 1, 2 and 3 (Table 1). If the occurrence of oestrus in ewes continuously associated with rams (Group 10) is used to define the limits of the breeding season, then it is clear that the treatments applied in Groups 1, 2 and 3 did not prolong the breeding season or reduce sexual activity during the periods following 30 days of isolation from rams.

TABLE 1.—Number of ewes exhibiting oestrus to teaser rams during each month of observation. Groups were isolated from rams during periods indicated and continuously associated with vasectomized rams at other times

TABEL 1.—*Getal ooie wat estrus getoon het in die teenwoordigheid van koggelramme gedurende elke maand van observasie. Groepe is van ramme afgesonder gedurende tydperke aangedui en te ander tye het hulle voortdurend by koggelramme geloop*

Treatment *Behandeling*	Group *Groep*	Number in group *Getal in groep*	30-day period commencing/30-*dae periode vanaf:*							
			March 1 *Maart* 1 1	March 31 *Maart* 31 2	April 30 *April* 30 3	May 30 *Mei* 30 4	June 29 *Junie* 29 5	July 29 *Julie* 29 6	Aug. 28 *Aug.* 28 7	Sept. 28 *Sept.* 28 8
Intermittent isolation from rams *Afwisselende afsondering van ramme*	1	26	Isolated *Afgesonder*	22	Isolated *Afgesonder*	22	24	19	18	14
	2	26	Isolated *Afgesonder*	23	Isolated *Afgesonder*	24	Isolated *Afgesonder*	15	14	11
	3	25	Isolated *Afgesonder*	23	24	25	Isolated *Afgesonder*	17	12	10
Isolated from rams for 30 days *Vir 30 dae van ramme afgesonder*	4	26	24	Isolated *Afgesonder*	25	25	24	22	15	11
	5	26	25	24	Isolated *Afgesonder*	23	24	23	19	8
	6	27	25	25	24	Isolated *Afgesonder*	25	21	8	3
Isolated from rams for 60 days Vir 60 dae van ramme *afgesonder*	7	27	26	Isolated *Afgesonder*	Isolated *Afgesonder*	27	26	26	19	10
	8	26	24	23	Isolated *Afgesonder*	Isolated *Afgesonder*	24	22	16	10
	9	27	26	27	25	Isolated *Afgesonder*	Isolated *Afgesonder*	14	9	2
Continuously with rams *Voortdurend by ramme*	10	26	25	25	23	24	24	19	12	9

Isolation for 30 *or* 60 *days:* Compared to Group 10, isolation for 30 days during April, May or June, or for 60 days during April and May, or May and June had no significant effect on the occurrence of overt oestrus before the end of August (Period 6). Thirty-day isolation during Period 4, however, resulted in less overt oestrus during September (Period 7, $P < 0\cdot01$) and October (Period 8, $P < 0\cdot05$) than isolation during Periods 2 and 3 respectively—apparently a delayed effect of isolation. Isolation for 60 days during June and July resulted in significantly fewer ewes showing heat during August (Period 6) than in Group 10 ($P < 0\cdot05$) and than in Groups 7 ($P < 0\cdot001$) and 8 ($P < 0\cdot01$) during June (Period 4) and July (Period 5) respectively.

Trends in the occurrence of overt oestrus during each 30-*day period*

In order to determine whether stimulation of oestrus occurred when the isolated groups were reassociated with rams, the ewes exhibiting heat during each period were grouped into those which exhibited heat during the first 17 days of each 30-day period and those showing their first oestrus of the period after the 17th day of each period. The latter group includes any ewes stimulated by joining with rams as well as some in which cycle length was greater than 17 days or in which overt oestrus was not observed during the first 17 days. The total number of ewes exhibiting oestrus for the first time during the first 17 days or 18 to 30th days of each 30-day period is presented in Table 2, and the daily cumulative totals of ewes showing their first heat in each 30-day period is presented graphically in Fig. 1.

Intermittent isolation: Significantly fewer ($P < 0\cdot05$) ewes were on heat in Groups 3 and 2 during the 17 days commencing March 31 and July 29 respectively, than in Group 10. It is clear from Fig. 1 that practically all ewes in Group 10 that exhibited oestrus in each period did so during the first 17 days, while following isolation of a group a number of ewes did not come on heat until after the 17th day of reassociation with rams.

Isolation for 30 *or* 60 *days:* By comparison with the incidence of oestrus in Group 10, 30 days of isolation from rams did not significantly reduce oestrous activity during the following 17 days. However, the number of ewes on heat during the first 17 days following isolation for 60 days was significantly smaller in Groups 7 ($P < 0\cdot01$), 8 ($P < 0\cdot02$) and 9 ($P < 0\cdot01$) than in Group 10. During September and October (Periods 7 and 8) when the ewes in Group 10 were going into anoestrus, some delayed effects of treatment on the number of ewes showing heat during days 1 to 17 of these periods, became apparent. During the first 17 days of the period commencing August 28 the number of ewes on heat was significantly greater in Group 5 than in Group 6 ($P < 0\cdot01$) and also greater in Group 7 than in Group 9 ($P < 0\cdot01$). Similarly during the first 17 days of the period commencing September 28, significantly more ewes were on heat in Group 4 than in Group 6 ($P < 0\cdot01$). Furthermore, during this same period significantly fewer ewes were on heat in Groups 6 and 9 than in Group 10 ($P < 0\cdot02$). From Table 2 and Fig. 1 it is again apparent that while the majority of the ewes continuously present with rams had exhibited oestrus by the 17th day, in the groups that had been isolated a number of ewes exhibited first heat only after the 17th day.

TABLE 2.—Number of ewes exhibiting oestrus for the first time during days 1–17 and days 18–30 of each 30-day period
TABEL 2.—*Getal ooie wat vir die eerste maal estrus getoon het gedurende dae 1–17 en dae 18–30 van elke 30-dae periode*

Treatment *Behandeling*	Group *Groep*	Number in group *Getal in groep*	30-day period commencing/30-*dae periode vanaf:*															
			March 1 *Maart* 1		March 31 *Maart* 31		April 30 *April* 30		May 30 *Mei* 30		June 29 *Junie* 29		July 29 *Julie* 29		Aug. 28 *Aug.* 28		Sept. 28 *Sept.* 28	
			1		2		3		4		5		6		7		8	
			Days/*Dae*		Days/*Dae*		Days/*Dae*		Days/*Dae*		Days/*Dae*		Days/*Dae*		Days/*Dae*		Days/*Dae*	
			1–17	18–30	1–17	18–30	1–17	18–30	1–17	18–30	1–17	18–30	1–17	18–30	1–17	18–30	1–17	18–30
Intermittent isolation from rams *Afwisselende afsondering van ramme*	1	26	Isolated *Afgesonder*		19	3	Isolated *Afgesonder*		19	3	23	1	17	2	16	2	13	1
	2	26	Isolated *Afgesonder*		21	2	Isolated *Afgesonder*		19	5	Isolated *Afgesonder*		9	6	12	2	9	2
	3	25	Isolated *Afgesonder*		16	7	24	0	22	3	Isolated *Afgesonder*		12	5	11	1	7	3
Isolated from rams for 30 days *Vir 30 dae van ramme afgesonder*	4	26	21	3	Isolated *Afgesonder*		21	4	23	2	22	2	21	1	15	0	11	0
	5	26	21	4	22	2	Isolated *Afgesonder*		17	6	23	1	21	2	18	1	5	3
	6	27	23	2	25	0	22	2	Isolated *Afgesonder*		20	5	20	1	8	0	1	2
Isolated from rams for 60 days *Vir 60 dae van ramme afgesonder*	7	27	22	4	Isolated *Afgesonder*		Isolated *Afgesonder*		12	15	24	2	23	3	19	0	4	6
	8	26	22	2	20	3	Isolated *Afgesonder*		Isolated *Afgesonder*		14	10	19	3	14	2	6	4
	9	27	26	0	26	1	23	2	Isolated *Afgesonder*		Isolated *Afgesonder*		7	7	9	0	1	1
Continuously with rams *Voortdurend by ramme*	10	26	22	3	24	1	22	1	23	1	23	1	18	1	11	1	9	0

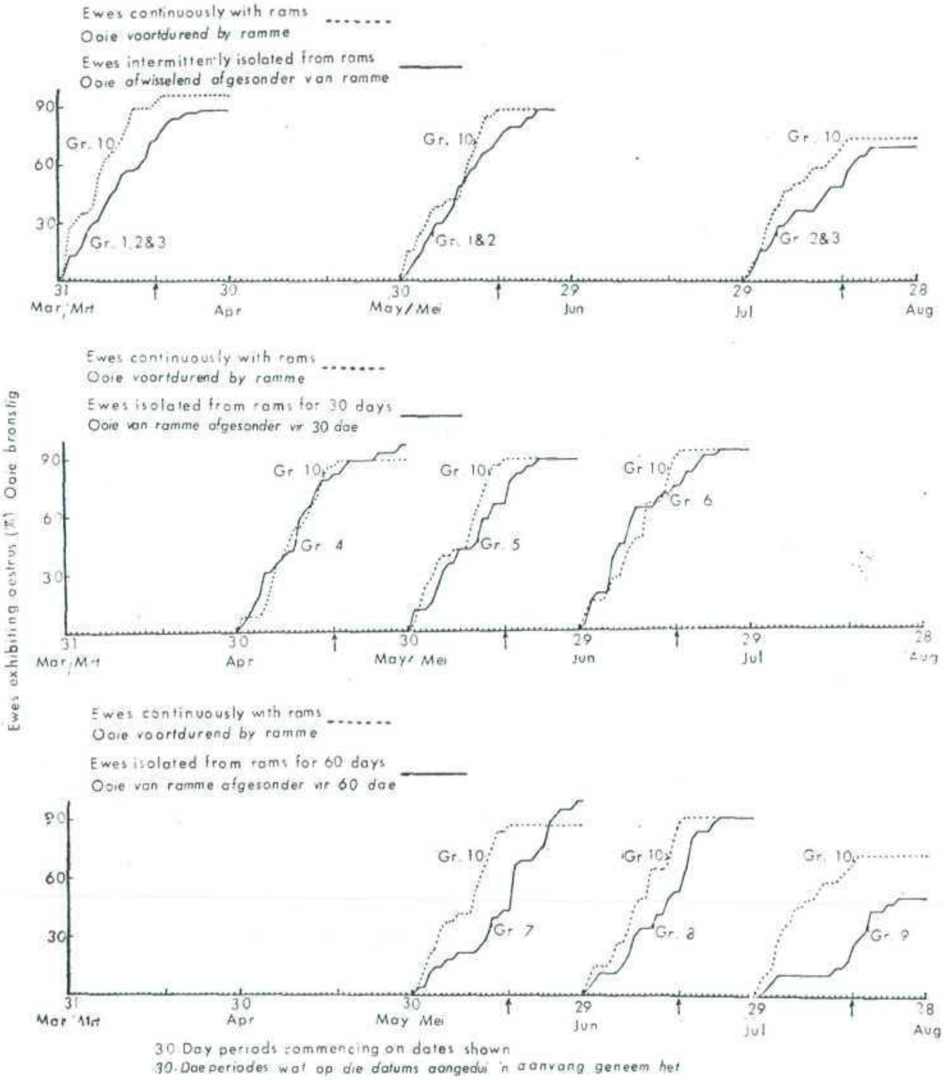

FIG. 1.—Daily cumulative totals of ewes exhibiting oestrus during 30-day periods. The graph for the ewes isolated from rams (—) represents the data obtained during the 30 days after reassociation with rams and that for Group 10 represents ewes which were continuously associated with rams (......)

↑ = 17th day after commencement of a 30-day period.

FIG. 1.—*Daaglikse toenemende getal ooie bronstig gedurende* 30-*dae periodes. Die grafiek vir die ooie wat van ramme afgesonder is* (—) *verteenwoordig die data verkry gedurende die* 30 *dae na bybring van ramme en die vir Groep* 10 *verteenwoordig ooie wat voortdurend by ramme geloop het* (......)

↑ = 17*de dag na aanvang van 'n* 30-*dae periode*

DISCUSSION

The reduction in the plane of nutrition, brought about by discontinuing the feeding of maize meal after June, may have reduced the occurrence of oestrus thereafter since the duration of the breeding season has been shown to be influenced by the plane of nutrition (Hunter, 1961, 1962; Smith, 1962, 1964). Interaction between the feeding level and the treatments applied may thus have occurred.

The results presented here suggest that particularly as the season advances, the sexual activity of the ewe is to some extent maintained by continued association with rams. Although isolation from rams may depress the level of sexual activity, it would appear that at the peak of the breeding season the seasonal stimuli are capable of supporting continued oestrous activity during a 30-day period of isolation from rams. However, if isolation continues for 60 days ewes may only show oestrus after stimulation by rams and if isolation for 30 days occurs during the declining stages of the breeding season, a marked reduction in the incidence of oestrus may occur. The results observed when isolation occurs during the breeding season are thus in accordance with those recorded when isolation occurs during the anoestrous period (Lishman, 1965). The results presented by Barrett, Reardon & Lambourne, (1962) and Watson (1962) support the contention that the ram stimulus plays an important role at the approach of anoestrus. Owing to the fact that Barrett *et al.* (1962) did not exclude oestrus initiated following joining with rams and since there were strain differences in the ewes used by Watson (1962), comparisons with the present work are difficult. The results presented by Lamond (1962a; 1964) also favour the hypothesis that the ram stimulus is necessary at the beginning and end of the breeding season. His observations obtained following progesterone suppression of oestrus, indicated that variations in the stage of introduction of rams could influence the results. The male apparently also plays a role in maintaining continued oestrous activity in the mouse where the anoestrus induced by caging females in large groups can be terminated by the introduction of the male stimulus (Lamond, 1959; Whitten, 1959; Marsden & Bronson, 1965).

In this experiment the periods of intermittent isolation were not effective in inducing a high level of oestrous activity during the anoestrous season. This was in contrast to the results reported by Lamond *et al.* (1963), possibly because the isolation periods were too long. A period sufficient to counteract habituation to the ram stimulus would appear to be required.

Further investigation is necessary to determine whether isolation from rams during the breeding season (*a*) depresses ovarian activity, (*b*) reduces the intensity and/or duration of oestrus thereby suggesting that silent heat occurred during the first 17 days after reassociation with rams, or (*c*) causes persistence of the corpus luteum and hence longer cycles, with the result that first heats following rejoining occur between the 18th and 30th days. Any of the above factors could account for the reduced incidence of oestrus during the first 17 days after rejoining with rams, although it must be borne in mind that Schinckel (1954a) observed the occurrence of silent ovulation within six days after introduction of rams at the commencement of the breeding season. Additional aspects which require elucidation are the nature and duration of oestrus during isolation and the sequence of events after reassociation of the sexes which occur in ewes that would not have shown heat if isolation had continued for a longer period.

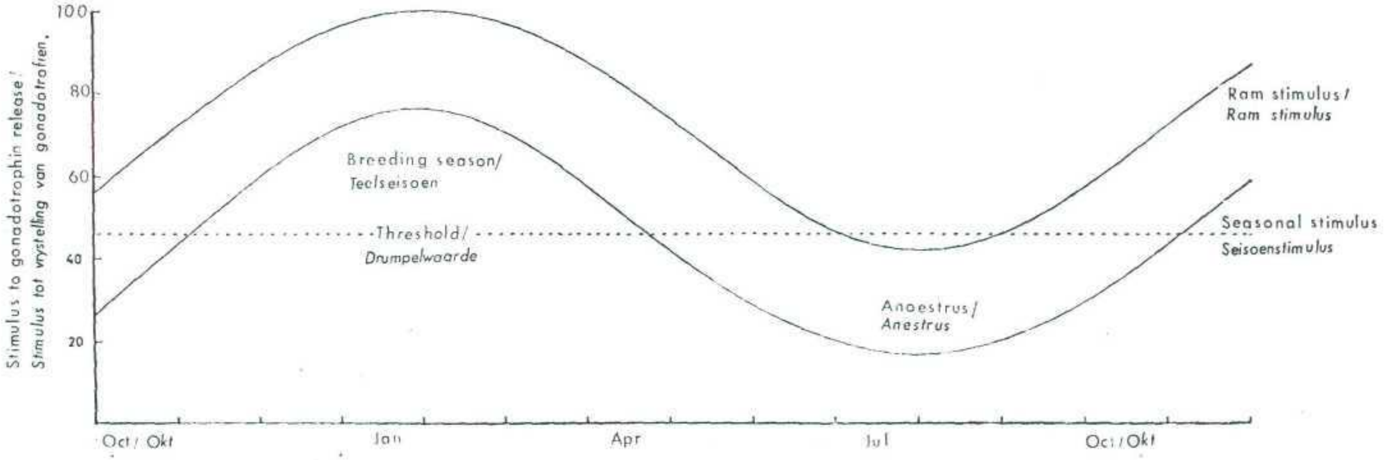

FIG. 2.—Changes in the stimuli which induce gonadotrophin release sufficient to result in follicle maturation and ovulation
FIG. 2.—*Veranderinge in die stimuli wat die vrystelling van voldoende gonadotrofien om rypwording en ovulasie van follikels teweeg te bring*

It might be speculated that the interaction between seasonal and ram stimuli in a particular ewe could be schematically represented as in Fig. 2. In this scheme it is assumed that:

(1) Since the level of sexual activity varies during the breeding season when all other factors appear to be constant, the seasonal stimuli which result in cyclic oestrous activity also vary.

(2) The ram stimulus is relatively constant, but it is of insufficient magnitude to meet the threshold requirements on its own. The latter is supported by the finding that when the seasonal stimuli can be expected to be low, the ram is incapable of inducing sexual activity (Riches & Watson, 1954; Lishman, 1965).

(3) The threshold for follicular maturation and ovulation remains relatively constant throughout the year. Seasonal variations in the stimuli which initiate sexual activity (Hart, 1950; Bunding, Schooley, Bock & Steelman, 1953; Braden, Lamond & Radford, 1960; Lamond, 1962b) have not been demonstrated to be due to variations in the threshold requirements. When rams are continuously present during anoestrus no ram stimulus is apparent at the commencement of the new breeding season (Schinckel, 1954b ; Lishman, 1965) probably because of habituation to the ram stimulus. If habituation is shown to be the strongest of a number of stimuli, this would suggest that during anoestrus the seasonal stimuli are of a lower magnitude than the ram stimulus. The annual mating and lambing rhythm can be expected to modify the scheme which has been suggested.

The accumulated evidence on the induction of out-of-season breeding and on the so-called natural breeding season of sheep requires re-appraisal in the light of the male effect demonstrated here. The need for further research is indicated, particularly in other seasonal breeders, where a similar set of circumstances may apply.

Opsomming

GESLAGSAKTIWITEIT BY OOIE NA AFSONDERING VAN RAMME GEDURENDE DIE TEELSEISOEN

Volwasse Merino-tipe ooie wat gedurende die periode vanaf November 1964 *tot einde Februarie* 1965, *estrus vertoon het, is in tien behandelingsgroepe van* 27 *ooie elk verdeel, met die doel om die invloed van* (a) *afwisselende periodes van afsondering van ramme (drie groepe), en* (b) 30-*dae (drie groepe) en* 60-*dae (drie groepe) periodes van afsondering van ramme op die voorkoms van bronstigheid te bestudeer.*

By ooie wat voortdurend by koggelramme geloop het, (een groep), was daar 'n daling in die getal bronstige ooie gedurende die 30-*dae periode wat op* 29 *Julie 'n aanvang geneem het. Die periodes van afwisselende afsondering het nie die teelseisoen, soos bepaal deur die voorkoms van estrus by ooie wat voortdurend by ramme geloop het, verleng nie. In vergelyking met ooie wat voortdurend by ramme geloop het, het afwisselende afsondering ook nie die geslagsaktiwiteit verminder gedurende die periodes na* 30 *dae van afsondering nie.*

Afsondering van ramme vir 'n enkele periode van 30 *dae, wanneer afsondering teen die hoogtepunt van die teelseisoen 'n aanvang geneem het, het nie 'n betekenisvolle verlaging in geslagsaktiwiteit in vergelyking met ooie wat voortdurend by ramme geloop het, teweeggebring nie. Afsondering gedurende die* 30-*dae periode wat op* 30 *Mei begin het is nietemin gevolg deur 'n verlaagde voorkoms van estrus gedurende September*

en Oktober in vergelyking met afsondering gedurende die periodes wat onderskeidelik op 31 Maart en 30 April begin het. Met die voortduur van afsondering vir 60 dae was die getal ooie wat gedurende die eerste 17 dae na heraansluiting by ramme bronstig was, betekenisvol laer as die by ooie wat nie afgesonder was nie.

Die ooie wat gedurende elke 30-dae periode bronstig was, is gegroepeer in diere wat gedurende die eerste 17 dae en dié wat na die 17de dag estrus getoon het. Na groepering op hierdie wyse was dit duidelik dat die meerderheid van die ooie wat voortdurend by ramme geloop het, bronstigheid vertoon het gedurende die eerste 17 dae, terwyl 'n aantal ooie in die groepe wat van ramme afgesonder was eers na die 17de dag bronstig was. Aansluiting by ramme na afsondering het sekere ooie skynbaar gestimuleer om estrus te vertoon.

Résumé

ACTIVITE SEXUELLE DES BREBIS APRES LEUR SEPARATION D'AVEC LES BELIERS PENDANT LA SAISON DE REPRODUCTION

Des brebis adultes du type Mérinos, en rut durant la période de novembre 1964 à fin février 1965, ont été divisées en dix groupes de 27 brebis chacun, ceci dans le but d'étudier l'influence (sur l'occurence du rut) de:

(a) *périodes intermittentes de séparation d'avec les béliers (trois groupes), et de*

(b) *périodes de 30 jours (trois groupes) et de 60 jours (trois groupes) de la saison de reproduction, pendant lesquelles les brebis ont été séparées d'avec les béliers.*

Parmi les brebis restées continuellement en présence des béliers (un groupe), on a constaté une diminution dans le nombre d'animaux en rut au cours de la pèriode de 30 jours commençant le 29 juillet. La saison de reproduction, comparée à la durée de la période de rut des brebis restées en contact continuel avec les béliers, n'a pas été prolongée par les périodes d'isolement intermittentes qui n'ont pas non plus diminué l'activité sexuelle des brebis, comparée à celle des brebis restées en contact continuel avec les béliers (celles du groupe-témoin).

La séparation d'avec les béliers pendant une seule période de 30 jours, commençant au point culminant de la saison de reproduction, n'a pas fait diminuer de façon significative l'activité sexuelle des brebis de ce groupe, comparée à celle des brebis du groupe-témoin, mais la séparation durant la période de 30 jours commençant le 30 mai a causé une diminution du rut au cours des mois de septembre et octobre, comparé aux périodes de séparation commençant le 31 mars et le 30 avril respectivement. Après une période de séparation de 60 jours, le nombre de brebis en rut au cours des 17 premiers jours après la réunion avec les béliers: était significativement moins élevé que dans le groupe-témoin.

Les brebis qui étaient en rut pendant chaque période de 30 jours ont été divisées en deux groupes. Faisaient partie du premier groupe, les brebis en rut pendant les 17 premiers jours, tandis que les brebis en rut après le 17me jour formaient le deuxième groupe. Ceci a permis de constater que la plupart des brebis du groupe-témoin étaient en rut pendant les 17 premiers jours, tandis que plusieurs brebis des groupes ayant été séparés d'avec les béliers étaient en rut après le 17me jour. Il semble que la réunion avec les béliers a stimulé le rut chez certaines brebis.

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REFERENCES

BARRETT, J. F., REARDON, T. F. & LAMBOURNE, L. J., 1962. Seasonal variation in reproductive performance of Merino ewes in Northern New South Wales. J. Exp. Agric. Anim. Husb. 2, 69–74.

BRADEN, A. W. H., LAMOND, D. R. & RADFORD, H. M., 1960. The control of the time of ovulation in sheep. Aust. J. Agric. Res. 11, 389–401.

BUNDING, I., SCHOOLEY, M. A., BOCK, F. C. & STEELMAN, S. L., 1953. Some effects of oestrogen, progesterone and gonadotrophin on the mating behaviour of ewes. J. Anim. Sci. 12, 943.

EDGAR, D. G., 1960. Talking about tupping. N.Z. Agric. 103, 209–213.

EYAL, E., 1958. The introduction of rams as a factor influencing sexual activity of ewes. Ktavin. Rec. Agric. Res. Sta. 8, 359–366. (Anim. Breed. Abstr. 27, 857).

HART, D. S., 1950. Photoperiodicity in Suffolk sheep. J. Agric. Sci. 40, 143–149.

HUNTER, G. L., 1961. Some effects of plane of nutrition on the occurrence of oestrus in Merino ewes. Proc. IVth Int. Cong. Anim. Reprod. The Hague 2, 197–201.

HUNTER, G. L., 1962. Observations on oestrus in Merinos. Proc. S. Afr. Soc. Anim. Prod. 1, 67–69.

LAMOND, D. R., 1959. Effect of stimulation derived from other animals of the same species on oestrous cycles in mice. J. Endocr. 18, 343–349.

LAMOND, D. R., 1962a. Anomalies in onset of oestrus after progesterone suppression of oestrus cycles in ewes associated with introduction of rams. Nature, 193, 85–86.

LAMOND, D. R., 1962b. Oestrus and ovulation following administration of placental gonadotrophins to Merino ewes. Aust. J. Agric. Res. 13, 707–717.

LAMOND, D. R., 1964. Seasonal changes in the occurrence of oestrus following progesterone suppression of ovarian function in the Merino ewe. J. Reprod. Fertil. 8, 101–114.

LAMOND, D. R., WELLS, K. E. & MILLER, S. J., 1963. Study of a breeding problem in Merino ewes in central Queensland. Aust. Vet. J. 39, 295–298.

LISHMAN, A. W., 1965. The influence of isolation from rams on sexual activity in ewes. M.Sc. Agric. Thesis, Univ. of Natal, Pietermaritzburg.

LYLE, A. D., 1965. The influence of rams on flock mating at the onset of the breeding season M.Sc. Agric. Thesis, Univ. of Natal, Pietermaritzburg.

MARSDEN, H. M. & BRONSON, F. H., 1956. The synchrony of oestrus in mice. Relative roles of the male and female environments. J. Endocr. 32, 313–319.

RICHES, J. H. & WATSON, R. H., 1954. The influence of the introduction of rams on the incidence of oestrus in Merino ewes. Aust. J. Agric. Res. 5, 141–147.

SCHINCKEL, P. G., 1954a. The effect of the presence of the ram on the ovarian activity of the ewe. Aust. J. Agric. Res. 5, 465–469.

SCHINCKEL, P. G., 1954b. The effect of the ram on the incidence and occurrence of oestrus in ewes. Aust. Vet. J. 30, 189–195.

SMITH, I. D., 1962. The effect of plane of nutrition on the incidence of oestrus in the Merino ewe in Queensland. Aust. Vet. J. 38, 338–340.

SMITH, I. D., 1964. The effect of seasonal variations in body-weight upon oestrous activity in Merino ewes. Proc. Vth Int. Cong. Anim. Reprod., Trento 3, 484–488.

UNDERWOOD, E. J., SHIER, F. L. & DAVENPORT, N., 1944. Studies in sheep husbandry in Western Australia. V. The breeding season of Merino, crossbred and British breed ewes in the agricultural districts. J. Dep. Agric. W. Aust. 21, 135–143.

WATSON, R. H., 1962. Seasonal variation in occurrence of oestrus in Merino ewes in Southern Victoria. Aust. Vet. J. 38, 310–323.

WHITTEN, W. K., 1959. Occurrence of anoestrus in mice caged in groups. J. Endocr. 18, 102–107.

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E SEASONAL PATTERN OF OESTRUS AMONGST EWES AS AFFECTED BY ISOLATION FROM AND JOINING WITH RAMS

(*Met opsomming in Afrikaans*)
(*Avec résumé en français*)

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ABSTRACT

During October 1966 a flock of Merino-type ewes was isolated from contact with rams. At 30 day intervals, commencing on February 1, 1966 a separate group of ewes was randomly selected from the isolated flock and joined with sterile teaser rams. A total of 12 groups of ewes was joined with rams and observations for oestrus were continued until March 26, 1967.

During the 17 day periods commencing March 3, April 3, October 29, November 28 and December 28, the level of oestrous activity amongst ewes which had been isolated from rams was significantly lower than that amongst ewes which had been associated with rams for at least 30 days. The conclusion that isolation from rams resulted in a reduction in the incidence of oestrus was further supported by the finding that after combining the data over all groups, the level of spontaneous oestrous activity following introduction of rams was significantly lower than the incidence of oestrus in the groups which had already been associated with rams for 30 days. The relative difference between these two categories of ewes showed a seasonal trend.

It was evident that the introduction of rams during the season of sexual quiescence was less effective in evoking oestrus in anoestrous ewes than at other times of the year since the proportion of ewes which exhibted heat following stimulation by rams was significantly smaller during July to early October than during February to June or late October to January. Nevertheless, the sudden introduction of rams was a more effective stimulus of oestrus in ewes, particularly during the anoestrous period, than was a prolonged association between the sexes. The seasonal changes in the proportion of ewes showing oestrus did not follow the same pattern in all groups of ewes.

The extent to which seasonal and ram stimuli are reflected in the incidence of oestrus at certain times following joining and the changes in the intensity and duration of the ram stimulus are discussed.

INTRODUCTION

The results reported by Yeates (1949, 1955), Hart 950) and Hafez (1952) support the conclusion that e breeding season in sheep is under photoperiodic ontrol. However, Radford (1961a, 1961b) has drawn ttention to the fact that in certain areas factors other an light may play an important role in the oestrous attern of Merino ewes.

In the Natal Region the majority of farmers breed heir ewes in spring (Adler, 1964) at a time when, ccording to Hunter (1959, 1962, 1964), sexual activity s still at a low level. However, as pointed out by iches & Watson (1954), studies of breeding activity n ewes are commonly based on the continuous ssociation (often for many months) of ewes with nfertile rams, whereas in farming practice the sexes re usually separated except during the limited mating period. The applicability, in farming practice, of experimental findings obtained under the aforementioned conditions, is questioned by the conclusion that fresh joining of the sexes in spring results in a stimulation of oestrous activity (Underwood, Shier & Davenport, 1944), while isolation of ewes from rams leads to a cessation of oestrous activity (Lishman & Hunter, 1966, 1967).

It has become increasingly evident that manipulation of the extent to which ewes are associated with rams can have a far-reaching influence on the reproductive activity of the females and the experiment reported here was aimed at obtaining additional information on this aspect and more particularly on the seasonal pattern of oestrus in ewes isolated from rams.

PROCEDURE

At present the only known method of detecting oestrus in the ewe is by the use of a sexually active ram. However, the incidence of oestrus amongst ewes isolated from rams can be measured by the occurrence of oestrus during the first 17 days following joining with rams. This procedure is based on the finding that at the onset of the breeding season, when ewes are joined with rams, the first ovulation of the breeding season occurs within a few days after introduction of rams (Schinckel, 1954a; Hunter & Lishman, 1967) and on the suggestion that in the absence of a waning corpus luteum from a previous ovulation oestrus is not displayed until one cycle (17 days) later (Schinckel, 1954b). Since the stimulated ovulation can occur nought to eight days following joining (Hunter & Lishman, 1967), oestrus can be expected 18 to 26 days after introduction of rams. Evidence to support this expectation has been obtained by Lyle & Hunter (1965).

In this study the occurrence of heat during the first 17 days following joining with vasectomized rams was used as a measure of spontaneous oestrus, i.e. oestrus not due to stimulation by joining with rams. Any ewes which did not exhibit heat during this period were then assumed to be in anoestrus at the time of joining with rams and where these ewes mated 18 to 30 days following joining, this was regarded as being due to stimulation by rams. In addition to the ewes stimulated by joining with rams the latter category may also include ewes in which, the cycle length exceeded 17 days, overt oestrus was not observed

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during the first 17 days or which exhibited heat at the first ovulation following a period of anoestrus.

A total of 228 adult Merino-type ewes which had not lambed for at least six months and which had been kept separate from rams as from October 1965 was used as experimental animals. During January 1966 the flock was transferred to a camp well isolated from other sheep and the ewes were placed on a daily ration of approximately 2½ lb unmilled lucerne hay. A salt-bone meal lick and water were freely available.

During the period February 1, 1966 to January 26, 1967 the oestrous pattern amongst ewes isolated from rams was determined by dividing the aforementioned interval into 12 periods of 30 days each and by joining a fresh group of ewes with rams on the first day of each 30 day period. Consequently, the first group (Group 1) joined the rams on February 1, and each subsequent group 30 days later than the previous group. Observations for oestrus in all groups continued until March 27, 1967, i.e. two 30 day periods after joining of the final group.

On the day of joining with rams the ewes, to be included in each group, were randomly selected from those remaining in the isolated flock and the number finally joined with rams is indicated in Table 1.

On being joined with vasectomized teaser rams the ewes were transferred to partly-roofed pens without any change in the feeding program. When associated with rams, observations for oestrus were made by teasing at eight-hour intervals with fresh vasectomized rams, but each group was also continuously associated with a teaser ram. The latter rams were changed weekly. All ewes were weighed from time to time as a check on adequate nutrition.

RESULTS

The first 30 day period following joining of each group with rams has been subdivided into days 1 to 17 and days 18 to 30 and the ewes classified according to the day on which they showed first heat. To facilitate presentation of results for all subsequent 30 day periods, only the ewes which exhibited heat during the first 17 days of each 30 day period have been considered. The total number of ewes which exhibited overt oestrus during the respective periods is presented in Table 1.

Incidence of oestrus amongst ewes isolated from or associated with rams

Comparison of the incidence of oestrus amongst ewes isolated from rams (isolated ewes) with that of ewes associated with rams (associated ewes), has been effected by comparing the group freshly joined with rams with the group that had already been associated with rams for 30 days, e.g. during the period commencing March 3, Group 2 was compared with Group 1. Each subsequent group was then compared with that introduced 30 days earlier. This procedure has been adopted so as to account for seasonal effects on the incidence of oestrus. The chi-square test was used throughout. The annual trend in the incidence of oestrus amongst isolated and associated ewes is illustrated in Fig. 1.

Table 1 and Fig. 1 show that the level of oestrous activity amongst the isolated ewes was lower or equal to that amongst the associated ewes, except during the period commencing September 29. The difference was significant during Periods 2 ($P < 0.05$), 3 ($P < 0.02$) 10 ($P < 0.001$), 11 ($P < 0.05$) and 12 ($P < 0.05$) and when the data were pooled a probability value of less than 0.001 was obtained. A significant heterogeneity

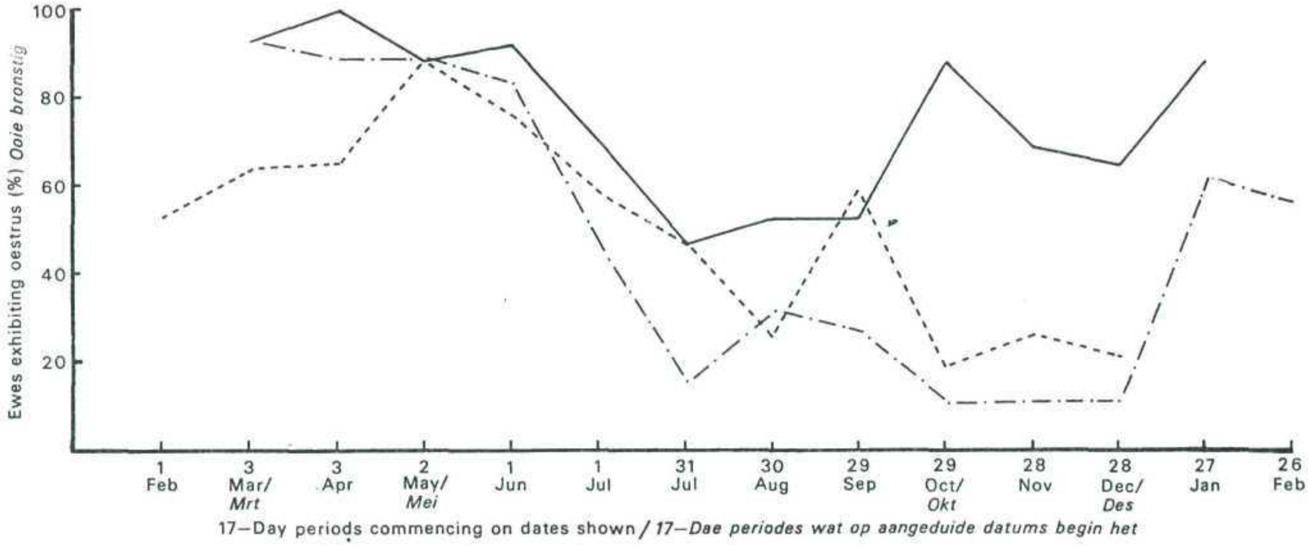

FIG. 1. — Seasonal variations in oestrous activity of groups of ewes either isolated from (..........) or associated with rams for 30 days (——) prior to the commencement of each 17-day observation period and the effect of prolonged association on the incidence of oestrus in Group 1 (—·—) which was joined with rams on 1 February 1966. Significant differences between (——) and (—·—) at 0·01 and 0·001 levels of P, respectively are denoted by ** and ***

FIG. 1. — Seisoensverskille in bronstigheid by groepe ooie wat van ramme afgesonder is (..........), of vir 30 dae met ramme geassosieer is (——) voor aanvang van elke 17-dae observasieperiode en die invloed van voortdurende assosiasie op bronstigheid in Groep 1 (—·—) wat op 1 Februarie 1966 by ramme aangesluit het. Betekenisvolle verskille tussen (——) en (—·—) teen 0·01 en 0·001 peile van P, onderskeidelik word deur ** en *** aangedui

TABLE 1. — Number of ewes exhibiting oestrus for the first time during days 1–17 and days 18–30 of the first and days 1–17 of subsequent 30 day periods following joining with vasectomized rams

TABEL 1. — *Getal ooie wat vir die eerste maal estrus getoon het gedurende dae 1–17 en dae 18–30 van die eerste en dae 1–17 van daaropvolgende 30 dae periodes na aansluiting by koggelramme*

Group *Groep*	Number in group *Getal in Groep*	30 day period commencing/30-*dae periode vanaf:* Feb. 1 *Feb. 1*		March 3 *Maart 3*		April 3 *April 3*		May 2 *Mei 2*		June 1 *Junie 1*		July 1 *Julie 1*		July 31 *Julie 31*	
		1		2		3		4		5		6		7	
		Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30
1	19	11	6	18	—	17	—	17	—	16	—	9	—	3	—
2	19			12	5	19	—	16	—	15	—	13	—	10	—
3	17					11	5	15	—	15	—	14	—	8	—
4	17							15	1	16	—	13	—	10	—
5	17									13	2	12	—	8	—
6	17											10	3	8	—
7	17													8	2
8	17														
9	17														
10	16														
11	17														
12	18														

Group *Groep*	Number in group *Getal in Groep*	30-day period commencing/30-*dae periode vanaf:* Aug. 30 *Aug. 30*		Sept. 29 *Sept. 29*		Oct. 29 *Okt. 29*		Nov. 28 *Nov. 28*		Dec. 28 *Des. 28*		Jan. 27 *Jan. 27*		Feb. 26 *Feb. 26*	
		8		9		10		11		12		13		14	
		Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30	Days/*Dae* 1–17	18–30
1	19	6	—	5	—	2	—	2	—	2*	—	11	—	10	—
2	19	8	—	6	—	6	—	2	—	1	—	5	—	8	—
3	17	8	—	7*	—	6	—	4	—	5	—	5	—	6	—
4	17	5	—	9	—	8	—	8	—	6	—	12	—	14	—
5	17	9	—	12	—	9	—	9	—	6	—	8	—	11	—
6	17	12	—	8	—	9	—	10	—	9*	—	10*	—	9	—
7	17	9	—	9	—	9	—	4	—	10	—	13	—	14	—
8	17	4	5	9	—	7	—	5	—	6	—	10	—	11	—
9	17			10	3	15	—	11	—	9	—	14	—	16	—
10	16					3	10	11	—	11	—	15	—	12	—
11	17							4	7	11	—	15	—	11	—
12	18									4	11	16	—	9	—

* Indicates one ewe died/*Dui aan een ooi dood*

χ^2 value substantiated the conclusion that the relative differences varied during the course of the observations. According to Fig. 1 initially (Periods 2 and 3) this difference was fairly large, but gradually decreased during Periods 4 to 7 (May to July) and rose markedly again during November, December and January. The high level of spontaneous oestrus in Group 9 would appear to be abnormal, but no explanation can be offered.

Seasonal effects on the stimulus due to joining, and the effect of prolonged association with rams

For each group the number of ewes which showed first heat 18 to 30 days after joining rams (Table 1) has been expressed as a percentage of the number which did not show oestrus during the first 17 days, and the results are presented in Fig. 2. There appeared to be a seasonal trend in the proportion of ewes exhibiting heat following stimulation by rams. In fact, significantly fewer ewes were stimulated during July to early October than during February to June ($P < 0.001$) or late October to January ($P < 0.01$). No significant difference was found between the latter two periods.

Ewes either continuously associated or freshly joined with rams

The effect of prolonged association with rams was measured by the incidence of oestrus amongst the ewes in Group 1, the results for the first 30 days after association with rams being excluded (Fig. 1). This group was selected since the ewes were associated with rams for longer than any other group. Similarly, the proportion in each group (Groups 1 to 12) exhibiting oestrus during the second 30 day period of association with rams represents the incidence of oestrus in ewes freshly joined with rams, but already stimulated by joining (Fig. 1). From the figure it is evident that fresh joining of ewes with rams maintained a higher level of overt oestrus, particularly during the "anoestrus" period (July to October), than prolonged association between the sexes. Furthermore, according to the results in Table 1, after Period 3 there was little difference between the incidence of oestrus

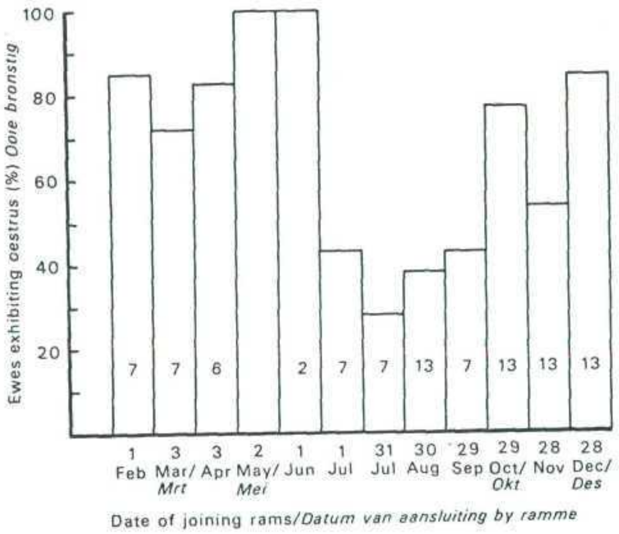

FIG. 2. — The influence of date of joining with rams on the number of ewes exhibiting first oestrus 18–30 days after introduction of rams. The figures within each column of the histogram denote the number of ewes which did not'show oestrus during the first 17 days following joining with rams

FIG. 2. — *Die invloed van datum van aansluiting by ramme op die getal ooie wat 18–30 dae na bybringing van ramme vir die eerste keer bronstig was. Die syfers binne elke kolom van die histogram dui aan die getal ooie wat geen estrus gedurende die eerste 17 dae na aansluiting by ramme getoon het nie*

amongst the ewes in Group 1 and the level of spontaneous oestrus in the unstimulated ewes.

Seasonal changes in the incidence of oestrus within each group

The percentage of ewes within each group which showed behavioural oestrus during the first 17 days of each 30 day observational period is presented in Fig. 3.

These results show that Group 1 followed the accepted pattern with a peak incidence of oestrus during March and a marked drop during July. However, the extension of the "anoestrous" period until January was unexpected. When the individual 30 day periods were compared, it was evident that in Group 1 significantly more ($P < 0.05$) ewes exhibited oestrus during March than during February (due to ram stimulus) and significantly less ($P < 0.05$) ewes during July than during June. The rise in the incidence of oestrus during February 1967 resulted in a significant difference ($P < 0.01$) between Periods 12 and 13.

In Groups 2, 10, 11 and 12 only the first and second 30 day periods differed significantly, while in the remaining groups no significant differences between any two successive periods were observed. Over all groups, Group 12 excluded, there was a significant rise in the proportion of ewes showing oestrus during February ($P < 0.001$).

From Fig. 3 it can be concluded that as the date of joining with rams was further delayed, the seasonal oestrous pattern tended to remain more constant, with very little difference between periods. There was also a similar tendency for the maximum level of oestrous activity to fall well below 100 per cent in Groups 5 to 8.

DISCUSSION

The results presented here support the conclusion (Lishman & Hunter, 1966, 1967) that isolation of ewes from rams tends to lead to a cessation of oestrous activity. The level of spontaneous oestrus and proportion of ewes stimulated by joining with rams during the period September to November were similar to the results obtained by Lishman & Hunter (1967) for a corresponding period.

The results presented in Fig. 1 suggest that fresh joining of ewes previously isolated from rams can to some extent eliminate the anoestrous period, even though the stimulus due to joining was low during the accepted non-breeding period. These results are in accordance with those obtained by Riches & Watson (1954) and Lamond, Wells & Miller (1963).

Although it has long been known that fresh joining with rams, at the onset of the breeding season, is capable of initiating oestrous activity (Underwood, *et al.*, 1944), it has only recently been suggested that such stimulation may also occur at other stages of the breeding season (Lamond, 1964; Smith, 1965). From the results presented in Fig. 2 it is concluded that the ram stimulus can occur at all times of the year.

The incidence of oestrus amongst ewes isolated from rams, as measured by the level of spontaneous oestrus following joining with rams (Fig. 1), can probably be assumed to be a reflection of the seasonal stimuli. Similarly, the level of sexual activity amongst ewes associated with rams reflects a continued ram stimulus in addition to the seasonal effects. Apparently this ram stimulus is a continuation of that which causes initiation of breeding activity, since removal of the stimulus, viz., by isolating ewes, tends to lead to a cessation of oestrous activity (Lishman & Hunter, 1966, 1967). If the abovementioned measures of seasonal and continued ram stimuli are considered in relation to the results depicted in Fig. 1, then it would appear that the ram stimulus is not equally effective throughout the season and the data in Fig. 2 support this contention. This conclusion is in accordance with that quoted by Lamond (1964), but contrary to the hypothesis proposed by Lishman & Hunter (1966). However, since the incidence of oestrus amongst ewes associated with rams for several months (Group 1) tended to drop to a lower level than amongst ewes freshly stimulated by joining (Fig. 1), adaptation to the continued ram stimulus does seem to occur.

Comparison between Fig. 1 and 2 revealed that the proportion of ewes showing first heat 18 to 30 days following joining with rams, fell markedly at about the same time as the significant drop in level of oestrous activity amongst ewes in Group 1 (viz. Period 6). However, the proportion apparently stimulated increased again approximately three months prior to the general rise observed in Groups 1 to 11.

This increase in incidence of oestrus in the aforementioned groups appeared to be independent of the length of the period for which the ewes were associated with rams and these results agree with those reported by Lishman & Hunter (1967). It is therefore concluded that association of the sexes for long periods does not lead to a deepening of anoestrus, but that the prolonged anoestrus observed by Schinckel (1954b), was merely due to the absence of the stimulus due to fresh association of the sexes.

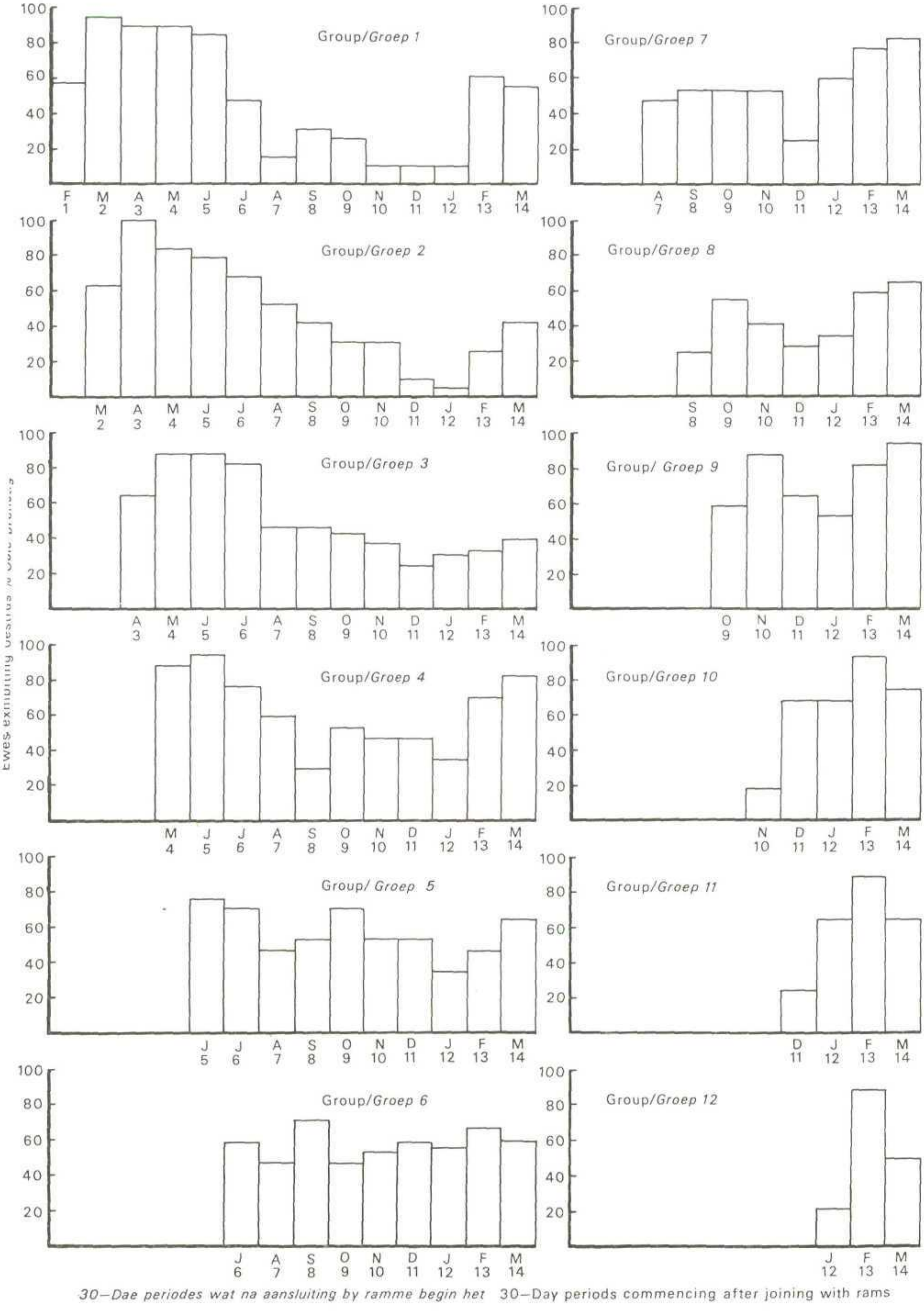

FIG. 3. — Seasonal variations in the level of oestrous activity amongst groups of ewes kept in isolation from rams prior to joining at different times of the year

FIG. 3. — *Seisoensvariasie in die peil van bronstigheid by groepe ooie wat van ramme afgesonder is voor aansluiting op verskillende tye van die jaar*

The results recorded in this experiment may not be the same as would have been obtained with ewes which lambed more regularly. Variations in the period of isolation from rams may also have influenced the incidence of oestrus in the various groups. Further investigation of these factors is indicated.

Opsomming

DIE SEISOENSPATROON VAN BRONSTIGHEID BY OOIE SOOS BEÏNVLOED DEUR AFSONERING VAN EN AANSLUITING BY RAMME

Gedurende Oktober 1966 is ’n kudde Merino-tipe ooie van kontak met ramme afgesonder. Met tussenpose van 30 dae vanaf 1 Februarie 1966 is ’n afsonderlike groep ooie uit die afgesonderde kudde ewekansig uitgesoek en by koggelramme gevoeg. Altesaam 12 groepe ooie is by ramme aangesluit en waarnemings van estrus het tot 26 Maart 1967 voortgeduur.

Gedurende die 17 dae periodes wat op 3 Maart, 3 April, 29 Oktober, 28 November en 28 Desember ’n aanvang geneem het, was die peil van bronstigheid by ooie wat van ramme afgesonder is betekenisvol laer as by ooie wat vir minstens 30 dae met ramme geassosieer is. Afsondering van ramme het dus ’n verlaging in die peil van bronstigheid teweeggebring. Hierdie gevolgtrekking is verder gestaaf deur die bevinding dat, nadat die data vir alle groepe saamgevat is, die peil van spontane bronstigheid na beëindiging van afsondering betekenisvol laer was as die peil van bronstigheid by groepe wat al vir 30 dae met ramme geassosieer is. Die relatiewe verskil tussen hierdie twee kategorieë van ooie het ’n seisoenstendens getoon. Dit is opgemerk dat aansluiting by ramme gedurende die nie-teelseisoen ’n geringer persentasie van die ooie wat in anestrum was gestimuleer het as aansluiting gedurende ander tye van die jaar aangesien die getal ooie wat na stimulering deur ramme bronstigheid getoon het betekenisvol kleiner was gedurende Julie tot vroeë Oktober as gedurende Februarie tot Januarie. Vars aansluiting van ooie by ramme was nietemin ’n meer effektiewe stimulus tot bronstigheid, veral gedurende anestrum, as langdurige assosiasie tussen die geslagte. Die seisoensveranderinge in die getal bronstige ooie het nie in alle groepe dieselfde patroon gevolg nie.

Die mate waartoe seisoens- en ramstimuli in die peil van bronstigheid op sekere tye na aansluiting weerspieël is, asook die veranderinge in die intensiteit en duur van die ramstimulus, word bespreek. Die gevolgtrekking word gemaak dat assosiasie tussen die geslagte vir lang periodes nie tot ’n verdieping van anestrus lei nie.

Résumé

LA DISPOSITION SAISONNIERE DE CHALEUR PARMI LES BREBIS AFFECTEES PAR L'ISOLATION ET LA MISE EN COMPAGNIE AVEC DES BELIERS

Durant octobre 1966, un troupeau de brebis du type Merino fut isolé de tout contact avec les béliers. A 30 jours d'interval, commencant le ler février 1966, un groupe séparé de brebis fut choisi au hazard dans le troupeau isolé et mis en compagnie de béliers amorces stériles. Un total de 12 groupes de brebis furent mises avec les béliers et les observations sur la mise en chaleur furent poursuivies jusqu'au 26 mars 1967.

Durant les périodes de 17 jours commencant le 3 mars, le 3 avril, le 29 octobre, le 28 novembre et le 28 décembre, le niveau d' activité d'entrée en chaleur parmi les brebis qui avaient été isolées des béliers fut significativement plus bas que celui parmi les brebis qui avaient été associées aux béliers pour au moins 30 jours. La conclusion que l'isolation des béliers résulta en une réduction de l'incidence de chaleur fut renforcée par la découverte que, en combinant les normes sur tous les groupes, le niveau d'activité de chaleur spontanée, consécutive à l'introduction des béliers, fut significativement plus bas que l'incidence de chaleur dans les groupes ayant déja été associés aux béliers pour 30 jours. La différence relative entre ces deux catégories de brebis montra une tendance saisonnière.

Il fut évident que l'introduction de béliers durant la saison de tranquilité sexuelle fut moins efficace dans l'émulation de chaleur chez les brebis non en chaleur qu'à d'autres périodes de l'année car la proportion de brebis qui se montrèrent en chaleur à la suite d'une stimulation par les béliers fut significativement plus petite de juillet à début octobre; que de février à juin ou de fin octobre à janvier. Nearmoins, la soudaine introduction de béliers fut une stimulation plus efficace de chaleur chez les brebis, particulièrement durant la période d'absence de chaleur que le fut une association prolongée entre les deux sexes.

Les changements saisonniers dans la proportion des brebis se montrant en chaleur ne suivirent pas le même modèle dans tous les groupes de brebis. L'intensité avec laquelle la stimulation saisonnière et la stimulation par béliers réflétée dans l'incidence de chaleur à certains moments suivant la mise en compagnie, ainsi que les changements en intensité et la durée de la stimulation-bélier sont discutées.

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REFERENCES

ADLER, E. D., 1964. Breeding practices, and the fertility of cattle, pigs and sheep in the Natal Region. *Proc. S. Afr. Soc. Anim. Prod.* 3, 13–25.

HAFEZ, E. S. E., 1952. Studies on the breeding season and reproduction of the ewe. Parts 1 – V. *J. agric. Sci.* 42, 189–265.

HART, D. S., 1950. Photoperiodicity in Suffolk sheep. *J. agric. Sci.* 40, 143–149.

HUNTER, G. L., 1959. A contribution to the study of the problem of low fertility among Merino ewes in South Africa. *J. agric. Sci.* 52, 282–295.

HUNTER, G. L., 1962. Observations on oestrus in Merinos. *Proc. S. Afr. Soc. Anim. Prod.* 1, 67–69.

HUNTER, G. L., 1964. The effect of season and mating on oestrus and fertility in the ewe. *Proc. S. Afr. Soc. Anim. Prod.* 3, 196–206.

HUNTER, G. L. & LISHMAN, A. W., 1967. Effect of the ram early in the breeding season on the incidence of ovulation and oestrus in sheep. *Proc. S. Afr. Soc. Anim. Prod.* 6, 137–139.

LAMOND, D. R., 1964. Seasonal changes in the occurrence of oestrus following progesterone suppression of ovarian function in the Merino ewe. *J. Reprod. Fertil.* 8, 101–114.

LAMOND, D. R., WELLS, K. E. & MILLER, S. J., 1963. Study of a breeding problem in Merino ewes in central Queensland. *Aust. vet. J.* 39, 295–298.

LISHMAN, A. W. & HUNTER, G. L., 1966. Sexual activity in the ewe following isolation from the ram during the breeding season. *S. Afr. J, agric. Sci.* 9, 993–1004.

LISHMAN, A. W. & HUNTER, G. L., 1967. Oestrus activity in the ewe following isolation from the ram during anoestrus. *S. Afr. J. agric. Sci.* 10, 747–752.

LYLE, A. D. & HUNTER, G. L. 1965. The effect on occurrence of oestrus in the ewe during spring of six and 14 day teasing periods prior to mating. *Proc. S. Afr. Soc. Anim. Prod.* 4 140–142.

RADFORD, H. M., 1961a. Photoperiodism and sexual activity in Merino ewes. I. The effect of continuous light on the development of sexual activity. *Aust. J. agric. Res.* 12, 139–146.

RADFORD, H. M., 1961b. Photoperiodism and sexual activity in Merino ewes. II. The effect of equinoctial light on sexual activity. *Aust. J. agric. Res.* 12, 147–153.

RICHES, J. H. & WATSON, R. H., 1954. The influence of the introduction of rams on the incidence of oestrus in Merino ewes. *Aust. J. agric. Res.* 5, 141–147.

SCHINCKEL, P. G., 1954a. The effect of the presence of the ram on the ovarian activity of the ewe. *Aust. J. agric. Res.* 5, 465–469.

SCHINCKEL, P. G., 1954b. The effect of the ram on the incidence and occurrence of oestrus in ewes. *Aust. vet J.* 30, 189–195.

SMITH, I. D., 1965. The influence of level of nutrition during winter and spring upon oestrous activity in the ewe. *Wld. Rev. Anim. Prod.* 1965 (4), 95–102.

UNDERWOOD, E. J., SHIER, F. L. & DAVENPORT, N., 1944. Studies in sheep husbandry in Western Australia. V. The breeding season of Merino, crossbred and British breed ewes in the agricultural districts. *J. Dep. Agric. W. Aust.* 21, 135–143.

YEATES, N. T. M., 1949. The breeding season of the sheep with particular reference to its modification by artificial means using light. *J. agric. Sci.* 39, 1–43.

YEATES, N. T. M., 1955. The effect of light on the breeding season, gestation, and birth weight of Merino sheep. *Aust. J. agric. Res.* 7, 440–446.

PAPER 4

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THE INCIDENCE OF OESTRUS IN MERINO EWES FOLLOWING INTRODUCTION OF BREEDING RAMS IN SUMMER AND SPRING

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Following the studies of Yeates (1949; 1955), Hart (1950) and Hafez (1952) it is generally accepted that the breeding season of the ewe is under photoperiodic control and that sexual activity is initiated by a decrease in the hours of daylight. The onset of sexual activity in Merino ewes in spring, as described by Radford & Watson (1957) and Hunter (1959; 1962; 1964), cannot be correlated with decreasing hours of daylight at that particular time. Furthermore, it has been found that ewes subjected to continuous light (Radford, 1961a) or a constant light rhythm (Radford 1961a; Clegg, Cole & Ganong, 1965) exhibited seasonal changes in sexual activity similar to those observed amongst ewes held in natural daylight. Hafez (1952) suggested that memory of an earlier light regime may be invloved in the initiation of the breeding season, while Dutt & Bush (1955) have recorded an early onset of breeding following low temperatures. However, according to Radford (1961a) it would be necessary to postulate an extended latent period, from stimulus to response, in order for such situations to apply to the Merino. Radford (1961a) therefore suggested that anoestrus followed suppression of sexual activity and that the breeding season was not produced by actual stimulation.

The ability of the ram to induce early onset of the breeding season is now well documented, but Smith (1965) suggested that the ram stimulus also occurs during the breeding season. Lishman & Hunter (1967) have subsequently demonstrated a lowered incidence of oestrus among ewes that had been isolated from rams and the observations reported here are considered in the light of these recent findings.

Procedure

A flock of slightly more than 250 Merino ewes, which had been purchased from farmers in the Natal Region, was joined with breeding rams during December, 1964, and the resulting lambs were weaned on September 14, 1965. On January 7, 1966, 12 breeding rams fitted with raddle harnesses (Radford, Watson & Wood, 1960) were joined with the same flock and the ewes marked were counted daily for 30 days. Weaning of lambs took place on September 20, 1966. In an effort to induce earlier lambing, eight breeding rams were subsequently introduced on November 1, 1966. Mated ewes were again counted as before.

During summer the ewes were run on kikuyu pasture, with only short periods on veld grazing. Winter feeding consisted of maize silage, *Eragrostis curvula* hay, lucerne hay and approximately three weeks oat grazing during the 1966 lambing period. During the non-mating season the ewe-flock was well isolated from rams.

Discussion of Results

The results obtained following mating both during January and November are presented as the cumulative percentage of ewes mated and the percentage mated each day. These results are presented in Figures 1A and 1B, respectively. It is clear that mating at these two times followed virtually identical patterns, with a low incidence of oestrus for the first 17 days followed by peaks on the 18th and 24th days. The low incidence of oestrus during the first 17 days following joining, indicated that many ewes were still in a state of anoestrus and the flush recorded after this day demonstrated the marked ram stimulus. According to Hunter (1959, 1962, 1964) the breeding season of the Merino, in South Africa, commences during November and the present observations, during a corresponding period, do not contradict this finding. However, the results obtained following January mating suggest that in the absence of rams the anoestrous period had not ceased, even by this date. Lyle & Hunter (1965) have suggested that the annual mating, lambing, weaning rhythm will determine the time when

PART B

UNDERNUTRITION DURING LACTATION AND THE EFFECT ON LAMBING AND CALVING RATES

INTRODUCTION

Research by G.L. Hunter in South Africa and by I.D. Smith in Australia, in the early to mid 1960's, had shown that a period of feed scarcity (continuing for some six months), which occurred during winter, delayed the onset of the subsequent spring breeding season. This work was important to the sheep industry as the extensive sheep farming areas were renowned for low quality grazing during winter, often compounded by feed scarcity due to summer droughts. These studies had been based on ewes exposed to sterile rams for long periods of time, without regular lambing occurring.

Paper 11

The findings presented in Part A had also demonstrated the important modifying effects of isolation from rams on seasonal breeding patterns in ewes. Consequently, the research described in Paper 11 incorporated sheep flocks that were exposed to a regular regime of mating and lambing and was designed to investigate the importance of food scarcity during an autumn lactation. In addition particular attention was given to absence of sterile rams. This research was initiated in the autumn of 1967 and continued for six years.

Findings :-

1. Underfeeding during lactation did not prolong lactation anoestrus.
2. Date of lambing was high correlated with duration of post-partum anoestrus.
3. The proportion of ewes exhibiting oestrus during the seasonal anoestrous period was reduced by underfeeding during lactation.
4. Due to a marked increase in oestrous activity after introduction of fertile breeding rams the depressing effects of undernutrition and isolation from rams were eliminated.

Implications/Significance :-

1. The two factors (undernutrition during autumn and isolation from rams) had little effect on the proportion of the flock mated during the annual, six-week breeding period.
2. Other researchers had accounted for the reduced lambing percentage (number of ewes lambing as a % of the number exposed to rams) observed following spring vs autumn mating in terms of a reduced incidence of oestrus during spring. In other words, the seasonal breeding period had only just commenced in spring whereas it had reached its peak in autumn. Our results (Paper 11) showed that few ewes were not served in spring.
3. Rejoining of ewes previously isolated from rams during anoestrus with rams at the onset of the annual breeding period obliterated the depressing effect (on mating) of lactational undernutrition. Why this should also have occurred in ewes regularly exposed to vasectomized rams during the winter was not clear. Perhaps a change of rams (vasectomized rams replaced with entire rams) constituted a new ram stimulus. However, a spontaneous initiation of the new breeding season amongst used that had become synchronized with an annual mating-lambing rhythm could not be excluded. The latter is not in accord with data presented in Papers 3 and 4 and the studies of D.M. Joubert and G.L. Hunter.

Paper I2

The results depicted in Paper I2 focus on the lambing performance of the animals incorporated in Paper 11.

Findings :-

1. Number of lambs produced in the subsequent lambing was positively correlated with bodymass at lambing, end of lactation and prior to mating. A negative correlation between productivity (0, 1 or 2 lambs/ewe) and weight loss during lactation was also obtained.
2. Litter size was not significantly influenced by flushing prior to mating or isolation from rams during anoestrus.

3. Twenty percent or more of the ewes mated did not produce a lamb, but the role of underfeeding during lactation or prior to mating was not clear cut.

Implications/Significance :-

1. Heavier ewes cycle more regularly and are more likely to be mated early in the breeding period and to produce more lambs per ewe per parturition than small individuals. Ewes, sub-standard in size, should thus be culled.

Paper 13

Based on research by others it was expected that undernutrition of lactating ewes during an autumn lactation would seriously reduce their conception rates during the following breeding period. However, even when as much as 20% of bodyweight was lost during a 12-week lactation period, this had a negligible effect on lambing rates (Paper 12).
In farming practice, if feed is scarce during autumn lactation then the situation is likely to continue into the winter. This would be due to two reasons, viz.,

1. Lactating ewes are commonly grazed on cereal crops such as oats, grown under dry-land conditions. When the expected late summer rains do not materialise then the lactating ewes are faced with feed scarcity.
2. Producers commonly believe that once lactation has been terminated by weaning of lambs then the ewes can subsist on low quality forage. Often the quality is overestimated, the quantity limited and the demands of wool production and replacement of body reserves ignored.

In Paper 13 the implications of a failure to allow recovery (during winter) from weight loss during autumn lactation was investigated. The possible stimulating effect of new rams (entire breeding rams) was also avoided.

Findings :-

1. Even though underfed ewes lost 17% of their postpartum body mass (PBM) and gained only 3% during the 14-week post weaning/pre-mating period this reduced the

lambing % (ewes lambing as a % of ewes mated) by only 12.9%. However the proportion of barren ewes (served but not lambing) was nearly 25% higher than in well-fed ewes.

Implications/Significance :-

1. Severe loss in bodyweight during autumn lactation without compensation during postweaning period seriously reduced lambing rates but not mating rates.

Paper 14

In seeking to increase the frequency of lambing to more than once every 12 months it may be necessary to give attention to aspects such as suckling a lamb, level of nutrition during lactation and stimulation by introduction of rams. Paper 14 looked at these factors in ewes lambing in spring (Oct/Nov).

Findings :-

1. Post-partum interval to ovulation, but not to oestrus was significantly correlated with ewe weight three days after lambing.
2. Neither weaning at three or 20 days post partum, supplementary feeding for 28 days during lactation nor joining with vasectomized rams influenced interval to oestrus.

Implications/Significance :-

1. Only weight at lambing influenced onset of ovulation post partum.

Paper 15

In attempting to explain why underfeeding reduced reproductive activity in ewes it was necessary to consider whether :-

a) the ability of the ovary to respond to gonadotropins would be reduced (Paper 15)

b) the quantity of gonadotropins reaching the ovary was compromised (Paper 16) and
c) the ability of the pituitary to release gonadotropin was reduced (Paper 17).

In the experiment detailed in Paper 15 the object was to determine whether undernutrition could affect sensitivity of the ovary to gonadotropic stimulation. Accordingly, pituitary release of LH was blocked by continuous administration of progesterone. Ovulation was then induced by injection of hCG at a level judged to be the threshold for ovulation induction.

Findings :-

1. Undernutrition (20% difference in bodyweight compared to well-fed animals) did not reduce ovulation rate, but weight of ovarian fluid tended to be reduced.
2. Ewes gaining in mass showed increased ovarian mass.

Implications/Significance :-

1. Limited evidence was obtained suggesting that underfeeding may reduce the sensitivity of the ovaries to gonadotropic stimulation.

Paper 16

At the time that the experiments detailed in Papers 12, 13 and 15 were being conducted it was clear that the quantity of gonadotropin reaching the ovaries needed to be measured. This was an important piece of information in elucidating the mechanism(s) whereby undernutrition prolonged seasonal anoestrus.
Unfortunately, at that time only expensive, time-consuming bioassays incapable of measuring the nanogram concentrations of LH and FSH in the circulation were available.

During 1970 steps were taken to set up a radioimmunoassay system for LH and in 1971 plasma samples (ovine) could be successfully assayed. This was a first for domestic species in Africa. The objective in Paper 16 was to measure the pre-ovulatory release of LH in ewes that had either been underfed or well-fed during autumn lactation. It was considered that treatment effects were most likely to be seen when the oestrous activity of ewes treated in this way had been shown to differ most. Accordingly, frequent blood samples were collected at oestrus during August, September and October.

Findings :-

1. A marked variation, within and between ewes, in the commencement (in relation to onset of oestrus) and magnitude of the pre-ovulatory LH release was seen.
2. No effect of underfeeding on LH release was detected.
3. Oestrus, without a detectable pre-ovulatory LH release, was observed in 13% of the oestrous periods studied.

Implications/Significance :-

1. Provided ewes are undergoing oestrous cycles, there would appear to be no deleterious effect of earlier underfeeding on pre-ovulatory LH release.

Paper 17

In view of the extreme variability of the results obtained in Paper 16 it was obvious that some form of synchronisation of the experimental animals was required. This could be achieved in two ways viz.,

1. By synchronising oestrus and ovulation through the use of frequent administration of progesterone.
2. By inducing a release of LH at a pre-determined time. This involved the administration of GnRH.

The object in Paper 17 was to determine whether underfeeding would affect the quantity of LH that could be released from the anterior pituitary. This was one of the forerunners of many experiments, subsequently performed throughout the world, along similar lines, .

Findings :-

1. Underfeeding reduced the sensitivity to GnRH.

2. Oestrogen priming increased LH release.
3. More LH was release on day 15 of the oestrous cycle than on day 3.

Implications/Significance :-

1. Restricted feeding during lactation may reduce pituitary stores of releasable LH or slow down rapid synthesis of LH.
2. Oestrogen priming appeared to eliminate the differences due tc underfeeding. Thus, a deepening of anoestrus in underfed ewes may be due, in part, to reduced oestrogen secretion and hence deficient priming. This is in agreement with the findings presented in Paper 15.

Paper 18

At this stage in the research program access was obtained to a number of beef herds and some of the principles that had been established using sheep could be tested in cattle. The first investigation (Paper 18) sought to determine whether supplementary feeding should be supplied to lactating beef cows or to their suckling calves. A secondary objective was to examine the role of time of calving within the annual 90-day spring/summer calving period.

Findings :-

1. Energy supplementation had no major effect on liveweight changes of cows or their reconception rates (CR) and intercalving intervals (ICI).
2. Variation in the ICI depended largely on the time of calving, but this was modified by the type of natural grazing.
3. Conception rates were closely associated with change in bodyweight of cows during the breeding period and the weight of the cows at the end of the breeding period.
4. Creep feeding of calves was beneficial for late born calves in the sweetveld areas, while all calves benefited in sourveld grazing.

Implications/Implications :-

1. Early calving did improve CR's but this depended on the opportunity to lengthen ICI.
2. The results obtained were the first indication that even when well-fed, Afrikaner cows are unlikely to exhibited conception rates much in excess of 50%, unless the mating period allows for extended ICI's.

Paper 19

Cows that are suckling their first calves are notoriously difficult to re-settle. Because spring grazing is unlikely to meet the demand for growth and lactation in such animals, there are good arguments for supplying additional energy during lactation. First calves commonly wean smaller calves than older cows and there is also justification for creep feeding where the milk supply is likely to be reduced. These concepts formed the basis for the treatments applied in the experiments described in Paper 19.

Findings :-

1. Supplemented energy had little measurable effect on weight loss during lactation or on CR's
2. The advantage of a high bodymass at parturition was again reflected in improved re-conception rates.
3. Early calving was decidedly beneficial as regards CR's, particularly in Afrikaner cows.
4. Creepfeeding of calves improved growth rates of the calves.
5. Supplementary feeding of both cows and calves improved CR's in the cows.
6. Sussex-Afrikaner first-calves showed re-conception rates that were 40% higher than straight bred Afrikaner or Hereford cows. The benefit of a higher bodymass at calving in the crossbred cows could not be ignored.

1. A 100% CR in first-calver Afrikaner cows was judged to be possible only if the interval between calving and introduction of bulls was extended to 133 days. The normal duration of the mating period (65 days in this case) would need to be added to this figure. Calving intervals approaching 365 plus 200 days are obviously not acceptable.

Paper 20

The objective in this study was to ascertain whether supplementing lactating cows, on summerveld, with protein would show greater benefit that energy alone.

Findings :-

1. An average of 797g of energy supplement increased the CR's by 23%. It also increased the % of cows calving within 30 days of onset of the calving season.
2. Protein (natural or non-protein nitrogen) was only about half as affective in improving CR's as the energy supplement.

Implications/Significance :-

1. In this experiment, lactating crossbred cows (Sussex X Afrikaner) required only additional energy during early lactation to improve their reproductive performance. The breed effects seen in Paper 19 were not contradicted by these findings.

Paper 21

The research described in Paper 21 was based on the finding that reconception rates and subsequent calving rates were markedly influenced by the weight of the cows at calving or at the end of the mating period.

Data collected over six years for Sussex-type cows was examined for relationships between conception rates and body weight of the cows at various stages of the production cycle.

Findings :-

1. Weight at the start of the annual breeding period had a marked effect on conception rates
2. Weight changes over the winter or during the breeding period had no effect on calving rates.

Implications/Significance :-

1. Breeding cows could be fed so as to achieve pre-determined calving rates.
2. The target weight which cows of different ages needed to achieve in order to secure an 80% calving rate could be pre-determined.

This research suffered from two limitations-

1. Bodyweight is not a very good measure of the physiological readiness of cows to breed. A better assessment would be to estimate the degree of fatness (Bodycondition scores) Such measures were used to great effect by H J Meaker and A van Niekerk in subsequent research.
2. The important modifying effect of date of calving was not examined.

Paper 22

D.H. Hale suggested that target mass at mating could be achieved either :

1. By allowing cows to lose weight during winter, followed by gain in weight during the subsequent summer.
2. By maintaining the desired condition throughout the year.

This study (Paper 22) examined the weights at which underfed, non-lactating cows, became anoestrus and at which they resumed oestrous cycles after good feeding. Two rates of loss and two of gain were applied.

Findings :-

1. Cows lost on average 100kg (21%) or 1.1 condition score points (1=thin, 5=fat) before they became anoestrus.
2. An additional 35-57kg (above anoestrous weight) was required for cows to resume ovarian activity.
3. Rate of loss or gain was unimportant.
4. Eighty-five percent of the cows conceived at the first mating after anoestrus.

Significance/Implications :-

1. The penalty to be paid when non-lactating, *Bos indicus* type cows are underfed, to the extent that they become anoestrus, is of the order of 25kg. The economics of feeding strategies which permit severe loss in bodyweight and condition, during winter, needs careful evaluation. This applies especially to *Bos indicus* type cattle which appear to be particularly sensitive (in terms of reduced calving rates) to periods of underfeeding. Perhaps this is part of the mechanism which permits survival under unfavourable environments.

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REPRODUCTION IN THE EWE IN RELATION TO PLANE OF NUTRITION, BODY MASS AND CHANGE OF BODY MASS. I. INCIDENCE OF OESTRUS BETWEEN LAMBING AND RECONCEPTION

(*Met opsomming in Afrikaans*)
(*Avec résumé en français*)

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ABSTRACT

Over a six-year period commencing 1967, a total of 761 Merino and 360 crossbred ewes which lambed in autumn were fed either 100% (High plane) or 50% (Low plane) of the estimated daily nutrient requirements during the 84-day lactation period. Twice-daily observations for oestrus were conducted until weaning of the lambs, at which stage half the ewes were isolated from rams (1968, 1969 and 1970 only) until one oestrous cycle prior to initiation of the annual breeding period. The low plane of feeding did not prolong the interval between parturition and first oestrus (average 39,2 to 52,8 days) or increase the number of ewes anoestrous throughout lactation. Body mass or change in body mass during lactation had no consistent effect on the interval to first oestrus. A highly significant association ($P=0,001$) between the date of lambing after 1 March (X) and the duration of the post-partum anoestrous period (Y) could be expressed by: $Y=58,0-0,23X$. Underfeeding during lactation significantly reduced the number of ewes exhibiting oestrus after the first week of August 1969, 1970 and 1971. For the 17-day periods commencing 8 and 25 August there was a significant association between the percentage ewes oestrous and their body mass at weaning (data for 1968–1971 pooled). During the subsequent four 17-day periods the oestrous activity was closely associated with the percentage change in body mass between parturition and weaning. Similarly, the number of oestrous cycles exhibited by each ewe from 8 August to 17 November was negatively correlated with the percentage loss in body mass to weaning. The ewes which mated early after 15 October had a greater pre-mating body mass than those which mated later. Nutrition and isolation from rams had no notable effect on the total number of ewes served during the annual breeding period of six weeks.

INTRODUCTION

The possibility of increasing meat supplies by lambing more frequently than once every 12 months is receiving world-wide attention and Hunter (1968) has reviewed the factors which influence the delay to rebreeding following parturition. According to Van Niekerk & Mulder (1965) the plane of nutrition during lactation is the greatest single factor affecting the duration of post-partum anoestrus.

In the grassveld regions of South Africa it is common practice to mate ewes in spring and early summer (Adler, 1964) so that the lambs are born at a time when their growth can be optimal (Reyneke, 1969). Such early-season mating results in a smaller lamb crop than where mating is delayed until autumn. A reduced incidence of oestrus at this early stage of the breeding season (Dun, Ahmed & Morrant, 1960; Shelton & Morrow, 1965; Watson & Radford, 1966) could be a factor contributing to the low lambing percentage.

Poor nutrition at some earlier stage can delay onset of the new breeding season (Hunter, 1962; Smith, 1964, 1965) and isolation of ewes from rams during anoestrus can produce a similar effect (Lishman & Hunter, 1967; Lishman, 1969).

The experiments of this study were designed to investigate to what extent under-nutrition and consequent loss in body mass of wooled sheep exposed to a regular regime of mating and lambing, influence the oestrous activity of breeding ewes.

PROCEDURE

Commencing in 1967, and continuing each year until 1972, ewes in good condition and which lambed in autumn were placed in pens three days after lambing. Until 1 November they were fed rations consisting of maize silage, milled lucerne hay and a concentrate mixture (90 parts maize meal : 10 parts whole carcase meal).

The experimental animals were drawn from flocks of Merino and crossbred (South African Mutton Merino × Merino) ewes and each ewe was allowed to suckle only one lamb.

The treatments applied are summarized in Fig. 1 and the factors studied were as described below.

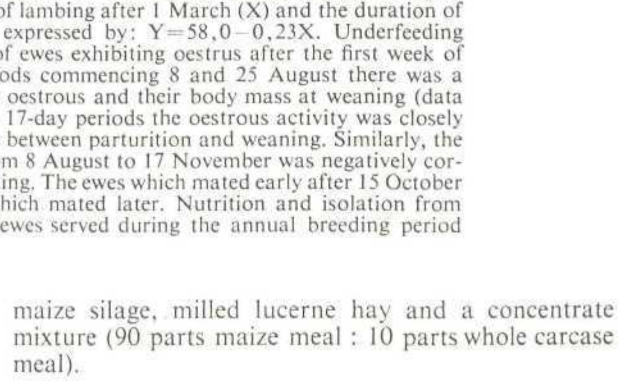

FIG. 1 Plan of the experimental treatments illustrating the expected changes in mass as a consequence of (i) high (——) and low (- - - -) planes of nutrition applied during lactation and (ii) the flushing effect (——•——, - - - • - - -) three weeks prior to joining with fertile rams
(1) Not applied during 1967
(2) Not applied during 1971 and 1972

FIG. 1 *Plan van die behandelings wat die verwagte veranderings in massa as gevolg van (i) hoë (——) en lae (- - - -) peile van voeding gedurende laktasie en (ii) invloed van prikkelvoeding (——•——, - - - • - - -) drie weke voor bybring van vrugbare ramme, weerspieël*
(1) *Nie toegepas gedurende* 1967
(2) *Nie toegepas gedurende* 1971 *en* 1972

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PAPER 16

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THE RELEASE OF LH AT OESTRUS IN EWES ON TWO PLANES OF NUTRITION DURING LACTATION

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The anoestrous condition which follows upon periods of underfeeding in ewes is apparently similar to the normal seasonal anoestrus and is usually manifested as a prolongation (Hunter, 1962) or precipitated onset (Smith, 1962) of the seasonal period of sexual rest. It has been suggested that the ovarian quiescence noted in underfed animals may be the result of pseudohypophysectomy (Mulinos & Pomerantz, 1940; Lamming, 1966; Leathem, 1966).

An acute release of LH at oestrus in ewes has been demonstrated by Niswender, Roche, Foster & Midgley, 1968; Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969; Wheatley & Radford, 1969) and it is possible that changes in the pattern of LH release may indicate functional changes in the pituitary of ewes which become anoestrous after periods of inanition. This phenomenon was studied in an attempt to elucidate the mechanism involved in a premature cessation of breeding amongst ewes previously subjected to underfeeding.

During August, September and October 1970 and 1971 and in October 1972, blood samples were obtained at oestrus from eighty-seven Merino ewes that had been fed either 100% (high plane) or 50% (low plane) of their estimated daily nutrient requirements during the preceding 84-day autumn lactation period. The experimental rations, changes in body mass and oestrous activity of the ewes have been described elsewhere (Lishman, Stielau & Botha, 1974). Jugular blood samples were obtained (indwelling cannulae) from at least five ewes, on each plane of nutrition, which exhibited oestrus after the commencement of a sampling period (i.e. August, September or October 1970 and 1971). The blood samples were taken at 2-hr (1970) or 1-hr (1971), intervals, commencing as soon as a ewe stood for service and sampling continued for up to 36 hr. During October 1972, the first blood was drawn approximately 12 hr before the expected onset of oestrus in thirteen ewes. Sampling was repeated at 4-hr intervals until mating commenced and thereafter at hourly intervals for 24 hr. A total of ninety-two oestrous periods were studied. In order to detect oestrus, vasectomized rams were placed with the ewes at intervals of not more than 2 hr, and the rams were frequently interchanged between various groups of ewes. As soon as a ewe permitted service, she was immediately removed from the presence of the ram.

The double antibody radioimmunoassay for ovine LH, as described by

Table 1. Variation in parameters associated with the preovulatory release of LH in ewes on high and low planes of nutrition during lactation

Year sampled	*Start of LH release relative to onset of oestrus (hr)*		*Duration of LH release (hr)**		*Maximum level of LH (ng/ml)*			*Amount of LH released (ng)*		
						Mean			*Mean*	
	High plane	*Low plane*	*High plane*	*Low plane*	*Total range*	*High plane*	*Low plane*	*Total range*	*High plane*	*Low plane*
1970	6·74	6·59	10·55	11·25	51·8–386·6	175·6	166·8	178·3–1460·0	743·5	677·0
1971	11·80	7·87	10·12	9·31	100·5–326·3	218·9	239·1	299·4–1519·0	852·1	804·9
1972	−0·86†	0·20	9·74	9·46	149·5–344·6	236·5	186·3	268·3–1115·1	856·1	556·2

* S.E. (all observations) = ±1·63.

† The negative value indicates that the release of LH commenced before onset of oestrus.

Niswender, Reichert, Midgley & Nalbandov (1969), was used to quantify LH levels in the plasma samples. As standard, NIH-LH-S16 was used and the initial dilution of anti-LH serum (Lishman, Stielau, Dreosti & Stewart, 1973) was 1:100,000. The duration of the LH surge (plasma levels equal to or greater than 10 ng/ml) was determined graphically, and the total quantity of LH released was estimated as the area under the curve.

The nature of the preovulatory LH release varied greatly (Table 1), and the quantity of LH released ranged between 178·3 and 1519·0 ng—more than an eightfold variation. Goding *et al.* (1969) reported a less than twofold variation in the magnitude of the LH surge, but Reeves, Arimura, Schally, Kragt, Beck & Casey (1972) noted considerable variation in the amount of LH released as well as in the shape of the release curve following administration of releasing hormones.

Goding *et al.* (1969) and Wheatley & Radford (1969) suggested that the LH surge which resulted in ovulation in the ewe did not commence before the onset of mating. This agreed with the time of LH release from the pituitary as observed by Robertson & Rakha (1966). The results obtained here, however, indicated that there was a marked variation in the timing of the LH peak in relation to the onset of overt oestrus and that in twenty-two oestrous periods (23·9%), it commenced at or before the onset of overt oestrus. Notable variations in the release of pituitary LH in relation to the initiation of mating have also been demonstrated by the earlier studies incorporating bioassay techniques (Santolucito, Clegg & Cole, 1960; Robertson & Hutchinson, 1962; Dierschke & Clegg, 1968).

There was no significant effect of either the plane of nutrition or the year of study on the latency to LH release (Table 1). The log transformation was applied to the data in Table 1 (duration of LH release excluded), but no significant differences were evident when comparisons were restricted to individual years.

In heifers, it has been concluded that underfeeding results in an increase in plasma LH (Gombe & Hansel, 1973), a result which is the reverse of the trend suggested by the present results. Gombe & Hansel (1973) proposed that a restricted intake of energy reduces the responsiveness of the ovary to LH and this finding has been confirmed by Lishman, Stielau, Swart & Botha (1974).

The occurrence of oestrus, without an LH release being detected, was highest during mid- to late anoestrus with a total of twelve (13·0%) oestrous periods in which no LH surge was observed during the sampling period. Previously, it had been assumed that when no LH surge was detected at oestrus, this was due to infrequent sampling (Goding *et al.*, 1969; Niswender *et al.*, 1968). Frequent sampling for 24 hr before and 48 hr after oestrus should exclude the possibility of non-detection of LH peaks.

The LH surges recorded at the last oestrus of the one breeding season or at the first oestrus of the new season did not differ distinctly in any respect from those observed in ewes which exhibited regular oestrus throughout the anoestrous season. Further, it was not possible to correlate the pattern of LH release with the body mass or with a change in the body mass of the ewes during lactation.

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REFERENCES

Dierschke, D. J. & Clegg, M. T. (1968) Studies on the relationship of serum and pituitary gonadotrophin levels to ovulation in the ewe. *J. Reprod. Fert.* **15,** 321.

Goding, J. R., Catt, K. J., Brown, J. M., Kaltenbach, C. C., Cumming, I. A. & Mole, B. J. (1969) Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during estrus and following estrogen administration in the sheep. *Endocrinology,* **85,** 133.

Gombe, S. & Hansel, W. (1973) Plasma luteinizing hormone (LH) and progesterone levels in heifers on restricted energy intakes. *J. Anim. Sci.* **37,** 728.

Hunter, G. L. (1962) Observations on oestrus in Merinos. *Proc. S. Afr. Soc. Anim. Prod.* **1,** 67.

Lamming, G. E. (1966) Nutrition and the endocrine system. *Nutr. Abstr. Rev.* **36,** 1.

Leathem, J. H. (1966) Nutritional effects on hormone production. *J. Anim. Sci.* **25,** Suppl. **1,** 68.

Lishman, A. W., Stielau, W. J. & Botha, W. A. (1974) Reproduction in the ewe in relation to plane of nutrition, bodymass and change in bodymass. I. The incidence of oestrus between lambing and reconception. *Agroanimalia,* **6,** 25.

Lishman, A. W., Stielau, W. J., Dreosti, I. E. & Stewart, A. M. (1973) Radioimmunological evaluation of rabbit anti-sera to ovine luteinizing hormone. *S. Afr. J. Anim. Sci.* **3,** 67.

Lishman, A. W., Stielau, W. J., Swart, C. E. & Botha, W. A. (1974) Nutrition of the ewe and the ovarian sensitivity to gonadotrophin. *Agroanimalia,* **6,** 7.

Mulinos, M. G. & Pomerantz, L. (1940) "Pseudohypophysectomy". A condition resembling hypophysectomy produced by malnutrition. *J. Nutr.* **19,** 493.

Niswender, G. D., Reichert, L. E., Midgley, A. R. & Nalbondov, A. V. (1969) Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology,* **84,** 1166.

Niswender, G. D., Roche, J. F., Foster, D. L. & Midgley, A. R. (1968) Radioimmunoassay of serum levels of luteinizing hormone during the cycle and early pregnancy in ewes. *Proc. Soc. exp. Biol. Med.* **129,** 901.

Reeves, J. J., Arimura, A., Schally, A. V., Kragt, C. L., Beck, T. W. & Casey, J. M. (1972) Effects of synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH) on serum LH, serum FSH and ovulation in anestrous ewes. *J. Anim. Sci.* **35,** 84.

Robertson, H. A. & Hutchinson, J. S. M. (1962) The levels of FSH and LH in the pituitary of the ewe in relation to follicular growth and ovulation. *J. Endocr.* **24,** 143.

Robertson, H. A. & Rakha, A. M. (1966) The sequence, time and duration of the release of follicle-stimulating hormone and luteinizing hormone in relation to oestrus and to ovulation in the sheep. *J. Endocr.* **35,** 177.

Santolucito, J. A., Clegg, M. T. & Cole, H. H. (1960) Pituitary gonadotrophins in the ewe at different stages of the oestrous cycle. *Endocrinology,* **66,** 273.

Smith, I. D. (1962) The effect of plane of nutrition on the incidence of oestrus in the Merino ewe in Queensland. *Aust. vet. J.* **38,** 338.

Wheatley, I. S. & Radford, H. M. (1969) Luteinizing hormone secretion during the oestrous cycle of the ewe as determined by radioimmunoassay. *J. Reprod. Fert.* **19,** 211.

PAPER 17

S. Afr. J. Anim. Sci. 7, 141–145 (1977)

THE EFFECTS OF FEED RESTRICTION, OESTROGEN PRIMING AND STAGE OF THE OESTROUS CYCLE ON GN-RH-INDUCED RELEASE OF LH IN EWES

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OPSOMMING: DIE INVLOED VAN VOERBEPERKING, VOORBEHANDELING MET ESTROGEN EN STADIUM VAN DIE ESTRUSSIKLUS OP DIE AFSKEIDING VAN LH NA GnVH TOEDIENING BY OOIE

Die invloed van ondervoeding gedurende 'n herfs laktasie, voorbehandeling met estrogeen en stadium van die estrussiklus op die afskeiding van LH na stimulering met GnVH is bestudeer by Merino-ooie. Die basale LH-konsentrasie voor GnVH-inspuiting is nie deur die behandelings beïnvloed nie. Voorbehandeling met estrogeen het die piek LH-konsentrasie betekenisvol verhoog beide op die derde ($P < 0{,}001$) en vyftiende dag ($P < 0{,}05$) van die estrussiklus. In vergelyking met piek LH-konsentrasie het die totale afskeiding van LH 'n minder konsekwente reaksie op voorbehandeling met estrogeen getoon. Ooie wat behandel is toe die reserwes van die hipofise hoog was (15de dag), het meer LH afgeskei (piek LH en totale LH) as wanneer die ooie vroeg in die siklus (3de dag) behandel is. Ondervoeding het die gevoeligheid van die ooie tot GnVH verminder, aangesien 'n laer piek LH-peil waargeneem is by hierdie ooie in vergelyking met ooie wat hul liggaamsmassa gedurende laktasie gehandhaaf het (15de dag; $P < 0{,}001$).

SUMMARY:

The effect of underfeeding during autumn lactation, oestrogen priming and stage of the oestrous cycle on the release of LH in response to GnRH was studied in Merino ewes. The basal LH level prior to GnRH administration was not influenced by the treatments applied. Oestrogen priming significantly increased the peak LH level, both on days 3 ($P < 0{,}001$) and 15 ($P < 0{,}05$) of the oestrous cycle. In comparison, the total LH release showed a less consistent response to oestrogen priming. Ewes treated when pituitary stores of LH were expected to be high (day 15) released more LH (peak LH and total LH) than when treated early in the cycle (day 3). Underfeeding reduced the sensitivity of the ewes to GnRH since a lower peak LH level was observed in such animals when compared to ewes which maintained their bodymass during lactation day 15; $P < 0{,}001$).

The decreased reproductive rate of underfed female mammals is considered to be due, at least in part, to changes in the synthesis and/or release of the hormones involved in reproduction (Mulinos & Pomerantz, 1940; Lamming 1960; Leathem, 1966; Howland 1972). When ewes were underfed during autumn lactation their oestrous activity declined to a minimum shortly before the onset of the new breeding season in spring (Lishman, Stielau & Botha, 1974). In an attempt to cast some light on the mechanism by which underfeeding reduces the incidence of oestrus, the Gn-RH-induced release of LH was measured during late September.

Procedure

The experimental ewes were from a flock of 120, two to seven-year old Merino ewes which had lambed between 14 March and 14 April 1974. Three days after parturition ewes, with single lambs, were randomly allocated to either the adequate or restricted levels of feeding. The former ration maintained the weight of the ewes during the 12-week lactation, while the latter resulted in a 23% loss during this time. After weaning of the lambs, all the ewes received a ration which maintained body condition in those ewes not fed a restricted diet during lactation. The composition of the rations fed has been described by Lishman *et al.* (1974).

The original intention was to utilize only ewes that were anoestrus during September and therefore to increase the proportion that became anoestrus the flock was kept isolated from rams. However, during August ovarian examination of 10 ewes, selected at random, revealed that 50% possessed an active corpus luteum. Consequently, the experiment was modified and the release of LH measured shortly after and before oestrus, when pituitary reserves were likely to be low and high, respectively (Roche, Foster, Karsch, Cook & Dzuik, 1970).

To simplify collection of blood samples the oestrous cycles of the ewes were synchronised by insertion of progesterone pessaries (G.D. Searle), followed on removal after 15 days by a single i.m. injection of 500 μg oestradiol benzoate (ODB). Observations for oestrus were then initiated using vasectomized rams twice daily. On day 3 of the second cycle following synchronisation, i.e. approximately 23 days after removal of the progestagen pessaries, 58 ewes were divided into 6 treatment groups (Table 1). Six hours prior to the i.m. injection of Gn–RH, (Abbott) on 24 September, 3 of these groups received a priming dose of 100 μg ODB. Directly thereafter, indwelling silastic jugular catheters (Portex) were inserted into all ewes and at two-hour intervals blood samples (5 ml) were withdrawn into heparinized syringes. At 12h00 the ewes were injected with the allocated dose of Gn-RH in saline, (Table 1) and for the next 8 hours, blood samples were obtained every 30 minutes. On day 15 of the cycle subsequent to that when Gn-RH was first administered 54 ewes from the flock of 120 were re-allocated, at random, to the treatments applied on day 3 of the previous cycle. However, in an attempt to improve the priming effect of oestrogen the dose of ODB was divided into 3 injections each of 30 µ g. These were administered at 8h intervals, commencing 22h before Gn–RH treatment. Blood samples were drawn as before, but at 15 minute intervals after Gn–RH injection. Following centrifugation the plasma samples were stored at -15°C until assayed for LH by the double-antibody radioimmunoassay of Niswender, Rechert, Midgley & Nalbandov (1969). This assay has been validated by Lishman (1972). NIH-LH-S16 was used as standard.

Results and Discussion

The data from the 2x2x2x3 factorial treatment arrangement were analyzed by least squares procedures appropriate for unequal subclass numbers. A model which accounted for the effects of day of the cycle, oestrogen priming, level of feeding and dose of Gn–RH used. Three characteristics of the LH release were measured viz., the basal level prior to Gn–RH, the highest level to which the hormone rose in the plasma following Gn–RH (peak LH) and the total release of LH (estimated from area under the LH release curve). The basal LH level (4,47 ± 1,16 ng/ml) was not significantly influenced by oestrogen priming or any of the treatments applied, whereas the least squares means in Table 1 indicate that oestrogen priming significantly increased the peak LH level both on day 3 (increase = 5,6 ng/ml; $P < 0{,}001$) and day 15 (increase = 10,6 ng/m; $P < 0{,}05$). This suggested that the divided priming regime applied on day 15 was more effective than the single dose given on day 3, but since the priming procedures varied the conclusion remains only tentative. The response to oestrogen priming, measured in terms of the total LH release (Table 1) was more variable than the peak LH levels and a significant positive response ($P < 0{,}05$), was obtained only in the restricted ewes on day 3 and the unrestricted animals on day 15. These results support the findings which indicate that oestrogen plays an important role in modifying the pituitary response to Gn-RH in the ewe, (Reeves, Arimura & Schally, 1971 a & b; Jackson, 1975; Coppings & Malven 1976), cow (Convey, 1973) and rat (Libertun, Cooper, Fawcett & McCann, 1974). In view of the observation that ODB elicits an LH surge within approximately 15 hours (Reeves, Beck & Nett, 1974; Jackson, 1975) and the present finding that 100 µg ODB does not elevate LH levels by six hours after administration, but does sensitize the pituitary to exogenous Gn-RH within this time, supports the hypothesis favouring a dual action of oestrogen viz., a quick action to sensitize the pituitary and a slower action on the hypothalamus to elevate the levels of Gn-RH. The conclusions of Nett, Akbar & Niswender (1974), Cumming (1975) and Jackson (1975) provide support for this hypothesis. However, Coppings & Malven (1976) proposed that the pituitary is sensitized, briefly, only 15 h after administration of oestradiol – 17β and that facilitated release of endogenous Gn–RH occurs 12 to 20h after administration of this oestrogen.

The ewes treated on day 15 of the oestrous cycle exhibited a significantly ($P < 0{,}01$) greater peak and total release of LH after 25 µg and 100 µg Gn–RH than those treated on day three (Table 2). This trend is in agreement with that reported by Hooley, Baxter, Chamley, Cumming & Findlay (1974) and Rippel, Johnson, Mauer & Webel (1974). If the quantity of LH released is related to pituitary reserves (Jenkin, Heape & Symons, 1977) then the 4-fold difference in pituitary content of LH between days 3 and 15 (Roche *et al.*, 1970) would be expected to result in a difference in peak LH values greater than the 24,6 ng/ml obtained in the present study. The difference, as a percentage of the value on day 3, was greatest at the lowest level of Gn–RH, hence the significant "day of cycle X dose of Gn–RH" interaction, when comparing days 3 and 15. This interaction was also reflected in the lower total LH release on day 15 than on day 3 when 50 µg GnRH was administered (Table 2).

Zolman, Gonvey & Britt (1974) proposed that the interaction between the dose of Gn–RH and the day of the oestrous cycle on which the releasing hormone was injected, was due to variations in the level of oestrogen. Our results are somewhat contradictory in that it could be expected that administration of a priming dose of 100 µg ODB would nullify any effect of differences in the level of endogenous oestrogen. However, a stage-of-cycle effect was still evident and if the circulating levels of progesterone and oestrogen levels had been measured, the results may have become clearer. In support of the present results is the finding that in rats the increased responsiveness to Gn–RH could not be correlated with the oestrogen levels at that time (Araki, Ferin, Zimmerman & Vande Wiele, 1975). Furthermore, Castro-Vazques & McCann (1975) demonstrated that ovariectomy did not block the increased responsiveness near the time of oestrus. Both Castro-Vazquez & McCann (1975) and Zeballos & McCann (1975) noted that priming with Gn–RH increased the responsiveness to subsequent releasing hormone.

Although the animals which received the maintenance diet (unrestricted) during lactation exhibited a higher peak LH value than those which were restricted at this time (Table 1) the differences were significant ($P < 0{,}001$) only on day 15. Beal, Kaltenbach & Dunn (1975) recorded a similar response regarding the total LH response, but not the peak LH level, in heifers fed 61% of their energy requirements. In an earlier study by Dunn, Rone, Kaltenbach, van der Walt, Riley & Akbar (1974) the peak LH was in effect higher in underfed beef cows. In the study reported here, on day 3 the total LH response was lower ($P < 0{,}001$) in the restricted ewes only, in those not primed with oestrogen. On day 15 this response was noted only in the primed ewes and those not primed exhibited the reverse trend.

The reduced LH release in the restricted ewes, as measured by peak LH levels, does not necessarily imply a deficiency of pituitary stores, since Rippel, Johnson & White (1974) demonstrated that the pituitary concentration of LH in anoestrous ewes was not influenced

Table 1

Least squares estimated release of LH (mean ± S.E.M.) after injection of Gn-RH in ewes as influenced by level of feeding, oestrogen priming and stage of the oestrous cycle

Level of feeding	Dose Gn–RH (µg)	Characteristic of LH release	Day 3 of oestrous cycle				Day 15 of oestrous cycle			
			n	Primed (100 µg ODB)	n	Unprimed	n	Primed (3 x 30 µg ODB)	n	Unprimed
Unrestricted	25	Peak[1]	4	82,1 ± 4,5	5	60,9 ± 4,5	4	113,8 ± 4,3	5	102,7 ± 4.3
		Total[2]		121,7 ±10,3		118,0 ±10,3		191,7 ±13.2		114.6 ±11.5
	50	Peak	5	156,9 ± 5,6	5	135,6 ± 5,6	5	179,6 ± 3,9	4	168,4 ± 4,4
		Total		284,6 ± 8,8		280,9 ± 8,8		277,9 ±12,7		200,9 ±11,7
	100	Peak	5	251,1 ± 3,8	5	239,8 ± 3,8	5	288,2 ± 4,5	4	277,1 ± 4,1
		Total		417,4 ± 8,8		413,7 ± 8,8		494,7 ±11,5		417,6 ±12,6
Restricted	25	Peak	5	75,8 ± 3,8	5	54,5 ± 3,8	5	92,0 ± 4,5	4	80,8 ± 4.0
		Total		142,6 ± 8,8		79,3 ± 8,8		130,8 ±11,4		145,2 ±13,3
	50	Peak	5	150,5 ± 3,8	5	129,3 ± 3,8	4	157,7 ± 4,5	4	146,6 ± 4,6
		Total		305,5 ± 9,7		242,2 ± 9,7		217,0 ±13,5		231,4 ±13,5
	100	Peak	5	244,7 ± 3,8	5	223,4 ± 3,8	5	266,4 ± 4,1	5	255,2 ± 4,0
		Total		438,3 ± 8,8		375,1 ± 8,8		433,8 ±11,3		448,1 ±11,3

[1] Highest concentration in plasma following Gn–RH (ng/ml)

[2] Derived from area under LH release curve (arbitrary units)

Table 2

Least square estimated release of LH (mean ± S.E.M.) after injection of Gn–RH in ewes as influenced by stage of the oestrous cycle in addition to level of feeding and oestrogen priming

Level of feeding	Dose GN–RH (µg)	Characteristic of LH release	Oestrogen primed				No oestrogen			
			n	Day 3	n	Day 15	n	Day 3	n	Day 15
Unrestricted	25	Peak[1]	4	89,0 ± 3,5	4	113,5 ± 3,5	4	72,6 ± 3,4	5	97,1 ± 3,1
		Total[2]		138,4 ± 9,7		165,9 ± 9,6		105,5 ± 9,8		133,0 ± 8,6
	50	Peak	5	159,4 ± 3,0	5	184,0 ± 3,0	5	143,0 ± 2,9	4	167.6 ± 3,4
		Total		301,1 ± 8,3		256,0 ± 8,7		268,2 ± 8,3		223,1 ± 9,9
	100	Peak	5	260,3 ± 3,1	5	284,9 ± 3,0	5	243,9 ± 3,0	4	268.5 ± 3,4
		Total		434,0 ± 8,3		472,5 ± 8,3		401,7 ± 8,3		439.5 ± 8,3
Restricted	25	Peak	5	71,9 ± 3,4	5	96,5 ± 3,1	5	55,5 ± 3,3	4	80,1 ± 3,4
		Total		125,7 ± 8,5		153,2 ± 8,5		92,7 ± 8,5		120,2 ± 9,6
	50	Peak	5	142,4 ± 3,0	4	166,9 ± 3,4	5	126,0 ± 3,3	4	150,5 ± 3,5
		Total		288,4 ± 8,3		243,3 ± 8,2		255,5 ± 9,3		210,4 ± 9,3
	100	Peak	5	243,3 ± 3,0	5	267,8 ± 2,9	5	226,9 ± 3,2	5	251,5 ± 3,1
		Total		421,3 ± 8,3		459,7 ± 8,3		388,3 ± 8,3		426,8 ± 8,3

[1] Highest concentration in plasma following Gn–RH (ng/ml)

[2] Derived from area under LH release curve (arbitrary units)

by successive injections of Gn-RH. Furthermore, Memon, Antoniewicz, Benevenga, Pope & Casida (1969) noted a decline in plasma LH concentrations without pituitary concentrations being affected in underfed ewes. Howland (1976) is of the opinion that synthesis of Gn-RH and the sensitivity of its target tissue are normal in the underfed rat. The similarity between the peak LH level in unprimed, well-fed ewes and primed, restricted ewes, obtained in the present study, suggests that anoestrus in underfed ewes (Allen & Lamming, 1961; Hunter 1962, Smith, 1962, Lamond, Gaddy & Kennedy, 1972, Lishman *et al.*, 1974) could be the result of an inhibited oestrogen secretion, and consequently inadequate steroid priming (Howland, 1976). Rawlings, Kennedy, Chang, Hill & Henricks (1977) have proposed a similar mechanism for the onset of seasonal anoestrus. Clearly, a deficiency in basal LH could be reflected in inadequate steroid production (Howland, 1976), but the present results do not suggest such a deficiency. An insensitivity to LH on the part of the ovary (Gombe & Hansel, 1973) is possibly one of the reasons why underfed females go into anoestrus. An aspect which requires testing, in the malnourished female, is the ability of the hypothalamo-hypophysial system to rapidly synthesise gonadotrophin just prior to oestrus (Roche *et al.*, 1970).

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References

ALLEN, D.M. & LAMMING, G.E., 1962. Nutrition and reproduction in the ewe. *J. agric. Sci., Camb.* 56, 69.

ARAKI, S., FERIN, M., ZIMMERMAN, A. & VANDE WIELE, R.L., 1975. Ovarian modulation of immonoreactive gonadotropins – releasing hormone (Gn-RH) in the rate brain: Evidence for a differential effect on the anterior and mid-hypothalamus. *Endocrinology* 96, 644.

BEAL, W.E., KALTENBACH, C.C. & DUNN, T.G., 1975. Effect of energy on LH response to consecutive Gn-RH injections. *J. Anim. Sci.* 41, 343.

CASTRO-VAZQUEZ, A. & McCANN, S.M., 1975. Cyclic variations in the increased responsiveness of the pituitary to luteinizing hormone-releasing hormone (LHRH) induced by LHRH. *Endocrinology* 97, 13.

CONVEY, E.M., 1973. Neuroendocrine relationships in farm animals: A review. *J. Anim. Sci.* 37, 745.

COPPINGS, R.J. & MALVEN, P.V., 1976. Biphasic effect of oestradiol on LH release mechanisms. *J. Anim. Sci.* 43, 279.

CUMMING, I.A., 1975. The ovine and bovine oestrous cycle. *J. Reprod. Fert.* 43, 583.

DUNN, T.G., RONE, J., KALTENBACH, C.C., VAN DER WALT, L.M., RILEY, M.L. & AKBAR, A.M., 1974. Hormone changes during underfeeding of beef cows. *J. Anim. Sci.* 39, 206.

GOMBE, S. & HANSEL, W., 1973. Plasma luteinizing hormone (LH) and progesterone levels in heifers on restricted energy intakes. *J. Anim. Sci.* 37, 728.

HOOLEY, R.D., BAXTER, R.W., CHAMLEY, W.A., CUMMING, I.A. & FINDLAY, J.K., 1974. Variations in LH release in response to Gn-RH during the oestrous cycle. *J. Reprod. Fert.* 36, 474

HOWLAND, B.E., 1972. Effect of restricted feed intake on LH levels in female rats. *J. Anim. Sci.* 34, 445.

HOWLAND, B.E., 1976. Gonadotrophin release induced by Gn-RH or progesterone in female rats maintained on high or low levels of feed intake. *J. Reprod. Fert.* 47, 137.

HUNTER, G.L., 1962. Observations on oestrus in Merinos. *Proc. S. Afr. Soc. Anim. Prod.* 1, 67.

JACKSON, G.L., 1975. Blockage of oestrogen-induced release of luteinizing hormone by reserpine and potentiation of synthetic gonadotropin-releasing hormone-induced release of luteinizing hormone by oestrogen in the ovariectomized ewe. *Endocrinology* 97, 1300.

JENKIN, G., HEAP, R.B. & SYMONS, D.B.A., 1977. Pituitary responsiveness to synthetic LH-RH and pituitary LH content at various reproductive stages in the sheep. *J. Reprod. Fert.* 49, 207.

LAMMING, G.E., 1966. Nutrition and the endocrine system. *Nutr. Abstr. Rev.* 36, 1.

LAMOND, D.R., GADDY, R.G. & KENNEDY, S.W., 1972. Influence of season and nutrition on luteal plasma progesterone in Ramboillet ewes. *J. Anim. Sci.* 34, 626.

LEATHEM, J.H., 1966. Nutritional effects on hormone production. *J. Anim. Sci.*, 25, Suppl. 1, 68.

LIBERTUN, C., COOPER, K.J., FAWCETT, C.P. & McCANN, S.M., 1974. Effects of ovariectomy and steroid treatment on hypophyseal sensitivity to purified LH-releasing factor (LRF) *Endocrinology* 94, 518.

LISHMAN, A.W., 1972. *The influence of variations in the level of nutrition on reproduction in the ewe.* Ph.D. thesis, University of Natal.

LISHMAN, A.W., STIELAU, W.J. & BOTHA, W.A., 1974. Reproduction in the ewe in relation to plane of nutrition bodymass and change in bodymass. I. The incidence of oestrus between lambing and reconception. *Agroanimalia* 6, 25.

MEMON, G.N., ANTONIEWICZ, R.J., BENEVENGA, N.J., POPE, A.L. & CASIDA, L.E., 1969. Some effects of differences in dietary energy and protein levels on the ovary and the anterior pituitary gland of the ewe. *J. Anim. Sci.* 28, 57.

MULLINOS, M.G. & POMERANTZ, L., 1940. "Pseudohypophysectomy" A condition resembling hypophysectomy produced by malnutrition. *J. Nutr.* 19, 493.

NETT, T.M., AKBAR, A.M. & NISWENDER, G.D., 1974. Serum levels of luteinizing hormone and gonadotropin-releasing hormone in cycling, castrated and anoestrous ewes. *Endocrinology* 94, 713.

NISWENDER, G.D., REICHERT, L.E., MIDGLEY, A.R. & NALBANDOV, A.V., 1969. Radioimmuno-assay for bovine and ovine luteinizing hormone. *Endocrinology* 84, 1166.

RAWLINGS, N.C., KENNEDY, S.W., CHANG, C.H., HILL, J.R. & HENRICKS, D.M., 1977. Onset of seasonal anoestrus in the ewe. *J. Anim. Sci.*, 44, 791.

REEVES, J.J., ARIMURA, A. & SCHALLY, A.V., 1971a. Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH-RH) at various stages of the oestrous cycle in sheep. *J. Anim. Sci.* 32, 123.

REEVES, J.J., ARIMURA, A., & SCHALLY, A.V., 1971b. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anoestrous ewes pre-treated with oestradiol benzoate. *Biol. Reprod.* 4, 88.

REEVES, J.J., BECK, T.W. & NETT, T.M., 1974. Serum FSH in anoestrous ewes treated with 17 β - oestradiol. *J. Anim. Sci.* 38, 374.

RIPPEL, R.H., JOHNSON, E.S., MAUER, R.E. & WEBEL, S., 1974. Response of the luteal-phase ewe to Gn-RH. *J. Anim. Sci.* 39, 224.

RIPPEL, R.H., JOHNSON, E.S. & WHITE, W.F., 1974. Effect of consecutive injections of synthetic gonadotropin releasing hormone on LH release in the anoestrous and ovariectomized ewe. *J. Anim. Sci.*, 39, 907.

ROCHE, J.G., FOSTER, D.L., KARSCH, F.J., COOK, B. & DZUIK, P.J., 1970. Levels of luteinizing hormone in sera and pituitaries of ewes during the oestrous cycle and anoestrus. *Endocrinology* 86, 568.

SMITH, I.D., 1962. The effect of plane of nutrition on the incidence of oestrus in the Merino ewe in Queensland. *Austr. vet. J. 38, 338.*

ZEBALLOS, G. & McCANN, S.M., 1975. Alterations during the oestrous cycle in the responsiveness of the pituitary to subcutaneous administration of synthetic LH-releasing hormone (LHRH). *Endocrinology* 96, 1377.

ZOLMAN, J., CONVEY, E.M. & BRITT, J.H., 1974. Relationships between the luteinizing hormone response to gonadotropin releasing hormone and endogenous steroids. *J. Anim. Sci.* 39, 355.

Conception rate of beef cows and growth of suckling alves as influenced by date of calving and supplemen- ary feeding

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e influence of energy supplementation (2,8 kg maize meal) of beef cows
ring early lactation and of creepfeeding their suckling calves was investi-
ted in two areas (Thornveld and Transitional Highland Sourveld). The cows
re bred in spring to calve during the first (early calvers) or last six weeks
e calvers) of a 90-day calving season.
The gains in bodymass of the cows which calved early were significantly
ater than for the late calvers. Energy supplementation had no major ef-
t on the bodymass gains or reconception rates (CR) and intercalving in-
vals (ICI). In the Thornveld only 14,8 % of the variation in mass gain dur-
early lactation was attributed to supplementation. Variation in the ICI
pended largely (70,5 %) on the time of calving. In the Sourveld only
9 % of the variation in ICI was due to this factor. Cows which recon-
ved were heavier at calving and when mating ceased, gained more during
breeding period and had calved earlier than cows which did not recon-
ve ($P \leqslant 0{,}01$). The CR was closely associated with change in bodymass
ornveld: $r = 0{,}72$; Sourveld: $r = 0{,}93$) and bodymass at the end of
ting (Thornveld : $r = 0{,}87$; Sourveld: $r = 0{,}98$). Early-born calves gained at
aster rate than those born late in the Thornveld, but not in the Sour-
d. Creepfeeding was beneficial for late-born calves suckled by cows
ich received energy supplements in the Thornveld. All creepfed calves
nefitted in the Sourveld. In the Thornveld the bodymass at weaning was
termined by the season of birth and creepfeeding (31,4 %) while in the
urveld creepfeeding and bodymass of the dam at calving were significant
ntributors (23,1 %) to variation in weaning mass.
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e invloed van energiebyvoeding (2,8 kg mieliemeel) van vleisbeeskoeie
durende vroeë laktasie en van kruipvoeding van hul kalwers is in twee
biede (Doringveld en Oorgangstipe Hoëlandsuurveld) ondersoek. Die koeie
in die lente gepaar om gedurende die eerste (vroeëkalwing) of laaste ses
ke (laatkalwing) van 'n 90-dae seisoen te kalf. Die koeie wat vroeg gekalf
t, het 'n betekenisvolle hoër massatoename gehandhaaf as dié wat laat
kalf het. Energiebyvoeding het geen noemenswaardige invloed op massa-
names, of herbesetting en interkalfperiode (IKP) gehad nie. Slegs 14,8 %
die variasie in massatoename gedurende vroeë laktasie in die Doring-
d kon aan byvoeding toegeskryf word. Variasie in die IKP was grootliks
,5 %) aan die kalfdatum te wyte. In die Suurveld kon hierdie faktor slegs
9 % van die variasie verklaar. Koeie wat herbeset geraak het was
aarder by kalwing en by beëindiging van paring, het meer in massa
geneem gedurende die paarseisoen en het vroeër gekalf as koeie wat nie
er beset is nie ($P \leqslant 0{,}01$). Die kalfpersentasie het 'n noue verband met
andering in liggaamsmassa (Doringveld: $r = 0{,}72$; Suurveld: $r = 0{,}93$) en
t liggaamsmassa teen die einde van paring (Doringveld: $r = 0{,}87$;
urveld: $r = 0{,}98$) getoon.
Kalwers wat vroeg gebore is het teen 'n vinniger tempo in massa
geneem as dié wat laat gebore is in die Doringveld, maar nie in die
urveld nie. Kruipvoeding was voordelig vir laatgebore kalwers van koeie
energiebyvoeding ontvang het in die Doringveld. Alle kruipgevoerde
wers in die Suurveld is bevoordeel. Die liggaamsmassa by speen is
paal (31,4 %) deur die seisoen van geboorte en deur kruipvoeding in die
ringveld. In die Suurveld was kruipvoeding en liggaamsmassa van die
er by kalwing belangrik (23,1 %) by variasie in speenmassa.
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ywords: Reproduction, beef cow, supplementary energy, time of calving,
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Introduction

The percentage calf-crop weaned is believed to be the most important single factor determining the profitability of the cow-calf operation (Snapp & Neumann, 1960). The calving rate may not be as important in the extensive ranching areas (Steenkamp, 1977) as in the more intensive, high rainfall areas; nevertheless, low calving rates are far too frequently encountered in such areas (Boulle, 1980).

In the absence of disease, inadequate feeding, particularly during the first few months after calving, is probably the main reason for the low reproductive rate encountered in farming practice (Joubert 1954; Warnick, 1959; Wiltbank, Rowden, Ingalls, Gregory & Koch, 1962; Bishop & Kotze, 1965). An energy deficiency at such times has been identified (Wiltbank *et al.*, 1962; Wiltbank, Rowden, Ingalls & Zimmermann 1964; Davis, Schalles, Kiracofe & Good, 1977).

Owing to the high cost of energy concentrates it is important to limit supplementation. This can be achieved by delaying extra feeding after calving until the reproductive mechanism becomes responsive to improved nutrition (Wiltbank *et al.*, 1964). This practice has the added advantage of increasing the cow's milk production at a time when the calf is most likely to be able to utilize the extra feed.

Inadequacies in milk production after the first three months of lactation can be compensated for by creepfeeding the calves (Heyns, 1960) so that the influence of "season of birth" on preweaning growth rate can be decreased (Marlowe & Gaines, 1958).

For the beef cow which grazes veld it is important to synchronize maximum need (early lactation) with the time when the grazing is at its best. Spring is therefore favoured as a calving time. However, because of the unpredictability of rains the quantity of the grazing is often limited, although the quality is good. Furthermore, when calving occurs four to six weeks prior to the onset of the spring grazing season, the growth of the calf is favoured. However, this situation is detrimental to reconception of the lactating cows (Harwin, Lamb & Bisschop, 1967; Bosman & Harwin, 1969). Where a 90-day mating season is followed, as many farmers do, the early calves commonly arrive at a time which results in the heaviest weaners. However, for several reasons many cows become pregnant towards the end of the mating period. Their calves are then dropped at a time which does not allow the calves to gain the maximum benefit from the early summer grass (Bosman & Harwin, 1966; Bonsma & Skinner, 1969).

Selection of a suitable calving season becomes a matter of optimizing preweaning growth of suckling calves and reconception of the lactating cow. The possible benefit of (i) supplementing the energy supplies of the cow which calves early and of (ii) offering a creepfeed so as to augment the lowered milk supply of the cow which calves late, formed the basis of this study.

Procedure

The effects of time-of-calving within the calving period (*i.e.* early or late), supplementary energy feeding of the lactating cow and creepfeeding of the calf were investigated in a 2^3 factorial experiment conducted at two localities (Thornveld or Transitional Highland Sourveld).

Calving times

At each locality the herd of Afrikander cows was randomly subdivided and the cows were mated to calve either during the first six weeks (early calvers) or last six weeks (late calvers) of a three-month calving season (Table 1). Afrikander bulls were used at the Onverwacht Research Station (Thornveld) while at the Kokstad Research Station (Transitional Highland Sourveld) the sires were Herefords.

The date of onset of the calving seasons in each area was, according to recommendations, based on information available at the time from the National Beef Performance Testing Scheme.

Energy supplementation

The energy concentrate consisted of 2,8 kg maize meal per day which was calculated to be the quantity required to satisfy the daily requirements of a lactating beef cow grazing spring grass. This supplement was supplied 30 days after parturition and continued until 60 days after the onset of the subsequent 90-day mating period. Group-feeding was followed, with each cow and her calf being drafted into the relevant group at the appropriate stage post-partum.

Creepfeeding of calves

The concentrate offered to the calves consisted of 80 parts maize meal and 20 parts peanut oilcake meal. It was fed from four months of age until the calves were weaned at 210 days. The daily allowance did not exceed 0,9 kg per calf per day.

General management

The lactating cows with their calves were grazed on summerveld and at weekly intervals the various treatment groups were interchanged within the available camps to minimize camp effects. Prior to calving the cows received a urea block in addition to the winter Thornveld (May to September). In the Sourveld the pregnant cows were overwintered on a daily ration of 13,5 kg maize silage, 4,5 kg *Eragrostis curvula* hay and a urea block. Normal practices of disease control, castration of male calves at three months of age and provision of calcium-phosphate licks were followed.

Breeding bulls were again joined with the cows for 90 days commencing on a date similar to that used for the early calvers (Table 1). During this breeding period the bulls were rotated weekly between the various subgroups.

The bodymass of each cow and that of her calf were recorded on the day of parturition and at four-week intervals until weaning. Reconception was based on calving data and not on pregnancy tests.

Statistical analyses

Least squares procedures were used to examine the changes in bodymass of cows and the growth of calves. Analyses of covariance were employed to correct for differences in cow mass at calving.

Treatment effects on the calving rate were tested by chi-square tests. Classification of cows into selected categories after pooling data over all treatments, permitted further examination of factors related to reconception. A stepwise regression (stepdown) procedure was applied to such data as well as to the change in bodymass of the cows during lactation and to the growth of the calves during the preweaning period.

Results

Lactating cows

Changes in bodymass according to time of calving

The changes in bodymass which occurred during the period when half the cows received the maize supplement (30 days post-partum to 60 days after initiation of breeding) indicated that the seasonal effects were minor (Table 2, Table 3). The average duration of this period was 84,8 days for the early calvers and 32,8 days for late calvers in the Sourveld, while in the Thornveld the respective periods were 85,2 and 28 days. Contrary to expectation, the average daily gains of the cows over the entire 210-day suckling period suggested that it was the late calvers which were under the greatest stress, both in the Thornveld and in the Sourveld (Table 3).

Supplementary feeding and bodymass change

In the Thornveld, provision of the energy supplement improved ($P \leqslant 0,01$) the average daily gains in bodymass during early

Table 1 Limits of the calving period for early and late calvers and timing of the subsequent mating period in two ecological areas of Natal

Locality	Time of calving within the season	*n*	Initial breeding period	Calving occurred	Subsequent breeding period
Thornveld	Early	43	16 December to 26 January	2 October to 14 November	20 December to 19 March
	Late	28	7 February to 19 March	20 November to 4 January	
Sourveld	Early	33	16 October to 26 November	31 July to 3 September	15 October to 12 January
	Late	47	7 December to 17 January	16 September to 10 November	

Table 2 Mean bodymass during the lactation period of beef cows calving at two stages of the season when fed an energy concentrate with suckling calves offered a creepfeed

	Feeding treatment				Average bodymass (kg)			
Locality	cow	calf	Time of calving	n	Parturition	Weaning	Parturition + 30 days	Onset mating + 60 days
Thornveld	Suppl.	Creepfed	Early	12	379,6	447,7	370,7	438,8
			Late	7	373,7	374,7	395,0	416,1
	Suppl.	Control	Early	12	378,7	421,9	363,1	420,8
			Late	10	393,5	399,5	408,7	430,7
	Control	Creepfed	Early	13	378,6	421,3	364,1	410,1
			Late	7	392,2	417,3	417,3	428,4
	Control	Control	Early	8	386,6	439,8	379,1	431,1
			Late	9	402,1	405,6	427,9	434,2
				SD	39,4	43,8	44,9	39,2
Sourveld	Suppl.	Creepfed	Early	9	466,9	482,5	451,8	455,5
			Late	13	405,8	395,4	390,3	393,8
	Suppl.	Control	Early	7	469,1	489,2	450,8	461,0
			Late	12	404,4	409,2	400,8	404,4
	Control	Creepfed	Early	8	433,5	447,3	407,0	414,8
			Late	12	413,2	397,6	392,9	399,4
	Control	Control	Early	9	444,5	461,9	416,7	431,2
			Late	10	417,7	402,5	397,7	407,5
				SD	84,4	68,6	63.2	60,5

Table 3 The effect of time of calving on bodymass changes of lactating cows

			Average daily gain (kg):	
Locality	Time of calving within season	n	Parturition to weaning	Parturition + 30 days to onset mating + 60 days
Thornveld	Early	43	0,21^{a}	0,65^{c}
	Late	28	0,05^{b}	0,75^{c}
			SE = 0,05	SE = 0,27
Sourveld	Early	33	0,06^{a}	0,11^{c}
	Late	47	−0,04^{b}	0,18^{c}
			SE = 0,05	SE = 0,27

^{a,b,c}Means in the same column relating to the same locality with different superscripts differ ($P \leqslant 0,01$).

Table 4 The effect of supplementary feeding with 2,8 kg maize meal on bodymass changes of lactating cows

			Average daily gain (kg)	
Locality	Treatment of cow	n	Parturition to weaning	Parturition + 30 days to onset mating + 60 days
Thornveld	Supplemented	37	0,15^{a}	0,81^{b}
	Control	34	0,15^{a}	0,48^{c}
			SE = 0,05	SE = 0,27
Sourveld	Supplemented	41	−0,01^{a}	0,07^{b}
	Control	39	−0,07^{a}	0,22^{b}
			SE = 0,05	SE = 0,27

^{a,b,c}For each locality, means in the same column with different superscripts differ ($P \leqslant 0,05$).

lactation (Table 4). However, this response occurred mainly amongst the late calvers (Table 5). Averaged over the entire suckling period, the effect of the energy concentrate was non-significant (Tables 4 and 5). Surprisingly, during early lactation in the Sourveld, both the early and late calving cows which were supplemented showed poorer gains than the controls (Table 4, Table 5). These trends were not evident over the entire 210-day lactation period.

The ADG during the period of energy supplementation was corrected (covariance) for the bodymass at calving. The overall trend of gains did not change and none of the differences were significant.

Regression analysis

The stepwise regression analysis was used to identify and quantify the effect of those factors which modified the influence of supplemental energy and of time-of-calving. The dependent variables were the average daily change in bodymass of the cows from 30 days after parturition until 30 days before the end of the 90-day mating period (Y_1) and from parturition until weaning of the calf (Y_2). The independent variables considered were those pertaining to the initial status of the cows (*i.e.* bodymass at calving and parity status) and to their experimental treatments (supplementation and time-of-calving). The factors associated with the calves that may have influenced the performance of their dams were creepfeeding and growth rate.

For the cows in the Thornveld the only factor found to contribute significantly to the change in bodymass from 30 days after calving until 30 days before the end of the mating period (Y_1) was "supplementation of the cow" (X_1) and the equation was,

$$Y_1 = 0{,}494 + 0{,}316X_1.$$

Only 14,8 % of the variation in Y_1 was accounted for in terms of X_1. When the change in bodymass of the cows from parturition until weaning of the calf (Y_2) was considered, 31,3 % of the variation was due to "time-of-calving" (X_3) and X_2 (bodymass of the cow at calving) accounted for a further

Table 5 Influence of time-of-calving and supplementary feeding on gain in bodymass of lactating cows

			Average daily gain (kg):				
			Parturition to weaning			Parturition + 30 days to onset mating + 60 days	
Locality	Time of calving	n	Control	n	Suppl.	Control	Suppl.
Thornveld	Early	21	$0{,}20^{ad}$	22	$0{,}23^{ad}$	$0{,}54^{bd}$	$0{,}73^{bd}$
	Late	13	$0{,}07^{ae}$	15	$0{,}02^{ae}$	$0{,}36^{bd}$	$0{,}95^{cd}$
	SD			0,05		0,27	
Sourveld	Early	17	$0{,}08^{ad}$	16	$0{,}07^{ad}$	$0{,}17^{bd}$	$0{,}07^{bd}$
	Late	22	$-0{,}04^{ad}$	25	$-0{,}02^{ad}$	$0{,}26^{bd}$	$0{,}06^{cd}$
	SD			0,05		0,27	

[a,b,c] Means in the same row with the same superscript do not differ ($P \geqslant 0{,}05$).

[d,e] Means in the same column with different superscripts differ ($P \geqslant 0{,}05$).

12,6 % to yield a total of 43,9 %. The equation was,

$$Y_2 = 0{,}764 - 0{,}0018\ X_2 + 0{,}002\ X_3.$$

The significant relationship established for the results obtained in the Sourveld was;

$$Y_1 = 0{,}403 - 0{,}162X_1 - 0{,}243X_4.$$

The negative coefficients for X_1 and X_4 supported the earlier conclusion that the supplementary energy in fact resulted in poorer gains while first calvers gained more than multiparous cows (X_4). Parity status (X_4) accounted for only 8,7 % of the variation in Y_1 and supplementation a further 5,1 % (total = 13,8 %).

In view of the relatively strong association between "parity status" (X_4) and "bodymass of the cow at calving" (X_2) the former was omitted from the model to determine whether X_2 would contribute significantly as it did in the Thornveld. However, it was found that,

$$Y_1 = 0{,}84 - 0{,}149X_1 - 0{,}004X_6.$$

A total of only 11 % of the variation in Y_1 could be accounted for, with X_6 ("bodymass of the calf at four months of age" *i.e.*, when creepfeeding commenced) being responsible for only 6,7 % of the variability of Y_1.

An examination of the effect of the independent variables on the ADG of the cows from parturition to the end of the lactation period (Y_2) showed that,

$$Y_2 = 0{,}43 + 0{,}004X_3 - 0{,}004X_6.$$

Of the 29,9 % total variation in Y_2 accounted for, 15,4 % was due to "time-of-calving" (X_3) and 14,3 % derived from "bodymass of the calf at onset of the creepfeeding phase" (X_6). Since the latter was correlated ($r = 0{,}508$) with "bodymass of the cow at calving" (X_2), the equation which omitted X_6 and which approached that obtained for the Thornveld was,

$$Y_2 = 0{,}515 - 0{,}0007X_2 + 0{,}002X_3 - 0{,}35X_9.$$

This relationship was only marginally inferior to that using X_2 instead of X_6 (28,1 % of variation in Y_2 accounted for). In this equation X_9 represented the ADG of the calf from birth to weaning.

While confirming and extending the results presented in Tables 4 and 5, the trends indicated by the regression analyses

Table 6 Reconception rates and intercalving intervals (ICI) for cows calving at different stages of the season and fed and an energy concentrate during early lactation

			Conception rate following				ICI (days) for	
	Nutrition of		Early calving		Late calving			
Locality	Cow	Calf	*No.	%	No.	%	Early calving	Late calving
Thornveld	Suppl.	Creepfed	7/11	62,6	6/7	85,7	391,5	354,3
	Suppl.	Control	6/11	54,5	4/7	55,5	397,5	367,2
	Control	Creepfed	6/11	54,5	3/6	50,0	409,5	351,7
	Control	Control	4/8	50,0	4/7	55,5	407,6	356,8
							SD = 29,11	
Sourveld	Suppl.	Creepfed	8/9	88,9	4/13	30,8	398,5	383,3
	Suppl.	Control	5/7	71,4	3/10	30,0	398,5	376,0
	Control	Creepfed	4/8	50,0	3/11	26,4	417,0	388,7
	Control	Control	9/9	100,0	4/9	44,4	404,5	384,2
							SD = 22,95	

* Based on cows remaining in the herd at subsequent calving after subtraction of deaths, lost ear tags *etc*.

also contradict the generally accepted belief that late calving benefits the maintenance of body reserves in the lactating cow. The common finding of a negative association between the growth of the suckling calf and the gain in mass of the lactating cow was also substantiated.

Reconception rates and intercalving intervals (ICI)

The procedure whereby cows were randomly designated to early and late calving times and mated accordingly, could be expected to favour the conception rate of the early calvers when remated under the conditions of this experiment. The 90-day mating period would then cease at a later stage post-partum for the early calvers than for those which calved late and accordingly a greater proportion of the former would be expected to reconceive. However, the ICI would be expected to increase.

Thornveld

Since the provision of maize did not enable the early calvers to maintain bodymass more effectively (Table 5), the failure of extra feeding to increase conception rates (Table 6) was thus not surprising. The reduction of ICI in those cows receiving maize was also non-significant. Amongst the late calvers the improvement in calving rate, where both the cow and the calf were supplemented, followed the same trend as changes in bodymass, but again the difference was non-significant. A similar failure of supplementary feeding to improve either conception rates or ICI was also noted in the Sourveld.

Regression analysis of ICI

The stepwise regression analysis with "bodymass at parturition" (X_1), "change in bodymass during the breeding period" (X_2), "supplementation of the cow" (X_3) and "time-of-calving" (X_4) as measured by the interval (days) from calving to the onset of the subsequent breeding period, suggested that,

$$Y = 361{,}8 + 0{,}675X_4.$$

In this relationship 70,5 % of the variation in Y in the Thornveld was accounted for by variation in X_4. The early calvers thus appeared to be at a disadvantage as regards maintaining an ICI of 365 days. However, their conception rate was likely to benefit since they were exposed to bulls when the last of the early calvers were more than 120 days post-partum. This is contradicted by the failure to observe a difference in the number of days between calving and the onset of the mating period for cows which reconceived as compared to those which did not (Table 7).

There was a negative correlation ($r = -0{,}23$) between ICI and the bodymass at the initial parturition, whereas the association between "change in bodymass during the breeding period" (X_2) and the ICI was positive ($r = 0{,}461$). The implications of this correlation are that cows which gained more during the breeding period had a longer delay to conception than cows which exhibited smaller gains in bodymass.

For the cows in the Sourveld the ICI was dependant on the bodymass of the cows at parturition (X_1) and also on the "time-of-calving" (X_4). The relationship was,

$$Y = 449{,}7 - 0{,}07X_1 + 0{,}51X_4.$$

In contrast to the results obtained in the Thornveld, in this equation only 23,9 % of the variation in Y could be assigned to X_4, while X_1 contributed a further 8,3 %. The negative coefficient for X_1 confirms the trend observed in the Thornveld, although it was non-significant in that area. In the Sourveld, X_1 and X_4 appear to be acting in opposite directions. The cows which reconceived were heavier ($P \leqslant 0{,}001$) at calving then those which did not conceive (Table 6). However, the early calvers, which had the longer ICI, also had a greater bodymass than the late calvers (450 *vs* 410 kg).

Regression analysis of conception rate

From an examination of the factors which influenced conception rate (Table 8) it was evident that in the Thornveld "time-of-calving" did not affect the likelihood of cows reconceiving. Similarly, the bodymass of the cows at parturition was non-significantly associated with conception rate. However, both the change in bodymass during the breeding period and the bodymass of the cows when the mating period ended, significantly influenced the likelikhood of the cows reconceiving (Figure 1A, Figure 2A)

Correlations between any two independent variables in Table 8 were low except for "mass change" and "time-of-calving" ($r = 0{,}46$). Contrary to the effect on ICI, from the regression analysis it was evident that cows which made the greatest gains exhibited the highest conception rate (Figure 1A).

Sourveld

Although all the variables considered had a high correlation with conception rate (Table 8), only those found to be significant in the Thornveld are presented for the Sourveld data in Figures 1B and 2B. As in the Thornveld here too there was a positive association between "mass change" and "time-of-calving" ($r = 0{,}53$). Other correlations between independent

Table 7 Characteristics of cows that reconceived and of those which did not become pregnant during the 90-day breeding period

Locality	Result of subsequent breeding	Bodymass (kg) at Initial calving	Bodymass (kg) at End of breeding period	Average change in bodymass (kg) during breeding period	Interval (days) from calving to onset of breeding period
Thornveld	Calf	$393{,}3^a$ $\pm 6{,}3$	$422{,}1^a$ $\pm 6{,}4$	$25{,}1^a \pm 2{,}9$	$28{,}5^a \pm 3{,}9$
	No calf	$380{,}5^b$ $\pm 5{,}4$	$404{,}9^b$ $\pm 5{,}0$	$21{,}9^b \pm 2{,}2$	$27{,}33^a \pm 3{,}7$
Sourveld	Calf	$463{,}8^a$ $\pm 7{,}5$	$447{,}8^a$ $\pm 7{,}8$	$15{,}8^a \pm 2{,}3$	$44{,}4^a \pm 3{,}8$
	No calf	$391{,}4^b$ $\pm 8{,}1$	$382{,}5^b$ $\pm 7{,}8$	$2{,}3^b \pm 1{,}6$	$14{,}5^b \pm 3{,}3$

[a,b] Means in the same column with different superscripts differ ($P \leqslant 0{,}01$).

Table 8 The relative role of factors which could have influenced the conception rate (CR) of lactating bee cows

	Locality			
	Thornveld		Sourveld	
Factor	Association (*r*) with CR (Y)	% variation in Y due to X	Association (*r*) with CR (Y)	% variation in Y due to X
Interval (days) from calving to onset of breeding period	−0,07	0,50	0,98	96,3
Mass change (kg) during breeding period	0,72	51,6	0,93	87,1
Bodymass (kg) at previous calving	−0,02	0,03	0,98	95,4
Bodymass (kg) at end of breeding period	0,87	75,1	0,98	95,6

variates were low.

From the data in Tables 7 and 8 it was clear that the cows which gained the most ($P \leqslant 0{,}001$) during the breeding period were the ones that reconceived ($r = 0{,}93$). However, from the regression analysis there was a positive association between "gain during the breeding period" and the ICI ($r = 0{,}243$). Not only did the early calvers have the benefit (as regards conception rate) of exposure to bulls at a later stage of the lactation period, but they also gained more (22,5 *vs* 3,3 kg) during the breeding period than the late calvers.

From the foregoing it would appear that although the early calving resulted in a longer average ICI, the beneficial effect was to increase the conception rate. Similarly, increased bodymass was advantageous in reducing the ICI and increasing the conception rate.

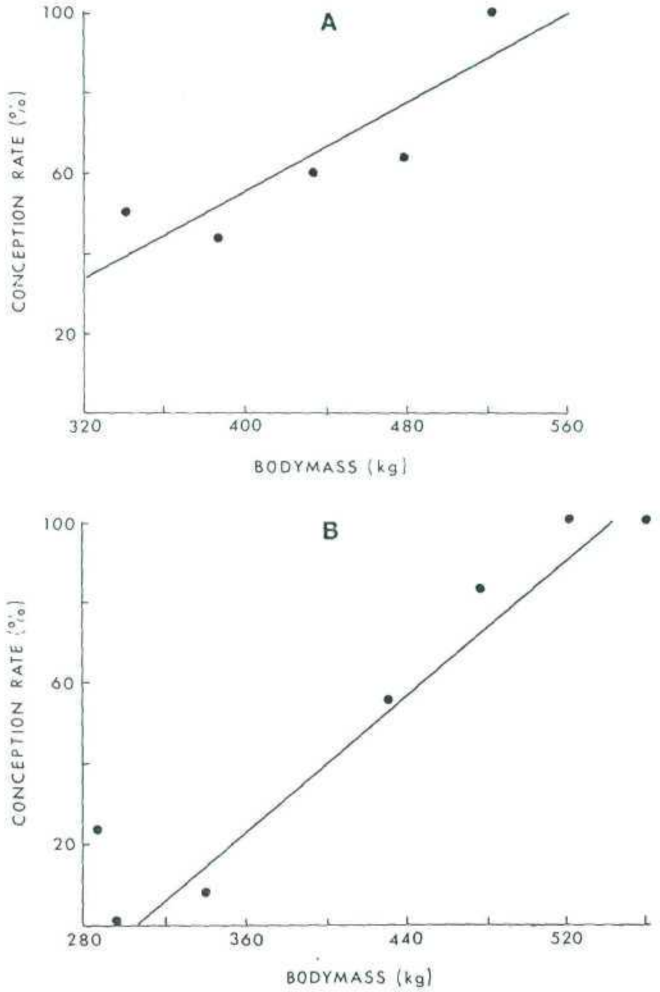

Figure 1 The association between change in bodymass during the mating season and conception rate of lactating cows. A. Thornveld, B. Sourveld.

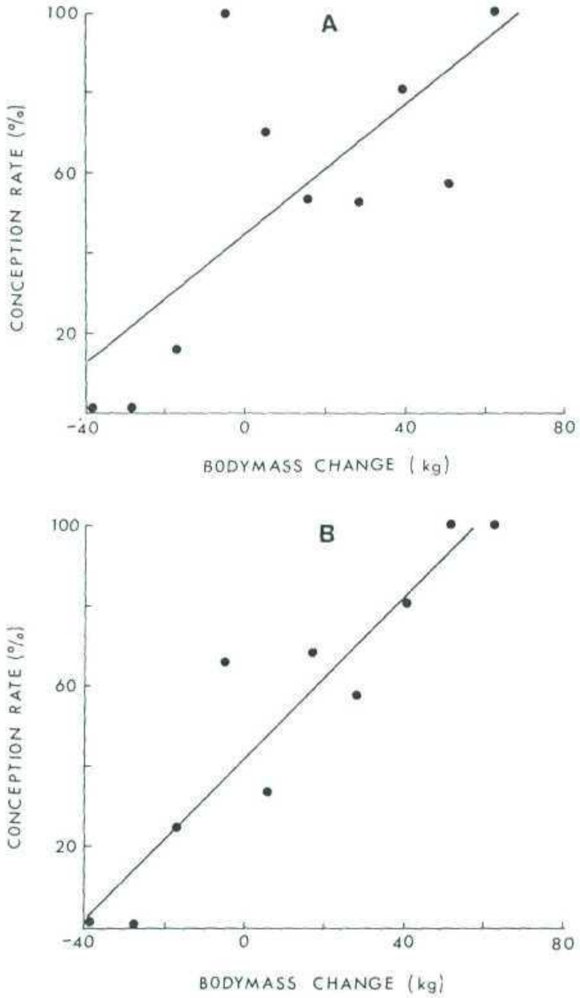

Figure 2 The relationship between bodymass at the end of the matin period and the conception rate of lactating cows. A. Thornveld, B Sourveld.

Growth of calves during the suckling period

The influence of time-of-birth within the calving period and of creepfeeding is illustrated by the results obtained by leas squares analysis of the average daily gains (Tables 9, 10 and 11)

As expected, the early born calves (*i.e.* born 2 October t 14 November) gained at a significantly greater rate ($P \leqslant 0{,}05$ than those born late (20 November to 4 January) in the Thornveld (Table 9). The birthmass had no significant effect on postnatal growth. By constrast, in the Sourveld the early born calve were not at an advantage in spite of the fact that their dam were considerably heavier than those which calved late (Tabl 2).

When the feeding treatment of the dam was ignored it wa evident that although there was a very definite advantage i 'able 9 Influence of season of birth on the prewean-ng gain in bodymass of suckling beef calves

			Daily gain (kg)	
.ocality	Time of birth	n	Birth to weaning	4 months to weaning
hornveld	Early	45	$0,60^{a}$	$0,52^{a}$
	Late	33	$0,53^{b}$	$0,40^{b}$
	SD		0,01	0,04
ourveld	Early	33	$0,79^{c}$	$0,77^{c}$
	Late	47	$0,80^{c}$	$0,77^{c}$
	SD		0,02	0,02

^{b,c}Means within the same column with different superscripts differ ($P \leqslant 0,05$).

he Thornveld in favour of the early born calves this could e reduced, but not eliminated, by creepfeeding (Table 10). However, when the data were reclassified so that the effect f supplementing the lactating cow on the growth of the calf ould be examined, it then became evident (Table 11) that reepfeeding of the late born calves was of benefit only where he dams also received an energy supplement during lactation. n these cows, supplementary feeding did reduce the bodymass loss during lactation. Although the growth rate of the calves was almost doubled, the benefit was considerably reduced when the ADG over the entire suckling period was evaluated (Table 10).

Provision of a creepfeed significantly ($P \leqslant 0,01$) improved the growth rate of both the early and the late born calves in the Sourveld. Taken over the entire suckling period of seven months, the benefit was significant ($P \leqslant 0,01$) only for the late born calves (Table 10). Although the difference was of the same order for the early born calves, the smaller number of animals in this group resulted in a non-significant t-test.

When considering the results in terms of the treatment to which the cows were subjected (Table 11), creepfeeding of the calf in the Sourveld was beneficial (early born: $P \leqslant 0,01$; late born: $P \leqslant 0,05$) whether the dam was supplemented or not. For the entire preweaning period the differences were significant, again only for the late born calves, although those recorded for the calves born early approached significance ($P \leqslant 0,10$; $P \geqslant 0,05$).

The growth rate of the calves was examined using the independent variables X_1 — supplementation of calf (creepfeeding), X_2 — bodymass of calf at birth, X_3 — bodymass of calf at four months of age (onset of creepfeeding phase), X_4 — season of birth, X_6 — bodymass of dam at calving, X_7 — bodymass change of dam from calving to wean-

Table 10 The effect of creepfeeding calves born at two stages of the calving period

		Average daily gain (kg) from							
		4 months to weaning				Birth to weaning			
Locality	Nutrition of calf	n	Early born	n	Late born	n	Early born	n	Late born
Thornveld	Creepfed	25	$0,59^{ac}$	14	$0,41^{bc}$	25	$0,63^{ac}$	14	$0,53^{bc}$
	Control	20	$0,59^{ac}$	19	$0,28^{bd}$	20	$0,64^{ac}$	19	$0,46^{bd}$
	SD		0,04				0,01		
Sourveld	Creepfed	17	$0,84^{ac}$	25	$0,82^{ac}$	17	$0,82^{ac}$	25	$0,84^{ac}$
	Control	16	$0,71^{ad}$	22	$0,71^{ad}$	16	$0,77^{ac}$	22	$0,76^{ad}$
	SD		0,02				0,02		

^{a,b}Means in the same row with different superscripts differ ($P \leqslant 0,05$).

^{c,d}Means in the same column with different superscripts differ ($P \leqslant 0,05$).

Table 11 The effect of supplementary feeding of dams on the preweaning growth of creepfed calves born at two stages of the calving period

			Average daily gain (kg) from:							
	Feeding treatment		4 months to weaning				Birth to weaning			
Locality	Cow	Calf	n	Early born	n	Late born	n	Early born	n	Late born
Thornveld	Suppl.	Creepfed	12	$0,55^{ac}$	7	$0,46^{ac}$	12	$0,62^{ac}$	7	$0,55^{ac}$
	Control	Creepfed	13	$0,61^{ac}$	7	$0,36^{bce}$	13	$0,65^{ac}$	7	$0,52^{bce}$
	Suppl.	Control	12	$0,60^{ac}$	10	$0,24^{bdef}$	12	$0,64^{ac}$	10	$0,44^{bdef}$
	Control	Control	8	$0,56^{ac}$	9	$0,30^{bcef}$	8	$0,64^{ac}$	9	$0,49^{bcef}$
	SD			0,04				0,01		
Sourveld	Suppl.	Creepfed	9	$0,84^{ac}$	13	$0,82^{ac}$	9	$0,81^{ac}$	13	$0,84^{ac}$
	Control	Creepfed	8	$0,84^{ace}$	12	$0,82^{ac}$	8	$0,82^{ac}$	12	$0,86^{ace}$
	Suppl.	Control	7	$0,66^{adbg}$	12	$0,71^{a}$	7	$0,73^{ac}$	12	$0,77^{aceg}$
	Control	Control	9	$0,75^{aceg}$	10	$0,72^{a}$	9	$0,80^{ac}$	10	$0,75^{adfg}$
	SD			0,02				0,02		

^{ab}Means in the same row with different superscripts differ ($P \leqslant 0,05$).

^{cdefg}Means in the same column with different superscripts differ ($P \leqslant 0,05$).

ing, X_8 — bodymass change of dam from 30 days after calving to 30 days before end of mating period (period of supplementation), X_9 — supplementation of cow.

In the Thornveld only the "season of birth" (X_4) significantly influenced the ADG from four to seven months of age (Y_1). The equation was,

$$Y_1 = 0,335 + 0,252\ X_4;$$

X_4 accounted for 44,9 % of the variation in Y_1 and, although non significant, "supplementation of the calf" (X_1) could account for 5,6 % of the variation in ADG when all other variables were omitted.

Although Y_1 was associated with X_2 and also X_7 (X_2:r = 0,356; X_7:r = 0,305), because of their association with "season of birth" (X_4) their influence was accounted for by X_4.

The model used for the Sourveld data also included "parity status of dam" as X_{10}. In agreement with the results in Tables 10 and 11, creepfeeding of the calf (X_1) accounted for 12,6 % of the variation in Y_1. The equation obtained was,

$$Y_1 = 0,712 + 0,086X_1.$$

The bodymass at birth (X_2) was correlated (r = 0,443) with the bodymass at four months of age, but neither of these variables was closely associated with the rate of growth after four months (Y_1).

When attention was focussed on the ADG of the calves from birth to weaning (Y_2) and X_3 was omitted from the model, then here too the only significant factor in the Thornveld was "season of birth" (X_4). In the equation $Y_2 = 0,492 + 0,144X_4$ the independent variable accounted for 41,1 % of the variation in Y_2.

In the Sourveld only 16,5 % of the variation could be explained in terms of X_2 and X_7, with the former accounting for 11,6 %. The equation derived was $Y_2 = 1,699 + 0,125X_1 - 0,132X_7$. The nett result was that the final bodymass at weaning in the Thornveld was determined by (in order of importance) "season of birth" (X_4) and "creepfeeding" (X_1). In the equation $Y = 150,10 + 11,84X_1 + 22,96X_4$ only 31,4 % of the variation in Y could be accounted for. Similarly only 23,1 % of the variation in bodymass at weaning for the calves in the Sourveld could be explained in terms of X_1. In this case X_6 ("bodymass of dam at parturition") also contributed significantly. The equation was,

$$Y = 172,5 + 18,29X_1 + 0,05X_6.$$

Although "season of birth" (X_4) and "birth mass of calves" (X_2) were correlated with Y (X_4; r = 0,387; X_2; r = 0,459) these factors were both correlated with the bodymass of the cow at calving. Hence, their individual effects were accounted for by X_6.

When all other factors are held constant a creepfed calf could be expected to show an advantage of almost 12 kg at weaning in the Thornveld (Table 12). Similarly, the advantage of early-season calving was of the order of 23 kg. Accordingly, a calf born early and being creepfed could be expected to show a 35 kg advantage at weaning compared to one born late and not offered creepfeeding (Table 12). A somewhat similar advantage of 18,3 kg due to creepfeeding was noted in the Sourveld (Table 12).

Discussion

There is general agreement amongst research workers that lactating beef cows are likely to benefit (as regards reproductive performance) from supplementary feeding after calving. The question that needs to be answered is why the results reported here are not in accordance with the accepted belief? While bearing in mind that the response may vary from year to year, depending mainly on rainfall distribution, and that only a small number of animals were available, our results demonstrated that:

(1) Contrary to expectation early calvers showed no particular disadvantage as regards ADG during early lactation. Furthermore, over the whole lactation period late calvers were in fact exhibiting the poorest performance.

In the Sourveld, over all treatments, there was a small negative correlation between bodymass at calving and the ADG, both during the period 30 days post-partum to 60 days after onset of mating (r = 0,255) and also the ADG for the entire lactation period (r = 0,114). Furthermore, amongst the early calvers the bodymass at calving for those cows which subsequently received the energy concentrate was 28,6 kg less than for the unsupplemented controls. Perhaps, under certain circumstances the heavier cows lose more bodymass during lactation and the beneficial effects of supplement, if any, could be masked.

Amongst the late calvers the situation was reversed with the non-supplemented cows showing a bodymass advantage of 29 kg over the supplemented cows and yet they gained more than the latter. Bhalaru, Dhillon & Tiwana (1981) suggested that buffalo cows which had the greatest mass at calving also lost most thereafter, when under nutritional stress.

Table 12 Bodymass at weaning of beef calves as influenced by supplementary feeding of dams, creepfeeding of calves and time-of-birth

			Average bodymass (kg)			
	Feeding treatment		Early born		Late born	
Locality	Cow	Calf	Observed	Predicted	Observed	Predicted
Thornveld	Suppl.	Creepfed	179,6	186,1	169,7	160,2
	Control	Creepfed	181,7	183,6	160,4	163,4
	Suppl.	Control	180,9	175,1	144,1	148,9
	Control	Control	177,8	172,8	154,1	153,5
Sourveld	Suppl.	Creepfed	240,0	242,1	235,8	235,4
	Control	Creepfed	239,5	238,4	236,5	236,2
	Suppl.	Control	224,5	224,1	218,3	216,9
	Control	Control	225,0	221,3	213,1	218,4

2) The benefit of supplemental energy was decidedly quesonable as regards ADG and reconception. This confirms the results obtained by Bosman & Harwin (1969), Harwin & Venter, (1970) and by Wells, Hopley & Holness (1980).

In attempting to answer the question posed, besides the ossibility of substitution feeding (van Ryssen, Short & Lishman, 1976) and unequal competition at the feed trough Wells *et al.*, 1980), there is the possibility that the extra energy imply increased milk production without affecting body reserves (Bellows & Short, 1978).

In view of the time-of-calving in relation to the onset of the reeding period late calvers were fed for an average of only our weeks. However, the expectation was that early calvers would benefit from additional feed.

Although Davis, Schalles, Kiracofe & Good (1977) concluded hat bodymass changes were associated with reproductive function, they proposed that earlier rebreeding on higher energy ations, but similar cow masses, indicated that mass changes were not necessarily accurate indicators of future reproducion. Meaker (1978) has also observed that changes in bodymass after calving were not related to energy intakes.

From our results it would appear that because energy feeding was advantageous in the Thornveld as regards ADG and because the general conception rate was not much above 50 %, ome factor or factors were operating to limit reproductive perormance. Grosskopf (1978), Levine, Amezquita & Hohenboken (1980) and Meaker, Coetsee, Smith & Lishman (1980) mphasize the importance of a minimum bodymass that each ow must maintain during the breeding season in order to coneive. Gain or loss prior to breeding was of little consequence provided the bodymass was above a certain minimum Grosskopf, 1976). This is in accordance with Richardson, Oliver & Clarke (1975) who believe that bodymass *per se* and not gain or loss is important in determining whether a lactating ow will conceive or not. Clearly the apparent contradiction, where changes in bodymass are shown to be important in some tudies (Davis *et al.*, 1977) and not in others (Carstairs, Morow & Emery, 1980; Wells *et al.*, 1980), is due to variation n the physiological status of the animals from experiment to xperiment when the studies are initiated.

The data in Figure 1 and 2 and in Table 8 could be said o support both the "target mass', and the "gain during the breeding period" concepts. In both areas, a calving rate of 0 % would appear to be possible provided the cows gained an average of 40 kg during the breeding period, or attained an average mass of 500 kg by the end of the 90-day mating eason. The data in Table 7 suggest that the heavier cows also gain the most. Consequently they have a double advantage. The occurrence of first-calvers does however confuse the issue.

In the foregoing, the date of calving has been ignored. Grosskopf (1976) maintained that cows which calved less than 5 days before the start of the 90-day season, did not have a good chance of conceiving. Only the data obtained in the Sourveld confirmed this suggestion.

In attempting to account for the longer ICI amongst the arly calvers (Table 6), attention needs to be given to the timing of the subsequent mating period. The data show that the interval between the initial parturition and the onset of the next breeding period was longer ($P \leqslant 0{,}001$) for those cows which onceived than for those which did not (Table 6). Consequently when reconception is considered, the time of calving is important, but to achieve a short ICI early exposure to bulls becomes an advantage. Here it should be noted that the early alvers were already 76 days post-partum when bulls were introduced. In effect there was no "tail" of late conceptions amongst the late calvers since the bulls had already been removed. Amongst the early calvers this was possible.

In order to obtain a clearer understanding of the relative roles of "date of calving" and of bodymass criteria it would be necessary to combine these two factors in a single analysis. To date it has not been possible to select a continuous variate which would yield meaningful results.

When focussing attention on the preweaning growth of the calves the two questions posed were,

(1) Can creepfeeding compensate for the effect of being born late in the season?

(2) Is it more important to feed the lactating cow or the suckling calf?

In the Thornveld there was a clear seasonal effect on the growth rate of the calves. This finding supports the suspicion that some factor or factors were limiting performance of the animals in this area.

Harwin & Venter (1970) were able to increase the growth of late born calves by creepfeeding so as to eliminate the seasonal effect. In the present study however, this effect of time-of-birth could be reduced, but not entirely eliminated, by the creepfeed provided in the Thornveld.

For the results obtained in the Sourveld the least squares analysis revealed only a minor seasonal effect which had to be overcome. In this area, creepfeeding was beneficial to both early and late born calves. Consequently late born calves that received creepfeed achieved a higher weaning mass than early born calves not creepfed.

The foregoing results support the contention (Ochoa, Mangus, Brinks & Denham, 1981) that creepfeeding can decrease the effect of environmental factors on weaning mass. In particular the creepfeed should be of benefit to calves suckling dams with limited milk (Ochoa *et al.*, 1981) as suggested by Harwin (1963) and by Cooper (1965).

The advantage at weaning, due to creepfeeding, of between 12 kg (Thornveld) and 18 kg (Sourveld) agrees with the results obtained by Marlowe, Mast & Schalles, (1965); Cundiff, Willham & Pratt, (1966); Martin, Lemenager, Srinivasan & Alenda (1981) and Ochoa *et al.* (1981), but is of lower magnitude than the 27 – 34 kg reported by Scarth, Miller, Phillips, Sherritt & Ziegler (1968); Harwin & Venter (1970); Ferreira (1975); Martin, Perry, Beeson & Mohler (1977); Stricker, Matches, Thompson, Jacobs, Martz, Wheaton, Currence & Krause (1979). If creepfeeding is to be employed as a means of reducing seasonal effects, then cognizance must be taken of the superior response of males (Martin *et al.*, 1981) and the likelihood of a detrimental effect on the lifetime performance of creepfed heifers (Mangus & Brinks, 1971; Kress & Burfening, 1972; Holloway & Totusek, 1972; Ellicott, Holland & Neumann, 1970; Martin *et al.*, 1981).

From the viewpoint of increased calf growth rate there was little to be gained from providing the dam with an energy supplement. This agrees with the findings of Harwin & Venter (1970).

In considering the cow/calf enterprise as a whole and focussing attention on the need to achieve good conception rates from year to year and good preweaning growth, the practice of providing an energy concentrate during early lactation cannot be recommended. It would appear to be more profitable to focus attention on improving the general nutrition of the breeding herd as a whole rather than attempting to supplement only during periods of peak demand. With creepfeeding the benefit is real, but the cost must be related to the value of the additional return. Limited feeding during periods of stress should be considered.

References

BELLOWS, R.A. & SHORT, R.E., 1978. Effects of precalving feed level on birth weight, calving difficulty and subsequent fertility. *J. Anim. Sci.* 46, 1522.

BHALARU, S.S., DHILLON, J.S. & TIWANA, M.S., 1981. Effect of bodyweight at calving on bodyweight changes during early lactation and post partum reproductive performance in buffaloes. *J. Agric. Sci.* Camb. 97, 595.

BISHOP, E.J.B. & KOTZE, J.J., 1965. Good strategy with beef cows. *Farming S. Afr.* 41, 6.

BONSMA, J.C. & SKINNER, J.D., 1969. Factors influencing birth date, birth weight and preweaning growth rate of Afrikaner, Bonsmara and Hereford cattle in the subtropics. *Proc. S. Afr. Soc. Anim Prod.* 8, 161.

BOSMAN, D.J. & HARWIN, G.O., 1969. The occurrence of post partum oestrus in beef cows under ranching conditions. *Proc. S. Afr. Soc. Anim. Prod.* 8, 165.

BOSMAN, D.J. & HARWIN, G.O., 1966. Genetic and environmental factors affecting pre-weaning traits in beef cattle under extensive ranching conditions. *Proc. S. Afr. Soc. Anim. Prod.* 5, 153.

BOULLE, J.P., 1980. An evaluation of management expertise in Natal beef herds. *20th Annual Congr. S. Afr. Soc. Anim. Prod.* April, 1980.

CARSTAIRS, J.A., MORROW, D.A. & EMERY, R.S., 1980. Post partum reproductive function of dairy cows as influenced by energy and phosphate status. *J. Anim. Sci.* 51, 1122.

COOPER, C.R., 1965. Effects of environmental factors and their two-way interactions in Colorado beef herds. Ph. D. Thesis. Colorado State University.

CUNDIFF, L., WILHAM, R.L. & PRATT, C.A., 1966. Effects of certain factors and their two-way interactions on weaning weight in beef cattle. *J. Anim. Sci.*, 25, 972.

DAVIS, D., SCHALLES, R.R., KIRACOFE, G.H. & GOOD, D.L., 1977. Influence of winter nutrition on beef cow reproduction. *J. Anim. Sci.* 45, 430.

ELLICOTT, G.H., HOLLAND, L.A. & NEUMANN, A.L., 1970. Most probable producing ability of Hereford cows. *Proc. Western Sec. ASAS.* 21, 363.

FERREIRA, I., 1975. Kruipvoeding — Die ware Jakob. *Vleisnywerheid,* Jan. – Maart, 1975, p.14.

GROSSKOPF, J.F.W. 1976. Die herkonsepsie van lakterende vleisraskoeie as beperkende faktor by die praktiese toepassing van ovulasie-sinkronisasie. Ph. D. thesis, Universiteit van Stellenbosch.

HARWIN, G.O., 1963. The effect of inbreeding and environmental factors on the weaning weight and postweaning growth of range Hereford cattle. Ph. D. Thesis, Colorado State University.

HARWIN, G.O., LAMB, R.D. & BISSCHOP, J.H.R., 1967. Some factors affecting reproductive performance in beef females. *Proc. S. Afr. Soc. Anim. Prod.* 6, 171.

HARWIN, G.O. & VENTER, H.A.W., 1970. An investigation of the creep feeding of beef calves in the mixed Bushveld. *Proc. S. Afr. Soc. Anim. Prod.* 9, 161.

HEYNS, H., 1960. The growth of the Afrikaner calf in relation to the production and composition of the milk of its dam. II. The milk production of the dam and growth of the calf. *S. Afr. J. Agric. Sci.* 3, 517.

HOLLOWAY, J.W. & TOTUSEK, R., 1972. Early weaning *vs* normal weaning *vs* creep feeding of replacement heifer calves. *Oklahoma Agric. Exp. Sta. MP.* 87, 18.

JOUBERT, D.M., 1954. The influence of high and low nutritional planes on the oestrous cycle and conception rate of heifers. *J. Agric. Sci.* 45, 164.

KRESS, D.D. & BURFENING, P.J., 1972. Weaning weight related to subsequent most probable producing ability in Hereford cows. *J. Anim. Sci.* 35, 327.

LEVINE, J.M., AMEZQUITA, M.L. & HOHENBOKEN, W.D., 1980. Relationship of liveweight to calving rate of grade Zebu heifers and cows on the eastern planes of Columbia. *J. Anim. Sci.* 50, 1040.

MANGUS, W.L. & BRINKS, J.S., 1971. Relationship between direct and maternal effects on growth in Herefords. I. Environmental factors during pre-weaning growth. *J. Anim. Sci.* 32, 17.

MARLOWE, T.J. & GAINES, J.A., 1958. The influence of age, sex and season of birth of calf and age of dam on pre-weaning growth rate and type score of beef calves. *J. Anim. Sci.* 17, 706.

MARLOWE, T.J., MAST, C.C. & SCHALLES, R.R., 1965. Some non-genetic influences on calf performance. *J. Anim. Sci.* 24, 494.

MARTIN, T.G., LEMENAGER, R.P., SRINIVASAN, G. & ALENDA, R., 1981. Creep feed as a factor influencing performance of cows and calves. *J. Anim. Sci.* 53, 33.

MARTIN, T.G., PERRY, T.W., BEESON, W.M. & MOHLER, H.T., 1977. High urea supplements and pre-weaning creep fed as factors affecting post weaning performance of bulls. *J. Anim. Sci.* 44, 739.

MEAKER, H.J., 1978. The importance of age at first calving, relationship between bodymass and fertility and feeding systems on production in the beef female. Ph. D. Thesis. University of Natal.

MEAKER, H.J., COETSEE, T.P.N., SMITH, M. & LISHMAN, A.W., 1980. The relationship between bodymass and fertility of beef cows of different ages. *S. Afr. J. Anim. Sci.* 10, 83.

OCHOA, P.G., MANGUS, W.L., BRINKS, J.S. & DENHAM, A.H., 1981. Effect of creep feeding bull calves on dam most probable producing ability values. *J. Anim. Sci.* 53, 567.

RICHARDSON, F.D., OLIVER, J. & CLARKE, G.P.Y., 1975. Analyses of some factors which affect the productivity of beef cows and their calves in a marginal rainfall area of Rhodesia. *Anim. Prod.* 21, 41.

SCARTH, R.D., MILLER, R.C., PHILLIPS, P.J., SHERRITT, G.W. & ZIEGLER, J.H., 1968. Effects of creepfeeding and sex on the rate and composition of growth of crossbred calves. *J. Anim. Sci.* 27, 596.

SNAPP, R.R. & NEUMANN, A.L., 1960. Beef cattle. 5th ed. New York. John Wiley & Sons. Inc.

STEENKAMP, J.D.G., 1977. Cost of production of a kilogram of live weaned calf. *Meat Industry* 26, 34.

STRICKER, J.A., MATCHES, A.G., THOMPSON, G.B., JACOBS, V.E., MARTZ, F.A., WHEATON, H.M.N., CURRENCE, H.D. & KRAUSE, G.F., 1979. Cow-calf production on tall fescue-ladino clover pastures with and without nitrogen fertilization or creepfeeding spring calves. *J. Anim. Sci.* 48, 13.

VAN RYSSEN, J.B.J., SHORT, A.M. & LISHMAN, A.W., 1976. Carbohydrate supplementation of lambs on kikuyu pasture. *Agroanimalia* 8, 43.

WARNICK, A.C., 1959. Effect of a protein deficiency on reproduction. Beef cattle. *Fla. Agr. Exp. Sta. Mim. Series* 59 – 10.

WILTBANK, J.N., ROWDEN, W.W., INGALLS, J.E., GREGORY, K.E. & KOCH, R.M., 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. *J. Anim. Sci.* 21, 219.

WILTBANK, J.M., ROWDEN, W.W., INGALLS, J.E. & ZIMMERMAN, D.R., 1964. Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. *J. Anim. Sci.* 23, 1049.

WELLS, P.L., HOPLEY, J.D.H. & HOLNESS, D.H., 1980. The influence of concentrate supplementation during the post-partum period on ovarian activity and conception in Afrikaner cows. Zimbabwe Dept. Res. & Spec. Serv. Ann. Rep. Year ending 30 September, 1980, Salisbury, Rhodesia.

Reconception and body-mass changes of energy upplemented first-calver beef cows and growth of their reepfed calves

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ie effect of dietary supplementation using 2,3 kg maize meal per day
ı change in body mass during early lactation and on the calving rate
first-calf Afrikander, Sussex and Hereford type cows which grazed
e Tall Grassveld of Northern Natal was studied. Half of the suckling
ilves were also allowed access to a creepfed.
Provision of a dietary energy supplement reduced the body mass
ss by an average of only 0,02 kg per day and no consistent effect on
e reconception rate was observed. In general, those cows which produced a calf after being exposed to breeding bulls for 65 days showed
higher body mass at first calving and at both the onset and the con-
usion of the subsequent breeding period than cows which would not
ilve the following year. Amongst the Afrikander cows the interval
om calving to the onset of the next breeding period accounted for
,2 % of the variation in calving rate. For all breeds, the calving rate
creased by 4,7 % for every ten days earlier calving.
The average growth of the calves between the ages of two months
id weaning (seven months) was significantly improved by 120 g per
iy where 0,9 kg creepfeed was supplied daily. During this period the
iily gain was influenced by, in order of importance, the body mass at
/o months, the provision of creepfeed, and the breed-type of the
ım. Over the entire suckling period (birth to weaning) the growth rate
as affected by the body mass of the dam at parturition, the provision
creepfeed, the breed-type of the dam and the sex of the calf.
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e invloed van byvoeding met 2,3 kg mieliemeel per dag op die
ırandering in liggaamsmassa gedurende vroeë laktasie en op die
ırbesetting van eerste-kalf Afrikaner, Sussex en Hereford-tipe koeie
at in die Langgrasveld van Noord-Natal gewei het, is bestudeer. Die
ılfte van die kalwers het toegang tot kruipvoer gehad. Die
ıwigsverlies van die koeie is gemiddeld met slegs 0,02 kg per dag
ıur byvoeding verminder en geen duidelike herbesettingsvoordeel is
aargeneem nie.
Oor die algemeen het koeie wat herbeset is na 65 dae van paring,
hoër massa by eerste kalwing gehad en by beide die begin en
nde van die daaropvolgende paarperiode as koeie wat nie die
aaropvolgende jaar sou kalf nie. By die Afrikanertipes was die
ıriode vanaf kalwing tot aanvang van die volgende paringsperiode
ırantwoordelik vir 38,2 % van die variasie in herbesetting. Waar
sverskille geïgnoreer is, was die herbesetting 4,7 % hoër vir elke tien
ıe wat die koeie vroeër gekalf het. Die gemiddelde groeitempo van
e kalwers tussen twee en sewe maande is betekenisvol verhoog met
20 g per dag deur 'n daaglikse inname van 0,9 kg kruipvoer. Gedurende hierdie periode is die daaglikse massatoename beinvloed deur, in
ılgorde van belangrikheid, die liggaamsmassa by twee maande,
uipvoeding en rastipe van die moer. Die groeitempo vanaf geboorte
ıt speen is beinvloed deur die liggaamsmassa van die moer by kalw-
g, kruipvoeding, rastipe van die koei en die geslag van die kalf.
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eywords: Energy supplementation, first calvers, calving rates,
eepfeeding

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Introduction

It is generally accepted that beef heifers conceive at a high rate during their first breeding season provided they have grown sufficiently (Bellows, 1968; Arije & Wiltbank, 1974). However, when breeding takes place while they are suckling their first calf the pregnancy rate is low (Bauer, 1965; Harwin, 1967). The nutritional requirements for growth of the young cow in addition to those for lactation are not usually met by the natural grazing available. The resumption of reproductive cycles is therefore considerably delayed, so that many first-calvers show heat only after the mating period has ceased. Harwin (1967) suggested that the calving rate of the herd as a whole could be increased by 10 to 15 % simply by additional feeding after the cow has calved for the first time. The problem can also be overcome by mating heifers so that they calve some four to six weeks before the cow herd. Although this allows a longer recovery period after parturition, this practice does extend the calving period of the herd as a whole. Furthermore, the heifers then calve at a time which is more beneficial to the growth of the suckling calf than to the reconception of the lactating dam.

The purpose of this experiment was to determine whether increasing the dietary energy intake would reduce the loss in body mass during lacation and thereby increase the reconception of first-calvers and to determine whether creepfeeding of the calves would compensate for the low milk production of such cows.

Procedure

In the Tall Grassveld of Northern Natal (Dundee Research Station), Afrikander and Sussex-Afrikander type heifers were mated to Sussex bulls, and Hereford type heifers to a Hereford bull so that calving would commence in August.

At parturition, the cows were allocated randomly (within breed type) to two groups. One group of cows was fed 2,3 kg maize meal per day. This was supplied from calving until the end of the subsequent breeding period. The other group received no dietary energy supplement. From the age of two months until weaning at 205 days, half the calves in each group had access to creepfeed (80 parts maize meal: 10 parts fishmeal: 10 parts sunflower oilcake meal), fed at the rate of 0,9 kg per day. The remaining calves were suckled without creepfeed.

During the winter, prior to calving, the pregnant heifers received maize silage (13,5 kg/day), *Eragrostis curvula* hay (4,5 kg/day) and 0,7 kg urea block per day. The veld grazing utilized during summer was divided into four camps which were grazed in rotation for seven day periods. Each treatment group grazed in every camp. Breeding bulls (Sussex) were joined with the cows for 65 days starting on 17 October. The bulls were interchanged between the various groups each week. Reconception was based on cows calving and the date of service was derived from the calving date. All animals were weighed at four week intervals. The body mass of each cow and that of her calf were also recorded on the day of parturition.

Treatment effects on changes in body mass of the cows and on growth of the calves were tested by least squares analysis. The effect of cow mass at calving on cow gains during the post-partum period was corrected for by covariance. Differences in characteristics of cows which reconceived were compared to those of cows not recalving using Student's *t*-test. Association between selected variates was measured by regression analysis and a stepdown regression procedure was employed to examine the factors suspected of influencing post-partum body-mass change of cows, reconception and also growth of calves. Chi-square analysis was used to test treatment differences in conception rate.

Results

The average daily gain in body mass (ADG) of the cows from calving until the end of the subsequent mating period (Table 1) depended on cow mass at calving (Table 2). The adjusted gain in mass during this period (*i.e.* the period of supplementary energy feeding) indicated a significantly ($P \leqslant 0,05$) greater loss amongst the Hereford type cows (Table 2). However, body mass at parturition differed between breed types (Table 2), and because of limited animal numbers the covariance analysis was based on all three breeds. Consequently, the correction of the ADG is not entirely satisfactory. By limiting the regression analysis to the Afrikander type females only, a significant correlation between body mass at calving (X) and ADG (Y) was obtained. However, only 6,0 % of the variation in Y could be accounted for, and not much can be gained by correcting Y. The mean daily loss in mass for all supplemented cows was 0,10 kg while the non-supplemented females experienced a decrease of 0,12 kg per day. Clearly supplementation was of no value in terms of ADG (Table 2).

Table 2 Gain in body mass of lactating first-calvers adjusted for body mass at calving

Breed	Mass at parturition (kg)	Average daily gain, parturition to end of mating period (kg)
Afrikander	418,0^a	−0,10^a
Sussex-Afr.	440,2^b	−0,01^a
Hereford	370,0^c	−0,20^b
SE	±4,9	±0,03

^{a,b,c} Means in columns with different superscripts differ significantly ($P \leqslant 0,05$).

Reconception

Supplementary feeding appeared to benefit (non-significantly) the reconception of only the Afrikander type cows where the calves also had access to a creep ration (Table 3). Amongst the other two breed types the results were highly variable. The Sussex type cows were notably superior to the other two breed types.

The Afrikander cows that re-calved, consistently showed a greater body mass than those which did not calve again (Table 4), although the change in body mass was contrary to expectation. Classification of the cows (all breed types combined) according to the body mass at parturition, indicated that the calving rate was significantly dependent on the body mass (Figure 1) with 96,5 % of the variation being accounted for. This finding is biased since the Hereford type cows, which showed low calving rates, tended to have relatively low body masses, while the Sussex types, with relatively high body masses, also exhibited good reconception. Restricting the analysis to the 68 Afrikander type cows, revealed that 87,9 % of the variability in calving rate was due to variation in body mass at parturition.

There was no significant association between the body mass at calving ($\bar{x}$ = 430,3 kg) and the interval from parturition to conception ($\bar{x}$ = 79,3 days). Amongst the Afrikander type cows the relationship was significant ($P \leqslant 0,01$) although only 12,5 % of the variation in the interval to conception could be accounted for in terms of variation in body mass at calving. In addition to being of lower body mass at calving, those Afrikander and Hereford type cows which did not re-calve were joined with bulls at a shorter interval (Herefords: $P \leqslant 0,001$) after calving than the cows which re-calved (Table 5). Since the bulls remained with the cows for 65 days and those cows

Table 1 Changes in body mass of lactating cows as influenced by provision of an energy supplement

Feeding treatment				Average body mass (kg) at:		Average daily gain (kg)	Average body mass (kg) conclusion of lactation
Cow	Calf	Breed	*n*	Parturition	End mating period		
Supplemented	Supplemented	Afrikander	17	427,3	414,6	−0,14	408,0
		Sussex-Afr.	6	440,2	437,5	−0,03	425,4
		Hereford	6	368,7	360,8	−0,15	374,2
		SE		± 16,7	± 7,1	±0,17	± 6,8
Supplemented	Control	Afrikander	16	400,8	396,2	−0,07	388,7
		Sussex-Afr.	4	423,2	439,7	±0,11	422,1
		Hereford	5	375,2	355,3	−0,11	364,3
		SE		± 12,6	± 7,6	±0,30	± 7,3
Control	Supplemented	Afrikander	16	421,7	411,6	−0,09	399,4
		Sussex-Afr.	4	420,3	418,4	−0,02	409,2
		Hereford	5	301,0	374,1	−0,12	369,8
		SE		± 18,8	± 7,6	±0,24	± 7,25
Control	Control	Afrikander	19	420,6	407,9	−0,15	394,3
		Sussex-Afr.	5	470,4	448,9	−0,20	432,4
		Hereford	6	361,7	349,1	−0,15	346,9
		SE		± 20,0	± 6,9	±0,20	± 6,7

Table 3 Reconception of first-calvers when fed a maize supplement during early lactation

Feeding treatment			Cows:			
Cow	Calf	Breed	Joined	Calved	%	Intercalving interval (days)
Supplemented	Supplemented	Afrikander	17	11	64,7	376,0
		Sussex-Afr.	6	5	83,3	362,0
		Hereford	6	3	50,0	365,0
Supplemented	Control	Afrikander	16	2	12,5	365,0
		Sussex-Afr.	4	3	75,0	361,7
		Hereford	5	1	20,0	382,0
Control	Supplemented	Afrikander	16	4	25,0	352,5
		Sussex-Afr.	4	3	75,0	348,7
		Hereford	5	3	60,0	364,7
Control	Control	Afrikander	19	5	26,3	371,4
		Sussex-Afr.	5	3	60,0	351,0
		Hereford	6	0	0,0	–
						SE + 12,6

Table 4 Body-mass characteristics of cows which may be related to reconception

		Body mass (kg) at:					
Breed type	Reproductive status	Parturition	Onset of breeding period	Conclusion of breeding period	Average change in body mass (kg) during breeding period	Average maximum loss in body mass (kg) from calving to joining with bulls	Average daily gain in body mass (kg), calving to end breeding period
Afrikander	Calf	$^{a}442{,}0 \pm 9{,}1$	$^{c}440{,}4 \pm 11{,}1$	$^{e}432{,}7 \pm 10{,}3$	$^{g}-5{,}6 \pm 2{,}1$	$^{i}-25{,}6 \pm 2{,}6$	$^{j}-0{,}12 \pm 0{,}04$
	No calf	$^{b}404{,}4 \pm 5{,}2$	$^{d}394{,}3 \pm 5{,}5$	$^{f}395{,}7 \pm 4{,}6$	$^{h}+1{,}1 \pm 2{,}1$	$^{i}-31{,}8 \pm 3{,}1$	$^{j}-0{,}11 \pm 0{,}04$
Sussex-Afr.	Calf	$^{a}439{,}7 \pm 9{,}3$	$^{b}443{,}4 \pm 9{,}5$	$^{c}438{,}7 \pm 8{,}0$	$^{d}+0{,}6 \pm 5{,}6$	$^{e}-22{,}1 \pm 5{,}7$	$^{f}-0{,}02 \pm 0{,}04$
	No calf	$^{a}442{,}3 \pm 23{,}9$	$^{b}441{,}0 \pm 23{,}1$	$^{c}431{,}7 \pm 21{,}2$	$^{d}-5{,}2 \pm 2{,}6$	$^{e}-28{,}1 \pm 10{,}3$	$^{f}-0{,}10 \pm 0{,}09$
Hereford	Calf	$^{a}382{,}0 \pm 11{,}9$	$^{b}383{,}1 \pm 12{,}1$	$^{c}376{,}9 \pm 12{,}2$	$^{d}-6{,}9 \pm 1{,}8$	$^{e}-22{,}0 \pm 5{,}9$	$^{f}-0{,}06 \pm 0{,}06$
	No calf	$^{a}363{,}4 \pm 8{,}0$	$^{b}360{,}0 \pm 7{,}6$	$^{c}354{,}1 \pm 8{,}4$	$^{d}-6{,}1 \pm 3{,}6$	$^{e}-29{,}3 \pm 5{,}2$	$^{f}-0{,}18 \pm 0{,}08$

$^{a-j}$ Means in columns with a breed type having the same superscript do not differ ($P \geqslant 0{,}05$)

which re-calved did so on average 75,5 days after calving (range 28 to 126 days), it is possible that a delayed onset of oestrus after parturition might have contributed to the low reconception rates. Classification of all the cows according to the interval from calving to commencement of the next breeding period (X) suggested a trend towards increasing conception rates (Y) as the cows calved earlier (Figure 2). Although 30,5 % of the variability in Y could be accounted for the correlation coefficient of 0,55 was non-significant ($r = 0{,}58$ required for $P \leqslant 0{,}05$).

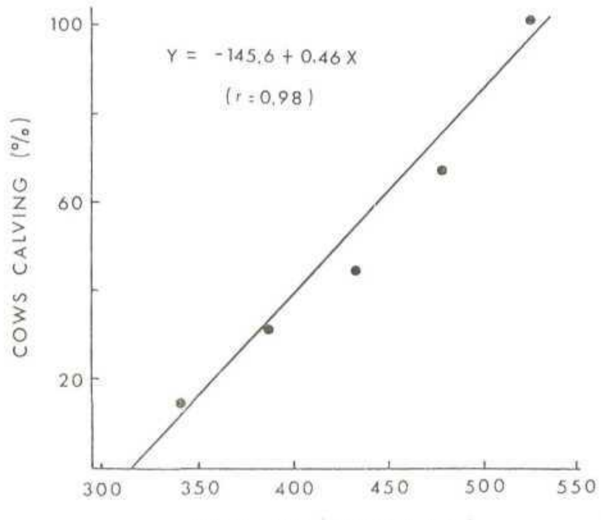

Figure 1 Changes in calving % of first calvers as influenced by body mass at parturition

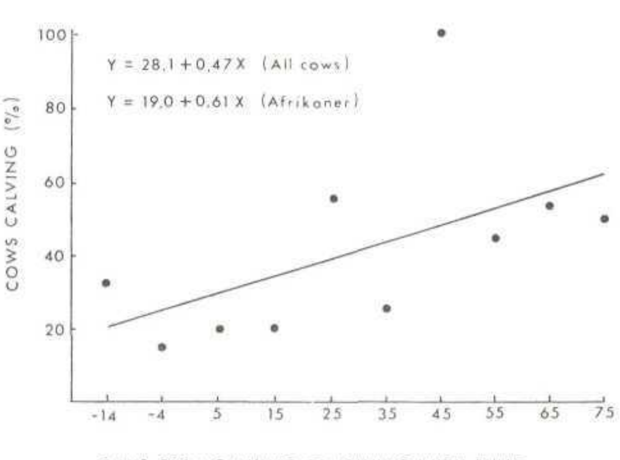

Figure 2 Calving rate as influenced by time of calving in relation to onset of subsequent mating period

Table 5 Reconception of different breed types and relationship of time of calving to onset of subsequent breeding period

Breed	Cows: Joined with bulls	Calved No.	Calved %	Interval (days) parturition to joining with bulls: For cows calving	For cows not calving	Interval (days) introduction of bulls to conception	Interval (days) parturition to conception	Intercalving interval (days)
Afrikander	68	22	32,3	$^{a}39,3 \pm 4,4$	$^{a}30,8 \pm 4,0$	$44,3 \pm 3,3$	$83,9 \pm 4,5$	$369,7 \pm 4,9$
Sussex-Afr.	19	14	73,4	$^{b}39,5 \pm 5,0$	$^{b}51,0 \pm 1,7$	$30,0 \pm 4,1$	$70,6 \pm 6,4$	$356,3 \pm 5,9$
Hereford	22	7	31,8	$^{c}36,2 \pm 7,3$	$^{d}\ 7,8 \pm 2,7$	$46,6 \pm 6,0$	$83,0 \pm 10,0$	$367,3 \pm 10,1$

^{a,b,c,d} Means in the same row within a breed with different superscripts differ significantly ($P \leqslant 0,01$)

Limiting the analysis to the Afrikander cows resulted in the regression:

$$Y = 19,02 + 0,61\ X$$

The regression was significant ($P \leqslant 0,05$) and 38,2 % of the variation in Y was explained in terms of X.

The use of conception rate as the dependent variable necessitates the grouping of animals into selected classes. To avoid this procedure the interval between the onset of the breeding period and the date of conception (calculated form the calving date) was used as the dependent variable (Y) and the interval from calving to the onset of the breeding period as the independent variable (X). Only by eliminating cows which had a body mass of less than 410 kg at parturition could a significant regression be established.

When the interval between calving and the introduction of bulls was considered in conjunction with the body mass of the Afrikander type cows at the onset of breeding (Table 6), it appeared that:

(a) For those cows which re-calved, if breeding commenced:
   (i) less than 45 days post-partum then 75 % of the cows had a body mass greater than 410 kg.
   (ii) more than 45 days post-partum then 60 % calved even though they had a mass of less than 410 kg.
(b) For cows which did not reconceive if breeding commenced:
   (i) more than 45 days post-partum, 80 % of the 15 animals had a mass of less than 410 kg.
   (ii) less than 45 days post-partum only 35,5 % of the 31 cows had a body mass of more than 410 kg. The difference was significant ($P \leqslant 0,05$).

Categorization of the cows on the basis of the interval from calving to the onset of breeding showed that:

(a) For the cows less than 45 days post-partum:
   Only 13 % having a body mass less than 410 kg conceived, but significantly more cows (45 %) conceived at a body mass exceeding 410 kg. This represents a factor of 3,5 times in favour of the cows with the greater body mass.
(b) For the cows more than 45 days post-partum:
   Of the cows only six (33,3 %) having a body mass below 410 kg conceived and the conception rate was 57,1 % when the body mass exceeded 410 kg. This difference was non-significant.

No reason could be found for the five Sussex type cows which did not re-calve, but the Hereford type cows were clearly at a disadvantage in view of the short period between calving and onset of the next breeding period (Table 5).

Suckling calves

During the creepfeeding phase (two to seven months) the average daily gain (ADG) of the calves (Y_1) was mainly dependant on the mass of the calves at the onset of this phase (Table 7). This variate was in turn correlated ($r = 0,41$) with the body mass of the dam at calving (Y_6) and the latter variate was

Table 6 Influence of body mass at the onset of the breeding period and post-partum interval to annual mating season on reconception of first-calf Afrikander cows

Average body mass of cows	< 45 days: Reconceiving No. of cows	%	Not reconceiving No. of cows	%	> 45 days: Reconceiving No. of cows	%	Not reconceiving No. of cows	%
< 410 kg	3	25,0	20	64,5	6	60,0	12	80,0
> 410 kg	9	75,0	11	35,5	4	40,0	3	20,0

Days from calving to onset of breeding period:

Table 7 Factors influencing the preweaning average daily gain (ADG) of calves

Variates included in regression analysis	Variates significantly influencing: ADG during creepfeeding phase Y_1	Order of importance	ADG birth to weaning Y_2	Order of importance
Creepfeeding of calf — X_1	X_1	2	X_1	2
Mass of calf at birth — X_2	–		–	
Mass of calf at onset of creepfeeding — X_3	X_3	1	excluded	
Sex of calf — X_4	–		X_4	4
Suppl. of dam — X_5	–		–	
Mass of dam at parturition — X_6	–		X_6	1
Av. daily mass change of dam during creepfeeding phase OR during preweaning phase — X_7	–		–	
Breed of dam — X_8; X_9	X_8; X_9	3	X_8; X_9	3

Regression equations: $Y_1 = 0,49 + 0,23\ X_1 + 0,001\ X_3 + 0,09\ X_4 + 0,04\ X$
$+ 0,13\ X_9$.
$Y_2 = 0,44 + 0,18\ X_1 + 0,12\ X_4 + 0,0003\ X_6 + 0,0$
$X_8 + 0,10\ X_9$.

therefore not shown to contribute significantly to the variation in Y_1. The factors shown to be significant, *i.e.* X_3, X_1, X_8, X_9 contributed 21,1, 15,1 and 8,3 % respectively to the 44,5 % variation in Y_1.

The ADG over the entire suckling period of seven months was markedly influenced by the 'body mass of the cow at calving' and variation in this factor explained 20,7 % of the accountable 46,3 % variation in Y_2. The remaining variables of significance *viz.* 'creepfeeding of the calf', 'breed type of the dam' and 'sex of the calf' contributed 13,5, 8, and 3,3 respectively to the variation in Y_2.

The influence of the breed type of the dam on the growth of the calf is reflected in the adjusted gains (Table 8). Over all breeds the 0,10 greater daily gain of the creepfed calves yielded an average 18,0 kg advantage in body mass at weaning over the unsupplemented animals. Although the steers gained only 0,005 kg per day more than the heifers during the creepfeeding period, the results have been presented for each sex (Table 8) in order to simplify comparisons. The adjusted daily gain in body mass of the calves from Hereford type dams (average = 0,69 kg) was 0,15 kg lower than that of calves from Sussex type dams (average = 0,84 kg) and 0,12 kg greater than that of calves suckled by Afrikander type cows (average 0,81 kg). Although the breed type effects were still evident amongst the creepfed calves, creepfeeding of the poorest calves enabled them to grow almost as quickly as the best calves that were not creepfed.

Table 8 Least squares means for adjusted preweaning growth of calves creepfed from two months until weaning five months later and 205-day weaning mass

		Average daily gain (kg) during:				
		Creepfeeding period		Period birth to weaning		
Treatment	Breed of dam	Steers	Heifers	Steers	Heifers	205-day corrected weanning mass kg
Creepfed	Afrikander	0,88	0,83	0,88	0,84	214,2
	Sussex-Afr.	0,93	0,87	0,92	0,85	215,9
	Hereford	0,75	0,79	0,75	0,71	189,6
Control	Afrikander	0,78	0,72	0,79	0,74	194,5
	Sussex-Afr.	0,81	0,76	0,85	0,77	201,7
	Hereford	0,63	0,58	0,68	0,61	164,4

Discussion

In view of the small number of animals representing the Sussex and Hereford types it would appear reasonable to focus attention on the trends observed amongst only the Afrikander type females. Here, the strikingly higher calving rate of the cows that were supplemented while their calves received creepfeed compared to the other treatment groups is of interest. It is difficult to find an explanation for the result obtained. Neither the date of calving, the body mass of the cows nor the preweaning growth of the calves were individually more to the advantage of cows in this treatment group than in any of the others.

The Afrikander cows which did not reconceive joined the bulls on average 30,8 days after calving (Table 5). With a mating season of 65 days this means that the cows would have had to conceive not later than 96 days after calving. There was a relatively even distribution of cows over the period −4 to 65 days between calving and joining with bulls. Furthermore, on the basis of the fitted line ($Y = 19,0 + 0,61X$) the conception rate improved by 6,1 % for every ten-day increase in the interval to joining. Assuming that the response remained linear, a calving rate of 100 % would have been achieved if the period from parturition to introduction of the bulls was extended to 133 days. The common conclusion that a short interval between calving and initiation of the next breeding season is an important factor contributing to low conception rates, requires more criticial evaluation.

From the data relating reconception rates to the body mass of the cows or the date of calving (Tables 5 and 6, Figures 1 and 2) it would appear justified to conclude that where cows calved more than 45 days prior to the onset of the next breeding period, body mass was of prime importance as regards recalving. Amongst cows which calved later, factors other than body mass may have exerted an influence. This conclusion is partially supported by Grosskopf (1976) who noted that many variables were associated with the likelihood of reconception. He drew particular attention to the body mass of the cow and the time of calving. The results in Table 4 confirm the finding that change in body mass during the breeding period is of relatively minor importance as regards reconception (Richardson, Oliver & Clarke, 1975; Carstairs, Morrow & Emery, 1980, Lishman, Lyle, Smith & Botha, 1983).

For each breed type of dam the benefit of creepfeeding (as reflected in 205-day mass) was proportional to the reputed milk production abilities of these types. Thus calves from Sussex-type dams showed the smallest benefit (14,2 kg) while calves suckled by Hereford-type females gained considerably (25,2 kg). This is in agreement with Hixon, Fahey, Kesler & Neumann (1982) who maintained that creepfeeding masked the milk production deficiency of Hereford cows. The present findings also support the contention (Harwin, 1963; Cooper, 1965; Ochoa, Mangus, Brinks & Denham, 1981) that creepfeed is best utilized by calves suckling cows with limited milk, due either to age or genetic make-up.

The magnitude of the benefit due to creepfeeding was similar to that recorded on the same veld type by Meaker (1978) and on other veld types by Lishman *et al.* (1982).

Conclusion

Supplementation of first-calf cows with a dietary energy concentrate from the time of parturition until the end of the breeding period did not consistently improve either maintenance of body mass or reconception. The preweaning gain of the suckling calves was influenced by creepfeeding and the body mass of the dam at parturition.

References

ARIJE, G.F. & WILTBANK, J.N., 1974. Prediction of age and weight at puberty in beef heifers. *J. Anim. Sci.* 38, 803.

BAUER, M., 1965. Five years study of ranch breeding stock, 1959–1964. *Rhod. Agric.* 62, 28.

BELLOWS, R.A., 1968. Prediction of age and weight at puberty in beef heifers. *A.I. Dig.* 16, 6.

CARSTAIRS, J.A., MORROW, D.A. & EMERY, R.S., 1980. Post partum reproductive function of dairy cows as influenced by energy and phosphate status. *J. Anim. Sci.* 51, 1122.

COOPER, C.R., 1965. Effects of environmental factors and their two-way interactions in Colorado beef herds. Ph.D. Thesis, Colorado State University.

GROSSKOPF, J.F.W., 1976. Die herkonsepsie van lakterende vleisraskoeie as beperkende faktor by die praktiese toepassing van ovulasie sinkronisasie. Ph.D. Thesis. University of Stellenbosch.

HARWIN, G.A., 1963. The effect of inbreeding and environmental factors on the weaning weight and postweaning growth of range Hereford cattle. Ph.D. Thesis, Colorado State University.

HARWIN, G.O., 1976. Cattle breeding without guesswork. *Fmr's Weekly*. 113, 40.

HIXON, D.L., FAHEY, G.C., KESLER, D.J. & NEUMANN, A.L., 1982. Effects of energy level and monensin on reproductive performance and lactation of beef cows. *Theriogenology,* 17, 515.

LISHMAN, A.W., LYLE, A.D., SMITH, V.W. & BOTHA, W.A., 1983. Conception rate of beef cows and growth of suckling calves as influenced by date of calving and supplementary feeding. *S. Afr. J. Anim. Sci.* 14, 10.

MEAKER, H.J., 1978. The importance of age at first calving, relationship between bodymass and fertility and feeding systems on production in the beef female. Ph.D. Thesis, University of Natal.

OCHOA, P.G., MANGUS, W.L., BRINKS, J.S. & DENHAM, A.H., 1981. Effect of creepfeeding bull calves on dam most probable producing ability values. *J. Anim. Sci.* 53, 567.

RICHARDSON, F.D., OLIVER, J. & CLARKE, G.P.Y., 1975. Analyses of some factors which affect the productivity of beef cows and their calves in a marginal rainfall area of Rhodesia. *Anim. Prod.* 21, 41.

S. Afr. J. Anim. Sci. 9, 231–236 (1979)

PAPER 20

PROTEIN AND/OR ENERGY SUPPLEMENTATION OF LACTATING BEEF COWS DURING THE EARLY SUMMER PERIOD IN NORTHERN NATAL

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(Key words: *Protein, energy, reproduction)*
(Sleutelwoorde: *Proteïen, energie, reproduksie)*

OPSOMMING: PROTEÏEN- EN/OF ENERGIEBYVOEGING AAN LAKTERENDE VLEISBEESKOEIE GEDURENDE DIE VROEË SOMERPERIODE IN NOORD NATAL

'n Studie is oor drie jaar uitgevoer om die invloed van proteïen- en/of energiebyvoeging aan lakterende vleisbeeskoeie oor 'n 100 dae periode, op die reproduksievermoë van hierdie koeie te bestudeer. Die gemiddelde herkalfpersentasie vir die koeie wat onderskeidelik energie-, natuurlike proteïen- en nie proteïen stikstofaanvullings ontvang het en vir die koeie in die Kontrole groep, was 96,0 persent, 85,8 persent, 85,8 persent en 73,1 persent. Die gemiddelde ru-proteïen (RP) inhoud van gesnyde grasmonsters vir die somer-, herfs-, winter- en lenteperiodes was onderskeidelik 5,88 persent, 4,20 persent, 3,80 persent en 5,67 persent. Die Ca en P inhoud van dieselfde monsters het geringe variasie getoon oor die vier seisoene en was gemiddeld 0,17 persent en 0,11 persent. Gebaseer op 'n aanname rakende die gemiddelde inname van 'n lakterende vleisbeeskoei en die chemiese samestelling van die grasbedekking, is dit bereken dat die weiding voorsien in 90 persent, 65 persent, 65 persent en 42 persent van die TVV, RP, Ca en P benodigdhede van die vleisbeeskoei (NRC, 1976).

SUMMARY:

An experiment was conducted to investigate the effects on the reproductive performance of lactating beef cows of supplementation with protein and/or energy during a 100 day period. Over three years the average recalving percentage for the cows receiving energy-rich-, natural protein-, and non protein nitrogen supplements and for the Control cows was 96.0 per cent, 85.8 per cent 85,8 per cent and 73,1 per cent respectively. The average crude protein (CP) content of clipped grass samples for the summer, autumn, winter and spring periods was 5,88 per cent, 4,20 per cent, 3,80 per cent and 5,67 per cent respectively. The Ca and P content showed no great variation over the four seasons and averaged 0,17 per cent and 0,11 per cent respectively. Based on an assumption concerning the average intake of a lactating beef cow and the chemical composition of the grass sward, it was estimated that the veld supplied 90 per cent, 65 per cent, 65 per cent and 42 per cent of the TDN, CP, Ca and P requirements (NRC, 1976) of the lactating beef cow.

The beef industry of Northern Natal is heavily dependent on the natural veld. Acocks (1953) refers to the veld in Northern Natal as Sour Grassveld and Mixed Grassveld and the natural vegetation consists mainly of *Hyparrhenia-Tristachya-Digitaria* spp., while large areas are devoid of trees. The quantity and quality of the vegetation varies mainly due to climatic and soil fertility conditions and is generally poor in both productivity and feed value. The summer veld in Northern Natal is characterized by the following:

(i) an imbalance between crude protein and energy content at the onset of the spring grazing season;

(ii) a drastic decrease in the total protein content of the veld as the grasses reach reproductive maturity;

(iii) a variable availability of plant material due to periodic droughts which in turn makes veld management difficult. The decrease in dry matter intake by the animal during droughts results in lowered animal production, and

(iv) a low calcium and phosphate content of the veld throughout the year.

These fluctuations in the natural vegetation impose limitations on the reproductive performance of the breeding cow and the live mass gain of the growing animal.

Conflicting results have been recorded when lactating beef cows were supplied with supplementary energy. Many studies have shown that when lactating cows are maintained on low levels of energy, irrespective of level of protein, ovarian inactivity results and consequently the interval to the first post partum oestrus is prolonged. (Bond, Wiltbank & Cook, 1958; Wiltbank,

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(*i*) *Plane of nutrition during lactation*

The ewes were randomly allocated to groups which were fed either 100% (High plane) or 50% (Low plane) of the estimated daily nutrient requirements (Morrison, 1956; Thomas & Aitken, 1959) during the 84-day lactation period.

(*ii*) *Association with rams after weaning*

Between parturition and weaning of the lambs vasectomized rams were used twice daily for observations of oestrus amongst all the ewes. After weaning, during 1968, 1969 and 1970, each group of ewes was randomly sub-divided and half the ewes were isolated from rams until one oestrous cycle prior to commencement of the annual breeding period. In the isolated group the observations for oestrus were resumed on 15 October. In the remaining sub-group, and for all the ewes studied during 1971 and 1972 twice daily observations for oestrus continued until 1 November.

After termination of the lactation period all the ewes received 100% of their estimated daily nutrient requirements. Feeding at this level continued until 11 October (1967, 1968 and 1969) and on this date each sub-group was again subdivided so that half the animals could be flushed while the remaining ewes continued on the post-weaning level of feeding. This treatment will be discussed in greater detail in a subsequent paper.

On 1 November all the experimental groups were combined into one flock. The ewes were placed on *Pennisetum clandestinum* pasture with entire breeding rams (3%) bearing raddle crayons (Radford, Watson & Wood, 1960) and marked ewes were noted daily until the end of the six-week mating period.

The body mass of each ewe was recorded three days after lambing and thereafter on every Wednesday.

RESULTS

Changes of body mass

The variations in average body mass of the groups of ewes on the different feeding levels are summarized in Fig. 2. During the six years of the study the average change in body mass between three days post-partum and weaning varied from −3,5 to 10,9% and −11,4 to −19,5% for the ewes on the High and Low planes of feeding, respectively.

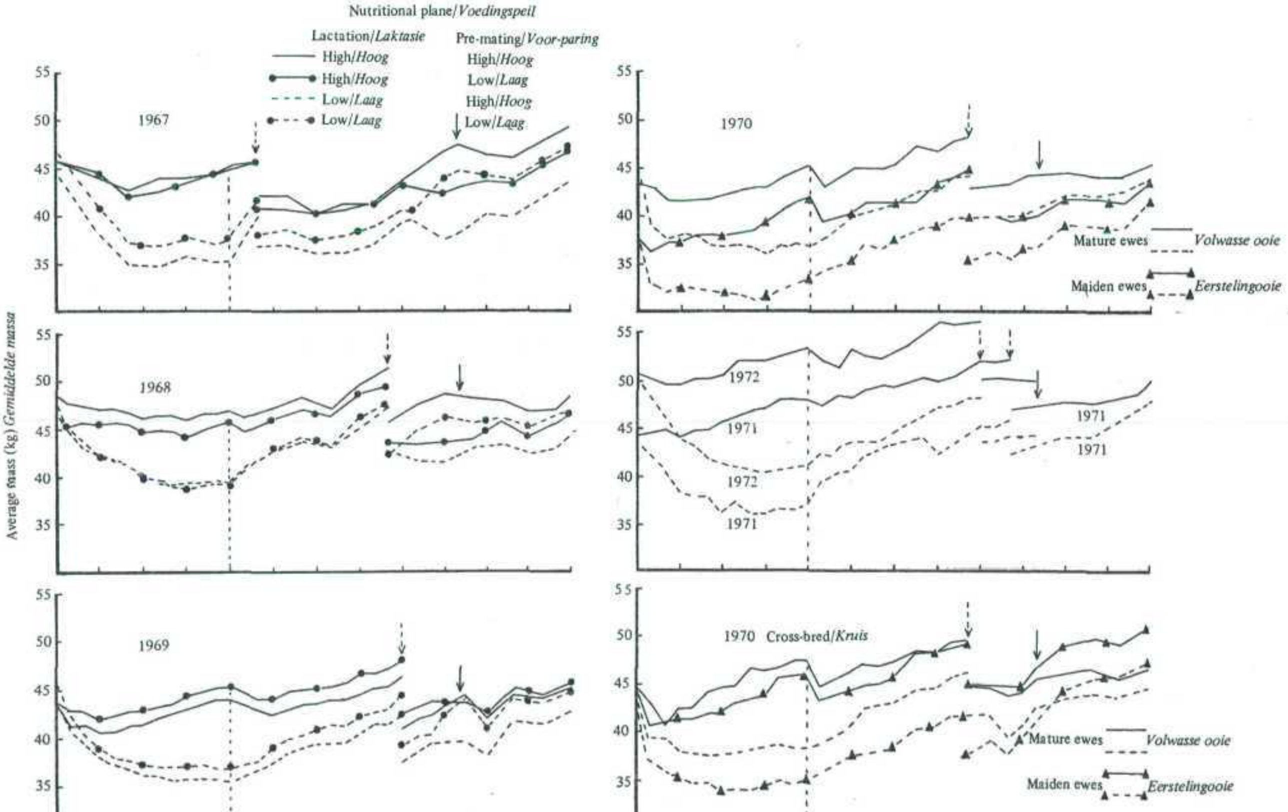

FIG. 2 Changes in body mass of groups of ewes subjected to high and low planes of nutrition during lactation and during a three-week pre-mating period

↓ Denotes joining with entire rams/*Dui aan bybring van ramme*

⇣ Denotes shearing/*Dui aan skeer*

FIG 2 *Veranderinge in liggaamsmassa van groepe ooie onderworpe aan hoë en lae voedingspeile gedurende laktasie en gedurende 'n periode van drie weke voor paring*

Rowden, Ingalls, Gregory & Koch, 1962; Wiltbank, Bond & Warwick, 1965; Dunn, Ingalls, Zimmerman & Wiltbank, 1969; Lamond, 1970). However, when Bellows, Gibson, Thomas & Pahnish (1968) fed a grain supplement to breeding cows during the annual breeding period, they obtained no improvement in the reproductive performance. The explanation offered was that adequate grass was available in that particular year. This was confirmed by the mass changes observed amongst the cows and calves. Similarly, Zimmerman, Clanton & Matsushima (1961) fed Hereford cows different levels of protein and energy and found that 83 per cent of the cows on a high energy-high protein diet conceived to first service compared to only 38 per cent for those on a high energy-low protein diet. This effect of protein on conception rate was confirmed by Wallace & Raleigh (1967).

In view of the lack of information on the early summer supplementation of lactating beef cows in the Northern Natal area, the reproductive performance of such cows supplemented with protein and/or ene during the early summer period was studied.

Procedure

This experiment was replicated during 1974, 19 and 1976 and the number of lactating Africander cr Sussex cows (aged between four and six years) randon allocated to the four treatments was 58 (1974), (1975) and 68 (1976), respectively. The followi treatments were applied:

Group 1 : Control – minerals only.
Group 2 : Minerals plus non protein nitrogen (NP
Group 3 : Minerals plus protein.
Group 4 : Minerals plus energy.

The different treatments commenced towards t end of October of each year and terminated appro mately 100 days later when the mating season ende The composition of the four supplements is shown Table 1.

Table 1

Composition of supplements fed to lactating cows grazing summer veld

		Supplements fed:			
		Minerals only	Minerals + NPN	Minerals + protein	Minerals + energy
Salt	%	33,3	31	25	25
Maize meal	%	–	34	–	60
Bone meal	%	66,7	20	15	15
Urea	%	–	15	10	–
High protein concentrate	%	–	–	50	–
CP	%	2,0	47,1	49,2	6,4
TDN	%	–	27,2	38,0	48,0
Ca	%	19,9	6,0	7,2	4,5
P	%	9,2	2,8	3,0	2,2

The cows in the four treatment groups had access to approximately 180 ha of veld which was divided into 16 camps of equal size. The different groups were rotated once weekly thereby ensuring that each treatment grazed each camp. This procedure was followed to limit possible camp effects.

All the cows were bred annually for 65 days, from approximately December 1 to February 4. The supplements were replenished weekly so as to ensure that the cows had access to adequate supplies. The body mass of the cows was recorded at 14 day intervals.

Only one camp, situated in the centre of the 1 camps used for this experiment, was used for the collec tion of grass samples. At 14 day intervals and over a 1 month period, 20 grab samples were collected at randon by clipping. The samples were then mixed together an subsampled for chemical analysis. Using standard pro cedures, the grass samples were analysed for CP, C and P.

Analysis of variance and least-squares analysis o variance were used to compare treatment means (Snede cor & Cochran, 1967).

Table 3

Average mass of cows, percentage recalving, weaning mass of calves and intercalving period of cows allowed access to four supplements

			Supplement:			
		Control	plus NPN	plus protein	plus energy	Sign
1973/74:						
Start	kg	438,7	462,0	464,8	462,7	NS
End	kg	466,8	497,1	505,9	508,3	2,3,4, > 1
Difference	kg	28,1	35,1	41,1	45,6	
Significance		NS	< 0,05	< 0,05	< 0,05	
Recalving	%	81,3	92,9	92,9	100,0	
Weaning mass calves	kg	188	191	197	198	
Intercalving period	days	381	367	368	375	NS
1974/75:						
Start	kg	474,0	475,9	473,0	475,4	NS
End	kg	487,2	500,1	487,6	479,1	NS
Difference	kg	13,2	24,2	14,6	3,7	
Significance		NS	NS	NS	NS	
Recalving	%	73,3	93,8	93,8	93,8	
Intercalving period	days	359	362	373	364	3 > 1
1975/76:						
Start	kg	408,8	413,1	425,1	424,2	NS
End	kg	427,5	455,1	469,2	465,1	2,3,4 > 1
Difference	kg	18,7	42,0	44,1	40,9	
Significance		NS	< 0,05	< 0,05	< 0,05	
Recalving	%	64,7	70,6	70,6	94,1	
Intercalving period	days	364	372	360	369	2 > 3

Start = Start of experiment
End = End of experiment
Difference = Difference in mass

The data on intercalving periods is deceptive as the cows receiving minerals only, returned similar or better intercalving intervals than the other groups while in fact, they produced the poorest reproductive performance (Table 3). This anomaly is illustrated in the histogram (Fig. 1) where the cumulative percentage of cows calving during ten day periods, commencing at the beginning of the calving season, is presented for the four groups. The figure shows that approximately 92 per cent of the cows receiving protein and energy supplements had calved within 30 days of the onset of the calving season while only 75,7 per cent and 82,5 per cent had calved during the same period in the Control and NPN supplemented groups (Fig. 1).

The CP, Ca and P content of grass samples collected manually from the experimental site over a 12 month period is persented in Table 4. The analyses indicated a pronounced change in CP content of the sward over the 12 months. An arbitrary division of the period into the four seasons, indicated that the CP content varied from 5,9 per cent in summer to 3,8 per cent in winter. The average Ca and P content of the samples showed no great variation over the period studied and the resulting averages were 0,17 per cent and 0,11 per cent for Ca and P, respectively (Table 4).

Discussion

The results of this trial demonstrate the beneficial effects which protein and energy supplementation can have on the lactating cow during a 100 day period *post partum.* The superior reproductive performance of the cows supplemented with energy, agrees with the results of Macfarlane, Somerville, Lowman & Deas (1977), Wiltbank *et al.* 1964) and Speth, Bohman, Melendy & Wade 1962).

Results

The intakes of the protein and energy supplements (supplements 3 and 4) were disappointingly low during the 1973/74 season (Table 2). This can be attributed largely to the supplements being spoiled by rain as uncovered containers were used. In subsequent years the troughs were supplied with covers. If the intakes of supplement for the first year are disregarded, the average intakes would be 201 g, 344 g, 913 g and 797 g per cow per day for Groups 1, 2, 3 and 4, respectively.

Table 2

Average intake of supplement, CP, TDN, Ca and P per cow for the four supplemented groups

		Supplement:		
	Control	Plus NPN	Plus protein	Plus energy
Intake of supplement (g/cow/day)				
1973/74	145	280	414	334
1974/75	172	290	801	692
1975/76	230	398	1024	902
Average intake of supplement●	201	344	913	797
Average feed intake●				
CP	4,0	162,0	449,2	51,0
TDN	–	93,6	346,9	382,6
Ca	40,0	20,6	65,7	35,9
P	18,5	9,6	27,4	17,5

●1973/74 intake of supplements excluded

The cows which received the energy-rich supplement (supplement 4) consistently produced the best reproductive performance followed by those cows receiving NPN and protein (supplements 2 and 3). These two treatments produced results which followed the same pattern during different seasons (Table 3). The average recalving percentages over the three seasons for the cows receiving supplements 1, 2, 3 and 4 were 73,1 per cent, 85,8 per cent, 85,8 per cent and 96,0 per cent, respectively. In the first and third seasons, the cows in Group 1 did not show significant increases in mass from the beginning to the end of the experimental period, whereas the breeding females in the other three groups were significantly heavier ($P < 0,05$) at the end than at the beginning of the experiment. Due to a shortage of Sussex bulls, some groups were mated to Drakensberger bulls in the second and third seasons. Data on the weaning mass of the calves is therefore available for one season only There was no significant treatment effect on the weaning mass of the calves, but a tendency for the calves from the protein and energy-enriched groups (supplements 3 and 4) to wean heavier than those from the Control and NPN supplemented groups (supplements 1 and 2) was evident (Table 3).

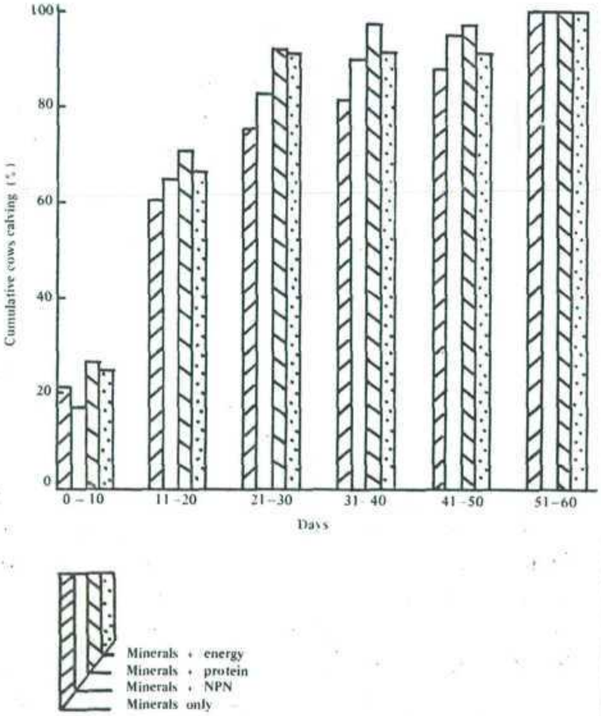

Fig. 1 *Cumulative percentages of cows calving in ten day increments from the beginning of the calving season for the four supplemented groups*

Table 4

Crude protein, calcium and phosphorus content of grass samples collected over 12 months from the experimental site

Month	CP (%)	Ca (%)	P (%)
December	7,30	0,17	0,11
January	5,67	0,19	0,10
February	4,66	0,18	0,15
March	4,48	0,16	0,12
April	4,24	0,20	0,11
May	3,88	0,16	0,09
June	3,35	0,15	0,07
July	4,27	0,17	0,11
August	3,77	0,15	0,09
September	4,66	0,15	0,10
October	5,17	0,16	0,13
November	7,19	0,18	0,11
Summer (December January February)	5,88	0,18	0,12
Autumn (March April May)	4,20	0,17	0,11
Winter (June July August)	3,80	0,16	0,09
Spring (September October November)	5,67	0,16	0,11

In order to assess the results of this trial in terms of satisfying nutrients requirements it was necessary to investigate the capacity of the veld to supply the suggested requirements for the lactating beef cow. The chemical analyses of the grass samples collected during the summer months from the area grazed, revealed that the average CP, Ca and P contents were 5,9 per cent, 0,18 per cent and 0,12 per cent respectively (Table 4). If it is assumed that a 450 kg lactating beef cow consumes approximately 9,5 kg DM per day through grazing during summer (NRC, 1976), such a cow will ingest 560 g CP, 17 g Ca and 11 g P which represents 65 per cent, 65 per cent and 42 per cent of the daily requirements of a lactating beef cow for CP, Ca and P (NRC, 1976). Furthermore, if the TDN content of summer veld is estimated at 48 per cent (Bredon, 1976), the cow will consume 4,5 kg TDN per day, or 90 per cent of the TDN requirements (NRC, 1976). From the above analyses and assumptions it would appear that lactating beef cows on veld similar to that grazed during the experiment will suffer from severe P, moderate to severe CP and Ca, and slight TDN deficiencies during the summer months. The CP deficiency may be merely theoretical as Bredon, Lyle & Swart (1970) suggested that the forage selected by cattle contained a higher percentage of CP and lower percentage of CF than indicated by analysis of clipped samples. They concluded that the difference in CP between grazed and clipped herbage was of the order of 25 per cent. Thus, from a theoretical point of view, it appears that lactating beef cows should be able to satisfy the majority of their nutritional requirements, except P, from veld grazing. However, the performance of the animals in this trial was not in keeping with this hypothesis since the cows receiving TDN or protein supplements consistently exhibited better reproductive performances than those cows receiving only mineral supplementation.

The difference in calving percentage between the Control group and the cows receiving the energy-rich supplement may be attributed to the effect of energy supplementation. The difference between the groups receiving the protein- and energy-rich supplements appear to be due to biological variation as both groups received virtually the same amount of TDN. On the other hand, the cows receiving NPN equaled the production of the cows in the protein supplemented group, but received far less CP and TDN than the cows in the latter group (Table 2). This phenomenon may have been due to the stimulatory effect of NPN supplementation on the activity of the rumen microflora which in turn stimulated a greater grass intake.

The exact reason for the increase in reproductive performance due to supplementation is not clear, but appears to be primarily due to energy supplementation, with protein supplementation being of lesser importance. The limitations of field experiments of this nature are clearly borne out by the difficulty encountered in interpreting the results and further studies should be carried out in order to establish the mechanisms which control the responses observed.

When the economic implications of this experiment were analysed, the results indicated that the cows receiving the energy-rich supplement returned the highest gross margin above cost of supplementation (R66,99 compared with R51,58 for cows receiving the protein-rich supplement). This was due to the cows having attained the highest reproductive rate at a relatively low cost (9,9c per kg) of the supplement per cow. On the other hand, the cows which received the protein supplement produced a favourable reproductive performance, but the high cost (16,8c per kg) of the supplement reduced its profitability. Hence, the latter group produced the poorest gross margin of all four groups. By continuing with the winter lick (NPN) until the end of the breeding season, and with a relatively low cost of supplementation, the gross margin for the cows supplemented with NPN (R62,96) compared favourably with the gross margin for the cows receiving energy. Except for supplementation with a natural protein, it was concluded that energy or NPN supplementation is a profitable practice.

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References

ACOCKS, J.P.H., 1953. Veld types of South Africa. *Mem. Bot. Surv. S. Afr.* 28.

BELLOWS, R.A., GIBSON, R.B., THOMAS, O.O. & PAHNISH, O.F., 1968. Grain supplement – forage production relationships in range cow reproduction. *Proc. Am. Soc. Anim. Sci. west. Sect.* 19, 313.

BOND, J., WILTBANK, J.N. & COOK, A.C., 1958. Cessation of estrus and ovarian activity in a group of beef heifers on extremely low levels of energy and protein. *J. Anim. Sci.* 17, 1211. (Abstr.)

BREDON, R.M., 1976. Guide to balanced feeding of dairy cattle. *Dept. Agr. Tech. Serv.*, Pietermaritzburg 1, 260.

BREDON, R.M., LYLE, A.D. & SWART, C.E., 1970. The use of oesophageal fistulated cattle on summer veld in East Griqualand. *Proc. S. Afr. Soc. Anim. Prod.* 9, 163.

DUNN, T.G., INGALLS, J.E., ZIMMERMAN, D.R. & WILTBANK, J.N., 1969. Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. *J. Anim. Sci.* 29, 719.

LAMOND, D.R., 1970. The influence of undernutrition on reproduction in the cow. *Anim. Breed. Abstr.* 38, 359.

MACFARLANE, J.S., SOMERVILLE, S.H., LOWMAN, B.G. & DEAS, D.W., 1977. Effect of nutrition and other factors on the reproductive performance of beef cows. *Anim. Prod.* 24, 131. (Abstr.)

NATIONAL RESEARCH COUNCIL, 1976. Nutrient requirements of beef cattle. No. 4 National Academy of Sciences, Washington, D.C.

SNEDECOR, G.W. & COCHRAN, W.G., 1967. Statistical methods. Iowa State Un. Press, Ames, Iowa.

SPETH, C.F., BOHMAN, V.R., MELENDY, H. & WADE, M.A., 1962. Effect of dietary supplements to cows on a semi-desert range. *J. Anim. Sci.* 21, 444.

WALLACE, J.D. & RALEIGH, R.J., 1967. Protein intake and exercise for pregnant heifers. *J. Anim. Sci.* 26, 931.

WILTBANK, J.N., BOND, J. & WARWICK, E.J., 1965. Influence of total feed and protein intake on reproductive performance of the beef female through second calving. *Anim. Breed. Abstr.* 34, 2072.

WILTBANK, J.N., ROWDEN, W.W., INGALLS, J.E. & ZIMMERMAN, D.R., 1964. Influence of post partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving *J. Anim. Sci.* 23, 1049.

WILTBANK, J.N., ROWDEN, W.W., INGALLS, J.E., GREGORY, K.E. & KOCH, R.M., 1962. Effect of energy level on reproductive phenonema of mature Hereford cows. *J. Anim. Sci.* 21, 219.

ZIMMERMAN, D.R., CLANTON, D.C. & MATSUSHIMA, J.K., 1961. Post partum reproductive performance in beef heifers as affected by protein and energy intake during gestation. *J. Anim. Sci.* 20, 957.

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THE RELATIONSHIP BETWEEN BODY MASS AND FERTILITY OF BEEF COWS OF DIFFERENT AGES

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(Key words: *Relationship, body mass, fertility, age)*
(Sleutelwoorde: *Verwantskap, liggaamsmassa, vrugbaarheid, ouderdom)*

OPSOMMING: DIE VERWANTSKAP TUSSEN LIGGAAMSMASSA EN VRUGBAARHEID BY VLEISBEESKOEIE VAN VERSKILLENDE OUDERDOMME

Liggaamsmassa — en reproduksiedata van 920 Sussex-tipe koeie, wat gewissel het in ouderdom van 1 jaar oud tot volwasse koeie (5 jaar en ouer), is ontleed vir verwantskappe tussen liggaamsmassa (X) en herkonsepsie (Y). Die gegewens is oor 'n 6-jaar periode ingesamel en het die volgende ingesluit: Liggaamsmassa aan die begin en aan die einde van die teelseisoen, persentasie massatoename oor die teelseisoen, liggaamsmassa aan die begin en aan die einde van die winter en persentasie massatoename of verlies oor die winterperiode. Betekenisvolle verwantskappe tussen liggaamsmassa aan die begin van die teelseisoen en herkonsepsie is by alle ouderdomsgroepe gevind, met die uitsondering van 2-jaar-oud diere wat vir die eerste keer gepaar is. Geen verwantskap is waargeneem tussen konsepsie en gemiddelde liggaamsmassa aan die begin van die winter, einde van die winter, persentasie massatoename of verlies gedurende die winter en persentasie massatoename oor die teelseisoen nie.

SUMMARY:

Records on body mass and reproductive performance of 920 Sussex type cows, ranging in age from yearlings to adults (5 years and older) were examined for relationships between body mass (X) and conception (Y). The data was obtained over a 6-year period and included: body mass at the start and at the end of the breeding season, percentage gain in mass over the mating season, body mass at the start and at the end of the winter and percentage gain or loss over the winter. Significant relationships were recorded for body mass at the start of the mating season and conception for nearly all the age groups with the exception of the 2-year-old animals mated for the first time. No relationship could be established for conception rate and mean body mass at the start of the winter, end of the winter, percentage gain or loss over the winter and percentage gain over the breeding season.

A considerable amount of research has been devoted to the effects of undernutrition on the reproductive performance of beef cows. Review articles by Lamond (1970), McClure (1970), Terblanche (1974) and Topps (1977) and additional information edited by Cunha, Warnick & Koger (1967) and Preston & Willis (1974) have dealt with this topic rather extensively. Throughout these reports there has been the inference that adequate nutrition, both *pre* and *post partum*, is of vital importance in order to sustain good reproductive performance in beef females.

Preston & Willis (1974) maintain that a rising plane of nutrition during the breeding season is the most important requirement to secure high calf crops. This contention is supported by McClure (1965), who supplemented cows with hay from calving until three weeks after service and recorded a 62% conception rate to first service, compared with 13% for unsupplemented animals. The data of Wiltbank, Rowden, Ingalls & Zimmerman (1964) and Dunn, Wiltbank, Zimmerman & Ingalls (1964) provide further evidence to support the belief that breeding cows should be improving in condition during the mating period.

Parker, Waldrip & Marion (1966) did not observe an effect of nutrition on calving performance and concluded that the failure to demonstrate a response was due to the overall fertility of the cows being very high. Meaker (1974) recorded a conception rate of only 25% among Africander cows that had gained 10 kg in body mass from 14 days *post partum* until the end of the mating season, whereas the conception rate was 87,5% when cows had lost 54 kg in body mass over the same period. The results achieved by Meaker (1974) are therefore at variance with the belief that a breeding animal must be gaining in mass in order to conceive. The results also suggested that body mass

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at the end of the breeding season influenced the rate of conception. It was found that as this body mass decreased from 435 kg to 378 kg, reconception decreased linearly from 87,5% to 25% (Meaker, 1974).

This relationship supports the contention that a critical or target mass must be attained before conception is likely to occur (Lamond, 1970). In more practical terms it means that the reproductive ability of cows will probably decline concurrently with a decrease in body mass below the target mass. Conversely, as body mass increases above the target mass, cows will tend to become subfertile due to excessive deposition of fat. Richardson, Oliver & Clarke (1975) examined the influence of body mass at the beginning and end of the mating season on conception rates. They concluded that the greater the loss in body mass over this period, the lower the subsequent calving rate. The hypothesis, as formulated by Lamond (1970), is supported by the findings of Ward (1968), Trail, Sacker & Fisher (1971), Meaker (1975), Steenkamp, van der Horst & Andrew (1975) and Buck, Light, Rutherford, Miller, Rennie, Pratchett, Capper & Trail (1976).

According to Broster (1973), a positive relationship between live mass and fertility in the cow has not been consistently demonstrated. Munro (1970, unpublished) analysed a large number of data and failed to produce a conclusive result regarding the relationship between fertility and live mass change for cattle (Broster, 1973). Similarly, Whitman, Remmenga & Wiltbank (1975) found that when they fed Aberdeen Angus and Hereford cows, aged 2-11 years, various levels of energy, before and after calving, the cows gaining in mass before calving had a greater likelihood of oestrus 50 days *post partum* than those which lost mass before calving. However, neither body mass change before breeding nor body condition at first breeding affected the incidence of pregnancy. Although numerous reports have demonstrated a positive relationship between body mass and fertility, much controversy still exists regarding the target mass concept. For instance, what is the target mass for a specific breed and age of cow? The objective of this study was therefore to determine whether body mass at the start and/or at the end of the mating season were related to conception in Sussex type cows.

Procedure

Records on the reproductive performances of 920 Sussex type cows, ranging in age from yearlings to adults (5 years and older), were examined. The data was obtained over a 6-year period. The animals were maintained on the Dundee Research Station where controlled summer mating seasons were used viz. 45 days for heifers and 65 days for all other females. Only Sussex bulls were used.

Body mass at the start and at the end of the breeding season was recorded for all the animals. Percentage gain in mass over the mating season, body mass at the start and end of the winter and percentage gain or loss over the winter were also recorded for cows 5 years and older.

In order to examine the relationships between body mass and fertility, the animals were divided into the following age groups (numbers in brackets refer to number of animals used in the calculations):

Age group:

1	Heifers 1-year-old and mated for the first time	(132)
2	Heifers 2-years-old and mated for the first time	(87)
3	Cows 2-years-old and mated for the second time	(47)
4	Cows 3-years-old and mated for the second time	(38)
5	Cows 3-years-old and mated for the third time	(36)
6	Cows 4-years-old, irrespective of the number of calves produced	(98)
7	Cows 5-years and older, irrespective of the number of calves produced	(482)

Within each age group, the cows were divided into equally spaced categories of body mass (X) and the percentage conception (Y) was calculated for each category. In all cases regression analyses were carried out using weighted means for Y. When animal numbers were limited (e.g. Groups 4 and 5), only a few categories of body mass were possible and only linear regressions could be fitted to the data. On the other hand, when numbers were not limiting (e.g. Groups 6 and 7), the animals were divided into a greater number of body mass categories and quadratic functions were fitted by least squares analysis.

Discussion of results

Yearlings

On average, when the mean body mass of the heifers at the start of the breeding season was 212 kg, 43% of them conceived whereas those with a mass of 337 kg exhibited a conception rate of 78% ($P < 0,10$). Thus, the conception rate rose at the rate of 3,0% for every 10 kg increase in body mass. However, when the mean body mass of the heifers exceeded 278 kg, the percentage conception tended to decrease, perhaps due to the heifers becoming too fat. Arnett, Holland & Totusek (1971) recorded a decrease in conception rate when cows became overfat. From this relationship it appears that the optimum mass at mating i.e. the target

Table 1

Relationship between body mass (X) at the start and at the end of the mating season and reconception (Y) for Sussex type cows of different ages

Age and number of times mated	n	Relationship at start/end of mating season	r	Sign
1 year — mated 1st time	132	Start $Y = -16{,}29 + 0{,}28X$	0,6930	$P<0{,}10$
		End $Y = -354{,}56 + 2{,}61X - 0{,}004X^2$	0,9652	$P<0{,}05$
2 years — mated 1st time	87	Start no significant relationship		
		End no significant relationship		
2 years — mated 2nd time	47	Start $Y = -51{,}97 + 0{,}37X$	0,8892	$P<0{,}05$
		End no significant relationship		
3 years — mated 2nd time	38	Start $Y = 30{,}86 + 0{,}16X$	0,8722	$P<0{,}05$
		End no significant relationship		
3 years — mated 3rd time	36	Start $Y = 42{,}55 + 0{,}12X$	0,9912	$P<0{,}01$
		End no significant relationship		
4 years — irrespective of matings	98	Start $Y = -1\,060{,}98 + 5{,}05X - 0{,}006X^2$	0,8992	$P < 0{,}05$
		End $Y = -1\,103{,}04 + 5{,}07X - 0{,}005X^2$	0,8912	$P < 0{,}05$
Adult cows — irrespective of matings	482	Start $Y = -415{,}58 + 1{,}94X - 0{,}002X^2$	0,9483	$P < 0{,}01$
		End $Y = -630{,}77 + 2{,}70X - 0{,}003X^2$	0,9838	$P < 0{,}01$

mass for yearling heifers, of Sussex type, is approximately 280 kg. In confirmation of this tendency, percentage conception and mean body mass of yearling heifers at the end of the mating season were significantly ($P < 0{,}05$) correlated (Table 1). Similar relationships have been recorded by Ellis (1974), Carter & Cox (1973), Young (1974) and Axelsen & Morley (1976).

Two-year-old

No significant relationship was observed between percentage conception and mean body mass at the start or at the end of the breeding season of 2-year-old heifers mated for the first time. Percentage conception did not vary greatly (85,7% — 100%) when the mean body mass of the heifers varied between 285 kg and 385 kg at the start of the mating season. These results tend to substantiate the findings of Broster (1973), with the exception that the present findings refer to a specific age group, while Broster studied dairy cows that received moderate to generous planes of nutrition. Harwin, Lamb & Bisschop (1967) found that mass at mating affected the reproductive rate of 2-year-old Africander heifers. They recorded a calving rate of 100% when the mass at mating exceeded 318 kg and only 60% when the mass was 250 kg and less. It can be concluded therefore that the mean body mass of the 2-year-old heifers used in this study was well above the minimum joining mass required for maximum conception. Although no definite joining mass for 2-year-old heifers mated for the first time was established, it would appear that the optimum for Sussex type beef heifers ranges between 320 kg and 340 kg.

A significant ($P < 0{,}05$) linear relationship was noted between conception and mean body mass at the start of the mating season of 2-year-old cows mated for the second time (Table 1). Although no decrease in conception was recorded above the maximum at 385 kg, it seemed pointless to feed these animals so as to exceed this mass as no extra benefit in conception could be expected. From the relationship demonstrated, it appears that approximately 390 kg would be the target mass for maximum conception to occur. No significant relationship was recorded at the end of the mating season, but it was evident that a tendency existed for conception to occur more readily as the body mass increased.

Three-year-old

Although animal numbers were limited for the 3-year-old cows mated for the second time, a significant ($P < 0{,}05$) linear relationship was recorded between conception and mean body mass at the start of the mating season. No relationship was noted for the same animals at the end of the mating season (Table 1). The results indicated that high conception rates were attained irrespective of whether the mean body mass at the start of the mating season was 350 kg (83%) or 450 kg (100%). From the results it would appear that the optimum mass at mating was approximately 410 kg.

The results of only 36 cows that were 3-years-old and had been mated for the third time were available. A significant ($P<0,05$) relationship was obtained between conception and mean body mass at the start of the breeding season (Table 1). It is obvious from previous discussions that with the greater animal numbers available these relationships tended to exhibit curvilinear responses. It can therefore be expected that, had there been more animals the same curvilinear relationship would apply for the group of 3-year-olds. However, the relationship recorded was of the order of only a 1,4% increase in conception for every 10 kg increase in body mass at the start of the mating season. Furthermore, according to the relationship it appeared that maximum conception occurred when the mean body mass at mating was approximately 440 kg.

Four-year-old

Significant ($P < 0,05$) quadratic relationships were recorded between conception and mean body mass at the start and at the end of the mating season of 4-year-old cows (Table 1). Both relationships followed the same trend, except that the body mass of the cows at the end of the mating season was approximately 11 kg greater than at the start. A marked decrease in conception rate, possibly due to overfatness at the onset of breeding, was observed with the 4-year-olds. When the mean body mass of the cows increased from 460 kg (where maximum conception was recorded) to 510 kg at the start of the mating season, the percentage conception decreased from 93% to 78%.

Adult cows

In accordance with the results obtained for the 4-year-old cows, highly significant ($P < 0,01$) relationships were recorded between weighted percentage conception and mean body mass at the start and at the end of the mating season of adult cows (Fig. 1). From the graphs it is evident that the optimum mass at mating, to achieve maximum conception, was approximately 510 kg. Body masses above this level resulted in no extra gain in conception rate and there was a tendency for conception to decrease with increasing body mass above the target mass. It was also evident that a gain of say 40 kg in body mass favoured conception rate more in thin cows than that it did in well fleshed cows. For instance, there was an increase of 28% and 12% in conception rates when the body mass of cows at the start of the breeding season increased from 320 kg to 360 kg and from 420 kg to 460 kg, respectively (Fig. 1).

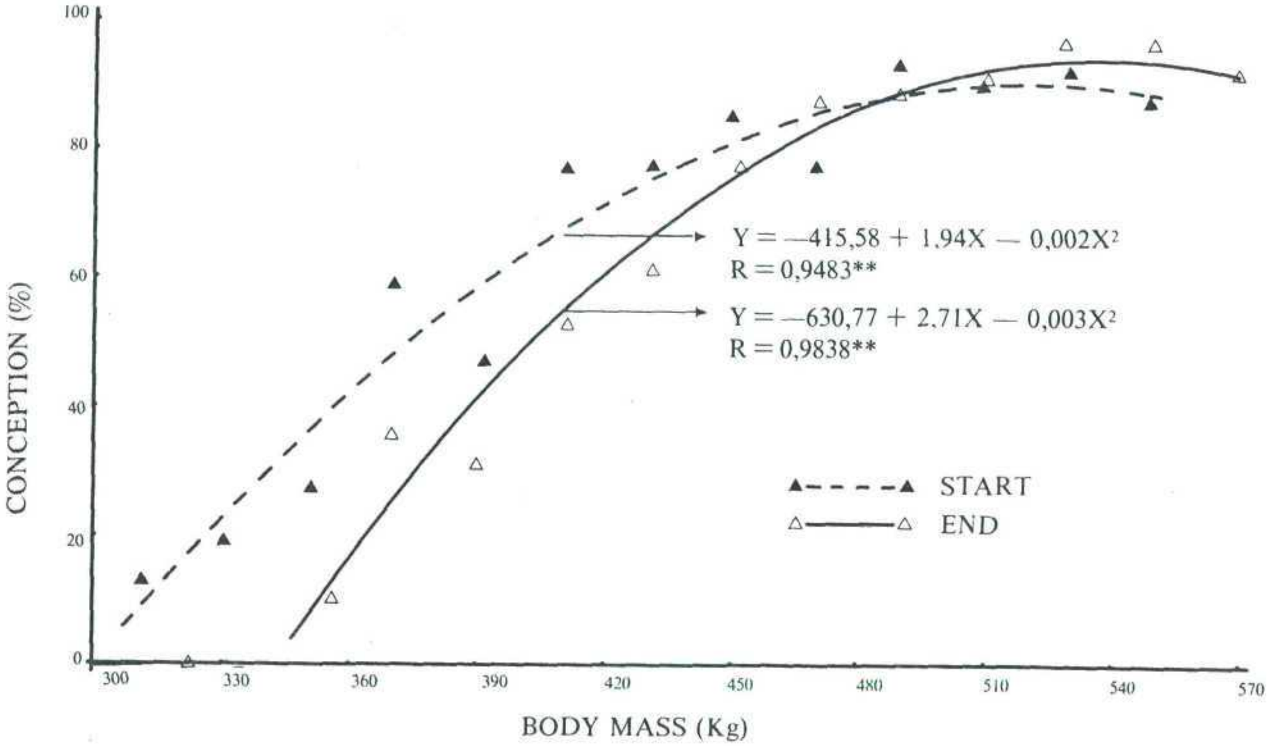

Fig. 1 *Relationship between conception and mean body mass at the start and at the end of the mating season of adult cows*

The calculated target masses to obtain maximum conception for the different aged animals (Table 2, Fig. 1) were used to establish a relationship between target mass and age of the cow (Fig. 2). This relationship (for cows calving for the first time at 2 years of age) was curvilinear and significant ($P < 0{,}05$). By using this regression (Fig. 2), the stockman would be able to determine what the target mass for any age of British beef type (Sussex) animals should be to ensure maximum conception. Obviously, the accuracy with which the target mass could be determined depends on the "correctness" of the calculated target masses for each age group.

Table 2

Suggested target masses for British beef type cows at joining to ensure maximum conception rates

Age group	Age and number of times mated	Suggested target mass
1	1 year — mated 1st time	280 kg
2	2 years — mated 1st time	330 kg
3	2 years — mated 2nd time	390 kg
4	3 years — mated 2nd time	410 kg
5	3 years — mated 3rd time	440 kg
6	4 years — irrespective of matings	460 kg
7	Adult — irrespective of matings	510 kg

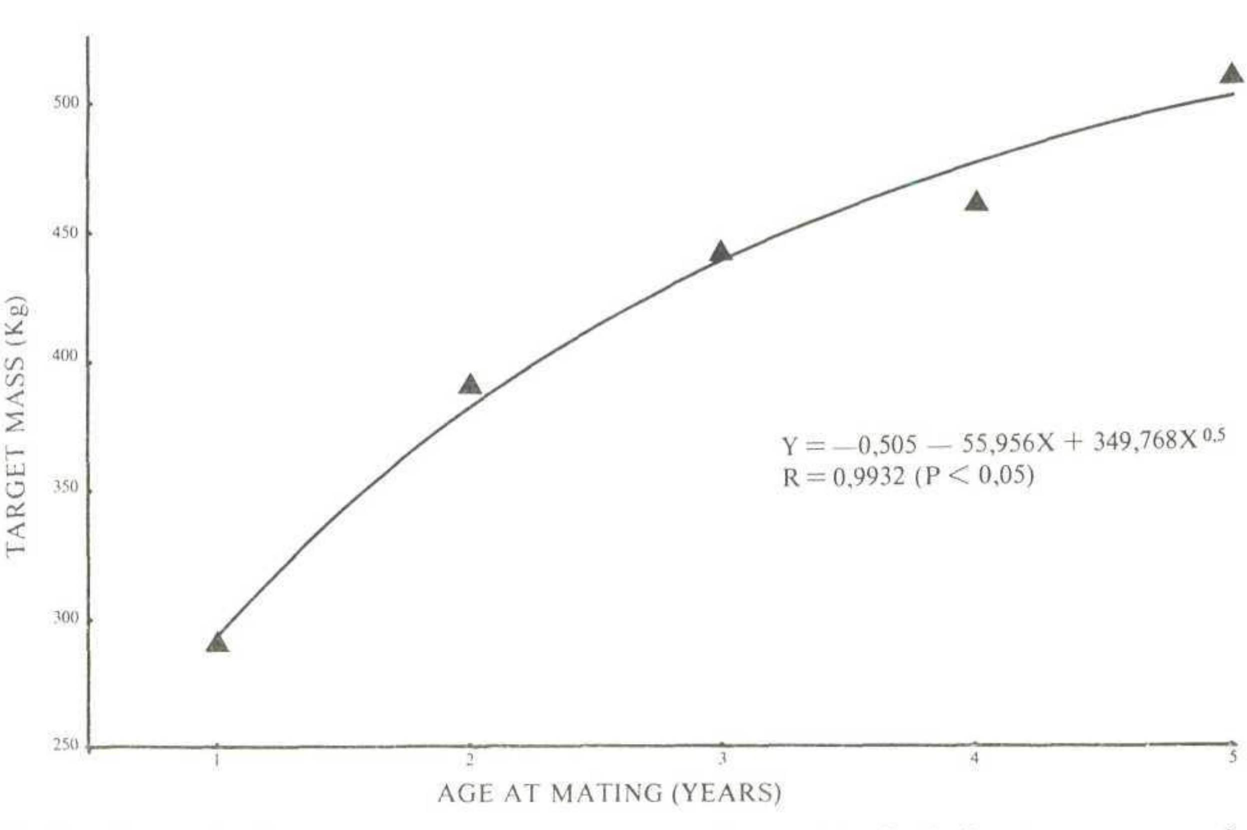

Fig. 2 Relationship between target mass and age at mating of cows calving for the first time at two years of age

No relationship could be determined between conception rate and mean body mass at the start of the winter, end of the winter, percentage gains or loss over the winter and percentage gain over the breeding season. The latter results support the findings of Richardson *et al.* (1975) and Steenkamp *et al.* (1975) who concluded that the ability to conceive is a function of body mass *per se* and not of gain during the post-partum period.

Conclusions

Lamond (1970) maintained that general application of the "target mass" concept was limited by the age and the breed of the beef female. The results of the present study have not only suggested a target mass for Sussex type cattle, but have also permitted the effect of age to be quantitated (Fig. 2). It is thus possible to recommend a target mass at joining for all age categories. There can be little doubt that the mass required to support maximum conception will vary between Zebu, dual-purpose and British beef types of beef cattle. However, the principle suggested by Lamond (1970) is likely to apply to most types. It is important that limits be set for the most common breeds and crosses encountered, since the stockman will then have a predetermined objective goal when selecting and applying feeding regimes. He will then no longer be solely dependant on subjective recommendations viz., that cows must be "well fed" after calving.

Should it become possible to establish a curvilinear relationship for all age-categories, then application of such guides can be expected to have a dramatic impact on the calving rate of the National herd. The producer would know well in advance of the breeding period what the target mass for each beef age group should be in order to maximize the probability of conception occurring.

Generally, at any given time, there is a relatively wide variation in body mass between individuals in a herd, even amongst cows fed similarly and being of similar age and productive status. It is thus necessary, to emphasize that although the target mass may be an absolute figure, because of individual variation the guide must be applied with discretion.

From the data presented it is clear that cows are able to conceive even when their body mass exceeds or is below the indicated target. Consequently, if the body mass of each cow is measured prior to the introduction of breeding bulls, then reference to appropriate figures (e.g. Fig. 1) will indicate just what the chances of conception for each individual are likely to be.

Another important aspect which emerged from the present results was the failure to show relationships between conception rate and body mass for all the age groups under discussion, viz, 2-year-old cows mated for the first time. These results are not unique since Broster (1975) also failed to show relationships between body mass change and fertility for lactating dairy heifers. Broster (1975) suggested that the cows may have been operating within an optimum range. In more simplistic terms, the heifers used in the trials reported here may have been fed on too high a plane of nutrition. This could have resulted in the mean body mass of the heifers being well above the suggested target mass, but not so high that it led to obesity and impaired fertility. The necessity for establishing a target mass for the 2-year-old heifers, mated for the first time, is clearly illustrated. Unnecessarily high planes of nutrition for this group, not only resulted in a wastage of expensive conserved winter feed, but also led to no improvement in the conception rate.

References

ARNETT, D.W., HOLLAND, G.L. & TOTUSEK, R., 1971. Some effects of obesity in beef females. *J. Anim. Sci.*, 33, 1129.

AXELSEN, A. & MORLEY, F.H.W., 1976. The effect of plane of nutrition on reproductive performance of early weaned heifers. *Proc. Aust. Soc. Anim. Prod.*, 11, 241.

BROSTER, W.H., 1975. Personal communication.

BROSTER, W.H., 1973. Liveweight change and fertility in the lactating dairy cow : A review. *Vet. Rec.*, 93, 417.

BUCK, N.G., LIGHT, D., RUTHERFORD, A., MILLER, M., RENNIE, T.W., PRATCHETT, D., CAPPER, B.S. & TRAIL, J.C.M., 1976. Environmental factors affecting beef cow reproductive performance in Botswana. *Anim. Prod.*, 23, 357.

CARTER, A.H. & COX, E.H., 1973. Observations on yearling mating of beef cattle. *Proc. N.Z. Soc. Anim. Prod.*, 33, 94.

CUNHA, T.J., WARNICK, A.C. & KOGER, M., 1967. Factors affecting calf crop. University of Florida Press, Gainesville.

DUNN, T.G., WILTBANK, J.N., ZIMMERMAN, D.R. & INGALLS, J.E., 1964. Energy level and reproduction in beef females. *J. Anim. Sci.*, 23, 594.

ELLIS, R.W., 1974. The relationships between percentage calving and weight at joining in yearling Hereford heifers. *Anim. Breed. Abstr.*, 42, 492.

HARWIN, G.O., LAMB, R.D. & BISSHOP, J.H.R., 1967. Some factors affecting reproductive performance in beef females. *Proc. S. Afr. Soc. Anim. Prod.*, 6, 171.

LAMOND, D.R., 1970. The influence of undernutrition on reproduction in the cow. *Anim. Breed. Abstr.*, 38, 359.

McCLURE, T.J., 1970. A review of developments in nutrition as it is related to fertility in cattle: 1964-69. *N.Z. Vet. J.*, 18, 61.

McCLURE, T.J., 1965. A nutritional cause of low non-return rates in dairy herds. *Aust. Vet. J.*, 41, 119.

MEAKER, H.J., 1975. Relationship between body mass and conception in beef cows. *S. Afr. J. Anim. Sci.*, 5, 45.

MEAKER, H.J., 1974. Maize silage and/or *Eragrostis curvula* hay for wintering pregnant beef cows. *S. Afr. J. Anim. Sci.*, 4, 175.

PARKER, E.E., WALDRIP, W.J. & MARION, P.T., 1966. Effects of grazing rates and levels of winter supplement on cow-calf performance. *J. Anim. Sci.*, 25, 599.

PRESTON, T.R. & WILLIS, M.B., 1974. Intensive beef production. Pergamon Press, New York.

RICHARDSON, F.D., OLIVER, J. & CLARKE, G.P.Y., 1975. Analyses of some factors which affect the productivity of beef cows and of their calves in a marginal rainfall area of Rhodesia. *Anim. Prod.*, 21, 41.

STEENKAMP, J.D.G., VAN DER HORST, C. & ANDREW, M.J.A., 1975. Reconception in grade and pedigree Africander cows of different sizes — post-partum factors influencing reconception. *S. Afr. J. Anim. Sci.*, 5, 103.

TERBLANCHE, H.M., 1974. Die invloed van voeding op die vrugbaarheid en voortplantingsvermoë van die koei. *J. S. Afr. vet. Ass.*, 45, 65.

TOPPS, J.H., 1977. The relationship between reproduction and under-nutrition in beef cattle. *Wld. Rev. Anim. Prod.*, 13, 43.

TRAIL, J.C.M., SACKER, G.D. & FISHER, I.L., 1971. Crossbreeding beef cattle in Western Uganda. *Anim. Prod.*, 13, 127.

WARD, H.K., 1968. Supplementation of beef cows grazing on veld. *Rhod. J. agric. Res.*, 6, 93.

WHITMAN, R.W., REMMENGA, E.E. & WILTBANK, J.N., 1975. Weight change, condition and beef-cow reproduction. *J. Anim. Sci.*, 41, 387. (Abst.).

WILTBANK, J.N., ROWDEN, W.W., INGALLS, J.E. & ZIMMERMAN, D.R., 1964. Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. *J. Anim. Sci.*, 23, 1049.

YOUNG, J.S., 1974. Reproduction and nutrition in the beef herd. *Proc. Austr. Soc. Anim. Prod.*, 10, 45.

PAPER 22

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The influence of loss and gain of body mass on ovarian activity in beef cows

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Ovarian activity was studied in 36 dry, *Bos taurus* cows fed to achieve different rates of body mass loss and gain in a 2 × 2 factorial experiment. Cows were fed hay to supply either 70% (Treatments 1, 2) or 40% (Treatments 3, 4) of their ME requirements for maintenance until they became anoestrus. Following a 90-day period during which body mass was maintained the animals were fed hay *ad lib.* and either 1 kg (Treatments 1, 3) or 4 kg (Treatments 2, 4) of maize per cow per day until they resumed luteal activity. During the initial 14 days after maize feeding commenced each cow was weighed every second day in order to obtain an estimate of gutfill. Oestrous activity was monitored twice daily by observing marker bulls joined with cows. Ovarian activity was assessed each week throughout the trial by palpation of the ovaries per rectum. Ovarian activity did not cease in four cows, despite a loss of about 31% of initial mass. One cow died during the course of the experiment. Thirty-one cows lost approximately 100 kg or 21% of their initial mass, and 1,1 condition score points over a period of about 5 months before they became anoestrous. Treatment did not influence mass and condition losses and gains and the time taken for cows to cease and resume ovarian activity. Gutfill was estimated at 25,9 ± 10,5 kg. Cows were between 35 and 57 kg heavier ($P < 0,01$) at the resumption than at the cessation of ovarian activity. The estimate of gutfill in each cow was subtracted from the actual mass at resumption of ovarian cycles. At the resumption of ovarian activity the mean adjusted mass (389,9 ± 44,9 kg) was significantly ($P < 0,01$) greater than that at the cessation of ovulatory cycles (368,6 ± 39,1 kg). The mean condition score at resumption of ovulation (2,35 ± 0,34) was also significantly ($P < 0,01$) higher than that at cessation of ovarian activity (1,72 ± 0,37). Treatment did not influence conception rates in cows. Over all treatments 85% of the cows conceived to service at the resumption of ovulatory activity.

In 'n 2 × 2-faktoriale proef is ovariale aktiwiteit ondersoek in 36 droë *Bos taurus*-koeie wat gevoer is om verskillende tempo's van massaverlies en massatoename te ondergaan. Koeie is hooi gevoer om 70% (Behandelings 1, 2) of 40% (Behandelings 3, 4) van hulle ME-behoeftes vir massa-onderhoud te voorsien totdat anestrus ingetree het. Na 'n periode van 90 dae waartydens massa konstant gehou is, is die koeie *ad lib.* hooi, en óf 1 kg (Behandelings 1, 3), óf 4 kg (Behandelings 2, 4) mieliemeel per koei per dag gevoer totdat eierstokaktiwiteit weer hervat is. Gedurende die eerste 14 dae van die periode waartydens mieliemeel gevoer is, is alle koeie elke tweede dag geweeg om 'n aanduiding van ingewandevul te kry. Eierstokaktiwiteit is vir die duur van die proef gemonitor deur daaglikse estruswaarnemings met behulp van merkerbulle, en weeklikse rektale ondersoeke van die eierstokke. In vier koeie het eierstokaktiwiteit nie gestaak nie, ten spyte van massaverliese van ongeveer 31% van aanvanklike massa. Een koei het gevrek gedurende die proef. Een-en-dertig koeie het ongeveer 100 kg of 21% van hul aanvanklike massa, en 1,1 van 'n kondisietelling verloor oor 'n periode van ongeveer 5 maande voordat anestrus ingetree het. Behandeling het nie massa- en kondisieveranderings en die tydsduur tot die staking en hervatting van eierstokaktiwiteit beïnvloed nie. Ingewandevul is op 25,9 ± 10,5 kg beraam. Koeie was tussen 35 en 57 kg swaarder ($P < 0,01$) tydens die hervatting as met die staking van eierstokaktiwiteit. Die beraming van ingewandevul is afgetrek van die werklike massa tydens die hervatting van eierstokaktiwiteit. Die gemiddelde gekorrigeerde massa by die hervatting van eierstokaktiwiteit (389,9 ± 44,9 kg) was betekenisvol ($P < 0,01$) groter as die met die intrede van anestrus (368,6 ± 39,1 kg). Die gemiddelde kondisiestelling by die hervatting van ovariale siklusse (2,35 ± 0,34) was ook betekenisvol ($P < 0,01$) hoër as toe anestrus ingetree het (1,72 ± 0,37). Behandeling het nie konsepsiesyfers in koeie beïnvloed nie. Oor al die behandelings het 85% van die koeie beset geraak met die hervatting van ovulasie.

Keywords: Beef cows, ovarian activity, body mass, anoestrus

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troduction

ithout adequate winter dietary supplementation beef ws can loose 10% or more of their autumn mass in the urveld areas of South Africa (Reyneke, 1971). Such sses in mass result in cows failing to achieve their rget mass' at the start of the mating season, and nsequently they are likely to achieve poor conception tes (Meaker, Coetsee, Smith & Lishman, 1980). Hale 975) proposed two strategies whereby cows can be fed achieve their 'target mass' at mating. Cows could be allowed to lose mass during winter, after which they are fed to regain this loss (during summer) before the bulling season. Alternatively, it may be economically and physiologically more efficient to maintain cows in good condition throughout the year. Results obtained by Hale (1975) favour the latter strategy. He found that following severe mass losses resulting in anoestrus, cows had to regain more mass than they had lost, before they resumed ovarian activity. Thus, 'interest was paid' on the mass lost before cows became anoestrus. Hale (1975) did not obtain an estimate of gutfill in his cows. While gutfill can account for 12 – 23% of live mass in well-nourished animals (Tayler, 1954; Hughes, 1976), the contribution to live mass is likely to be relatively small in animals subjected to severe feed restriction. Therefore, in the experiment conducted by Hale (1975) part of the observed increase in body mass between cessation and resumption of sexual activity may have been due to gutfill. The present study was initiated to determine changes in mass and condition required for cows to cease and then resume ovarian activity and to annul the contribution of gutfill to these changes. Furthermore, body condition changes and the rate of change in body mass was studied.

Procedure

During August 1983, prior to the start of the experiment, 36 mature, non-pregnant, beef cows (32 Simmentaler × Hereford and four Sussex) were fed maize silage and *Eragrostis curvula* hay until their condition score was approximately 3 (1 = thin; 5 = fat; Van Niekerk & Louw, 1982). During the pre-experimental period, cows were run continuously with vasectomized bulls fitted with chinball markers in order to allow detection of oestrus. All cows were found to be cycling normally. The experiment was conducted in feeding pens and comprised three phases: Phase 1, the induction of severe mass losses until cows became anoestrus; Phase 2, maintenance of body mass for a 90-day period; Phase 3, rapid mass gain until ovulatory cycles were re-established. The cows were allocated to one of four treatments in a 2 × 2 factorial design. During Phase 1 cows were fed hay to supply either approximately 70% (Treatments 1, 2) or 40% (Treatments 3, 4) of their metabolizable energy (ME) requirements for maintenance. Cows in Treatments 1 and 2 were all fed together during Phases 1 and 2, as were the cows allocated to Treatments 3 and 4. During Phase 3, two rates of body mass gain were imposed by feeding cows hay *ad lib.* and either 1 kg (Treatments 1, 3) or 4 kg of maize per cow per day (Treatments 2, 4). The quantities of roughage and grain fed during the three phases are summarized in Table 1. The initiation of Phases 2 and 3 did not occur simultaneously in all animals, but was staggered on an individual cow basis, according to the date on which each cow ceased ovulatory activity.

Table 1 Daily quantities of *Eragrostis curvula* hay and maize fed per cow during the three phases of the trial

Treatment	*n*	Phase 1 (Mass loss)	 2 (Maintenance)	 3 (Mass gains)
1	9	4,6 kg hay	Quantity of hay varied to result in mass maintenance	Hay *ad lib.* plus 1 kg maize
2	10	4,6 kg hay		Hay *ad lib.* plus 4 kg maize
3	10	2,9 kg hay		Hay *ad lib.* plus 1 kg maize
4	7	2,9 kg hay		Hay *ad lib.* plus 4 kg maize
Total	36			

During Phases 1 and 2 the cows were joined with vasectomized bulls fitted with chinball markers, and during Phase 3 they were run with fertile bulls. Each week for the duration of the trial all cows were rectally palpated for the presence of *corpora lutea*. Ovarian examination revealed that the mating of cows by teasers and/or fertile bulls was not an accurate indicator of ovulation. Thus, some cows were mounted by bulls midway through the 3-month period during which ovaries were quiescent. Consequently, the date on which cyclic activity ceased (end of Phase 1) was taken as being the last day on which a *corpus luteum* was detected, when followed by a 12-week period of ovarian quiesence. Similarly, the date on which a *corpus luteum* was first palpated during Phase 3 was regarded as that on which cyclic activity resumed. Both the calving dates and the dates on which ovulatory cycles were observed to have resumed were used to estimate the dates of conception.

Cows were weighed weekly, and their condition scored at fortnightly intervals during the trial. Water and feed were not withheld prior to weighing. An attempt was made to estimate gutfill in cows fed the *ad lib.* ration during Phase 3. For this purpose cows were weighed at 2-day intervals for the initial 14 days of Phase 3. Again, water and feed were not withheld prior to weighing.

Analysis of variance was used to test the influence of treatment on changes in mass and condition in cows. The Student's *t* test on paired observations was used to test for differences between masses and condition scores measured at different stages of the experiment. The influence of treatment on conception rate was examined by the chi-square test.

Results

One of the 36 cows died during the course of the experiment. This cow ceased ovulatory cycles 104 days after the start of the trial, having lost 98,6 kg (22%) of her initial mass (448,6 kg). Despite being given free access to feed this cow continued to lose body mass and died having lost an additional 44 kg over a 73-day period. At death her condition score was 1,0. Four of the 36 cows (11,1%) did not cease ovarian activity within 239 days of the start of the trial, despite drastic mass and condition losses. These cows lost 152,2 ± 14,9 kg (31,3 ± 5,1%) of their initial mass and their condition score declined to 1,1 ± 0,3, having been 2,94 ± 0,1 at the start of underfeeding. These animals were removed from the trial to avoid further deaths. Thirty-one cows completed the trial and only data of these animals are included hereafter.

During Phase 1 the cows lost approximately 100 kg or 21% of their initial mass, over a period of about 5 months, before ovulatory activity ceased (Table 2). Cows lost approximately 1,1 condition score points until they ceased ovulation, when their score was about 1,7. Sexual activity ceased earlier (15 days) and after greater loss of body mass (11 kg) in cows fed 40% of their maintenance requirements than in those fed 70% of requirements. However, these differences due to varying

ble 2 Mean mass and condition score changes ring Phase 1 (rapid mass loss)

	Treatments	
	1, 2 (70% of ME for maintenance)	3, 4 (40% of ME for maintenance)
	15	16
ial mass (kg)	472,4 ± 52,3	471,1 ± 50,0
ys to cessation of ovulation	168,5 ± 35,7	153,8 ± 35,9
ss at cessation of ovulation (kg)	375,2 ± 47,4	362,5 ± 29,6
ss loss to cessation of ovulation kg)	97,2 ± 24,4	108,6 ± 26,4
of initial mass lost to cessation f ovulation	20,7 ± 4,9	22,8 ± 3,7
erage daily mass change to essation of ovulation (kg/day)	-0,60 ± 0,20	-0,73 ± 0,20
ial condition score	2,77 ± 0,33	2,91 ± 0,26
re at cessation of ovulation	1,67 ± 0,37	1,77 ± 0,37
re loss to cessation of ovulation	1,1 ± 0,42	1,14 ± 0,34

trient intakes were not significant (Table 2).

During Phase 2 cows allocated to Treatments 1 and 2 t 0,2 ± 0,29 kg/day and cows in Treatments 3 and 4 t 0,09 ± 0,2 kg/day. The difference between these ns was not significant. Figure 1 illustrates changes in dy mass during the first 14 days of Phase 3. Mass reases of approximately 28 kg occurred within the st 4 days, and this gain represented most (94,2%) of e mass gained over the first 14 days. It is generally cepted that live mass gains of this magnitude, over a 4-y period, are not physiologically possible. It was erefore assumed that the rate of real change in body ss between days 0 and 4 was the same as that between ys 4 and 14. The balance of the gain measured tween days 0 and 4 was assumed to represent gutfill. all cows the mean estimate of gutfill was 25,9 ± 10,5 or 7,2 ± 2,9% of the mass measured on day 0 of Phase 3. Estimates of gutfill were not affected by treatment.

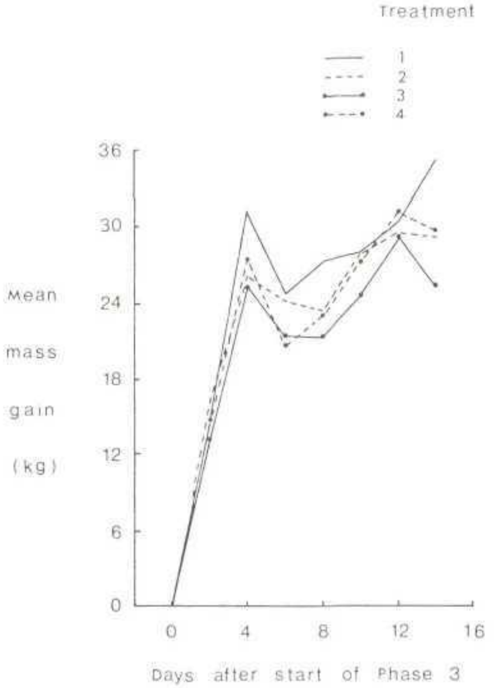

Figure 1 Mean mass gains in cows during the initial 14 days of Phase 3

Treatment did not significantly influence mass gains during Phase 3 (Table 3), although cows in Treatments 2 and 4 (which each received 4 kg of maize per day) gained between 0,1 and 0,2 kg/day more than those in Treatments 1 and 3 (fed 1 kg of maize per day). None of the cows experienced digestive upsets following realimentation. Cows fed 4 kg of maize per day during Phase 3 did not resume ovarian activity consistently earlier than those fed 1 kg of maize per day. Thus, cows in Treatment 2 resumed ovarian activity an average of 25 days later than those in Treatment 1, whereas cows in Treatment 4 recommenced ovulatory cycles 20 days earlier than those in Treatment 3 (Table 3). For each

ble 3 The influence of treatment on mean mass changes during Phase 3 (rapid mass gains), and masses at ssation and resumption of ovulation

	Treatment			
	1	2	3	4
	6	9	10	6
imated gutfill (kg)	30,4 ± 4,5	24,1 ± 16,1	25,0 ± 5,7	25,9 ± 11,6
ys from start of Phase 3 to resumption of ovarian activity	70,3 ± 39,3	94,7 ± 62,4	69,5 ± 37,9	49,2 ± 31,5
G from start of Phase 3 to resumption of ovarian activity (kg/day)^{ab}	0,33 ± 0,35	0,41 ± 0,38	0,24 ± 0,43	0,47 ± 0,41
ss when ovarian activity:				
Ceased (kg)	368,2 ± 48,2 } *	379,8 ± 49,1 } **	367,0 ± 32,4 } **	354,9 ± 24,9 } *
Resumed (kg)	424,8 ± 70,8	427,2 ± 53,0	402,4 ± 29,7	412,1 ± 42,3
Resumed minus gutfill (kg)	394,4 ± 70,2	403,1 ± 48,8	377,4 ± 27,6	386,2 ± 36,5
ss change from cessation to resumption of ovarian activity (kg)^{b}	26,2 ± 38,6	23,3 ± 35,0	10,4 ± 24,0	31,3 ± 34,3

DG: Average daily gain; ^{b}This gain excludes that due to gutfill;

ans joined vertically differ significantly from each other ($^{*}P < 0,05$; $^{**}P < 0,01$)

Table 4 The influence of treatment on mean condition scores at the cessation and resumption of ovarian activity

	Treatment			
	1	2	3	4
Score at cessation of ovulatory cycles	1,58 ± 0,49 }**	1,72 ± 0,29 }**	1,85 ± 0,44 }*	1,63 ± 0,14 }*
Score at resumption of ovulatory cycles	2,37 ± 0,22	2,39 ± 0,36	2,25 ± 0,26	2,46 ± 0,53
Score change from cessation to resumption of ovulatory cycles	0,78 ± 0,45	0,67 ± 0,39	0,4 ± 0,39	0,83 ± 0,52

Means joined vertically differ significantly from each other(*$P < 0,05$; **$P < 0,01$)

treatment, the mean mass at which cows resumed ovarian activity was significantly ($P < 0,05$) greater than that at which they become anoestrus. The magnitude of the difference between masses at the aforementioned stages was not influenced by treatment. Estimates of gutfill were subsequently subtracted from the actual masses, to obtain adjusted masses at the resumption of ovarian activity. For each individual treatment the adjusted masses at resumption did not differ from those at the cessation of ovulation. However, after pooling data from all cows the mean adjusted mass at resumption (389,9 ± 44,9 kg) was significantly ($P < 0,05$) greater than that at the cessation of ovarian activity (368,6 ± 39,1 kg).

Actual condition scores, and the magnitude of the difference between these at the cessation and resumption of ovarian activity were not influenced by treatment (Table 4). However, within each treatment the mean score at the re-initiation of ovulation was significantly ($P < 0,05$) higher than that at which cows became anoestrus. Scores obtained at the cessation of ovulatory activity were pooled, as were those measured in all cows at the resumption of ovulation. The mean score at the re-establishemnt of ovulation (2,35 ± 0,34) was significantly ($P < 0,01$) higher than that measured when cows became anoestrus (1,72 ± 0,37).

The number of cows which conceived during Phase 3, and the stage, mass and condition score at which conception occurred was not influenced by rate of mass loss and gain (Table 5). A notable feature of the results presented in Table 5 is that the vast majority of cows conceived at the resumption of ovulatory cycles. Thus, over all treatments 85% (22/26) of the cows which conceived did so at the reinitiation of sexual cycles. The four cows which conceived later became pregnant between 28 and 124 days after re-establishment of ovulatory cycles. Five cows failed to conceive, despite having been exposed to fertile bulls for at least 21 and at most 144 days after the resumption of ovarian cycles. After pooling data obtained in all animals, the cows (22) which conceived at the resumption of ovulation were compared to those (9) which failed to do so, in respect of

Table 5 The influence of treatment on conception rates in cows (see Table 1 for description of treatments)

	Treatment			
	1	2	3	4
n	6	9	10	6
Cows which conceived at resumption of ovulation				
No	5	6	6	5
Mean mass at resumption of ovulation (kg)	414,0 ± 73,4	420,2 ± 54,3	397,2 ± 19,6	433,3 ± 44.7
Mean condition score at resumption of ovulation	2,34 ± 0,23	2,5 ± 0,35	2,25 ± 0,27	2,25 ± 0.27
Cows which conceived after resumption of ovulation				
No	0	1	2	1
Days from resumption of ovulation to conception in individual cows	—	76	36; 124	28
Mean mass at resumption of ovulation (kg)	—	456,7	437,2 ± 50,1	376,8
Mean condition score at resumption of ovulation	—	2,0	2,25 ± 0,35	2,0
Mean mass at conception (kg)	—	536,2	480,8 ± 88,0	382,7
Mean condition score at conception	—	3,0	3,0 ± 0	2,0
Cows which did not conceive				
No	1	2	2	0
Days exposed to fertile bulls after resumption of ovulation in individual cows	92	21; 92	92; 114	—
Mean mass at resumption of ovulation	479,0	433,0 ± 80,0	397,5 ± 6,1	—
Mean condition score at resumption of ovulation	2,5	2,25 ± 0,35	2,0 ± 0,0	—
Mean mass at termination of trial (kg)	561,1	510,3 ± 28,2	468,5 ± 33,4	—
Mean condition score at termination of trial	3,0	3,0 ± 0,0	3,25 ± 0,35	—

Table 6 Mean mass and condition changes in cows that did and did not conceive at the resumption of ovulation

Parameter	Cows which conceived at resumption of ovulation	Cows which failed to conceive at resumption of ovulation
n	22	9
Mass at commencement of Phase 1 (kg)	455,3 ± 45,2^{a}	511,7 ± 39,8^{b}
Mass loss during Phase 1 (kg)	97,0 ± 25,0^{a}	117,9 ± 22,3^{b}
% of initial mass lost during Phase 1	21,2 ± 4,4	23,3 ± 4,1
Mass at cessation of ovulation (kg)	358,3 ± 36,1^{a}	393,8 ± 36,0^{b}
Ratio: $\frac{\text{Mass at resumption}}{\text{Mass at cessation}} \times \frac{100}{1}$	115,1 ± 7,3^{a}	107,9 ± 9,9^{b}
Condition score at commencement of Phase 1	2,78 ± 0,31	2,97 ± 0,23
Condition score loss during Phase 1	1,11 ± 0,42	1,13 ± 0,25
% of initial score lost during Phase 1	39,4 ± 13,6	37,2 ± 9,0
Condition score at cessation of ovarian activity	1,67 ± 0,4	1,84 ± 0,3

Within the same row means with different superscripts differ significantly ($P < 0,05$) from each other

umber of parameters (Table 6). Cows which failed to nceive were significantly ($P < 0,05$) heavier at the rt of the trial, lost more mass during, and were avier at the end of Phase 1, than those which imately became pregnant at the resumption of ovarian tivity. Corresponding measures of condition did not ffer between the two groups of cows. Cows which nceived were 15,1% heavier at the resumption than at e cessation of ovulation, as against a corresponding ass increase of only 7,9% in cows which failed to nceive (Table 6).

scussion

e main objective of this study was to establish to what tent severe undernutrition influences subsequent productive ability in beef cows. The results presented Tables 3 and 4 clearly indicate that cows were gnificantly heavier and in better condition when they sumed, relative to when they ceased ovarian activity. is finding, which concurs with that of Hale (1975) has portant practical implications. Dry, non-pregnant ws should be placed on winter feeding regimes which event mass losses resulting in cows becoming oestrus. This strategy will obviate the necessity of eding cows to gain mass and condition beyond that at hich anoestrus had previously set in. On the basis of sults obtained in this study it is interesting to speculate at the 'target mass' for a particular conception rate in ctating beef cows may be influenced by previous tritional history. Cows which undergo a period of oestrus induced by severe mass losses may later (when ctating) have a higher 'target mass' than cows not bjected to severe feed restriction. In this context it is teresting to note that heifers which experience a olonged period of undernutrition have a higher 'target ass' (for a particular conception rate) than their well-d contemporaries (Sparke & Lamond, 1968; Wiltbank, asson & Ingalls, 1969).

Hale (1975) and Richards, Wettemann & Schoenemann (1986) conducted similar, but not identical experiments to the one described in the present study. According to the limited information reported by Hale (1975) cows subjected to severe undernutrition lost about 68 kg or 21,2% of their initial mass over a period of about 135 days before they become anoestrus. This agrees closely with the 21,7% of initial mass lost over a period of about 150 days in the present study (Table 2). Richards, *et al.*, (1986) recorded mass losses of approximately 24% in underfed cows over a period of 182 days, at the end of which 91% of the animals had ceased luteal activity. In the study by Hale (1975) cows were 70 kg heavier at resumption than at cessation of ovarian activity, but gutfill was not estimated. When the estimate of gutfill obtained in the present study (about 25 kg; Table 3) is subtracted from the aforementioned increase in mass obtained by Hale (1985), the cows in his study would have been 45 kg heavier at resumption than at cessation of ovarian cycles. This mass increase is considerably greater than the 21 kg recorded in the present study (Table 3). The different breeds and rates of mass change used could account for this discrepancy but the principle of cows being heavier when they resumed relative to when they ceased ovarian activity is now clearly established. In the study by Richards, *et al.*, (1986) condition scores, but not masses were higher at the return to, than at the cessation of sexual cycles. Donaldson & Takken (1968) established the same principle for heifers, which, following severe feed restriction, were significantly lighter on becoming anoestrus than when later returning to oestrus following a period of *ad lib.* feeding.

Four of the cows used in the present study failed to become anoestrus, despite having lost about 152 kg or 31% of their initial mass. Breed-type could influence the magnitude of mass and condition losses that cows can tolerate prior to ceasing ovarian activity, a theory substantiated by the work of Hale (1975). Dry Africander cows lost 19%, and Mashona cows 32% of their initial mass before they stopped sexual activity. The majority of South African beef cattle are *Bos indicus* types. Differences in reproductive function between *Bos indicus* and *Bos taurus* cattle are well documented (Anderson, 1936; Plasse, Warnick & Koger, 1968; Randel, 1976; Rhodes, Randel & Harms, 1978). Further research is required to establish whether *Bos indicus* cows would cease sexual activity after losing more or less of their initial mass and condition than the *Bos taurus* cows used in the present study.

The estimate of gutfill obtained in this study can at best be regarded as an approximation, but an attempt to gauge its magnitude was considered essential. The mass increase of about 25 kg between stages at which cows ceased and resumed ovarian activity should be regarded as the minimum, because strictly speaking an estimate of gutfill should have been obtained, and subtracted from the masses at which cows ceased ovulatory activity. However, it was reasoned that the contribution of gutfill to live mass was relatively small at this stage, when each cow was fed only between 2,9 and 4,6 kg of hay per day. Gutfill was estimated to constitute about 7% of live mass at the commencement of 'full feeding' (Phase 3). This is a relatively small proportion when considering that Tayler (1954) and Hughes (1976) maintain that gut contents can constitute 12 to 22% of an animal's mass. It is likely though that a degree of 'rumen shrink' occurred during the months of feed restriction, which could explain the relatively small contribution of gutfill to live mass in this study.

A surprising feature of the results presented in Table 5 was that the majority of cows conceived immediately on resuming ovarian activity. A varying proportion of lactating cows commonly experience 'short' oestrous cycles during the transition from anoestrus to the resumption of normal ovarian activity (Short, Randel & Bellows, 1974) and in dairy cows conception rates at the first oestrus after calving are poorer than at subsequent heat periods (Olds & Cooper, 1970). Beef heifers have reduced follicle numbers and plasma progesterone levels when subjected to short-term undernutrition (Hill, Lamond, Henricks, Dickey & Niswender, 1970). On the other hand it was shown by Richards, *et al.* (1986) that undernourished anoestrous cows, on being fed to resume sexual activity, conceived as rapidly as dry cows fed to maintain mass over an extended period. This finding, together with that of the present study indicate that in the majority of dry cows the anoestrous state induced by severe undernutrition can be rapidly changed to one of normal fertility, once cows have regained sufficient mass and condition to reinitiate oestrous cycles. A small number of cows failed to conceive, despite having been exposed to fertile bulls for as long as 124 days after the reinitiation of ovulatory cycles (Table 5). These cows may have undergone undetected embryonic failure or abortion, or fertilization failure due to structural abnormalities such as blocked fallopian tubes. The data summarized in Table 6 suggest that size of cow may influence conception rates following severe feed deprivation. Cows which failed to conceive on resuming ovarian function were initially heavier than, but in similar condition to those which conceived, suggesting that the cows which failed to conceive were larger than those which became pregnant. Cows which experienced conception failure resumed ovarian cycles when only 7,9% heavier than when they became anoestrus, as against a corresponding mass increase of 15,1% in cows which conceived on returning to sexual activity (Table 6). Relatively large cows may thus experience lower conception rates following severe mass losses than smaller cows.

Treatment, that is the different rates of mass loss and gain, did not influence the length of the periods to, and the magnitude of mass and condition losses and gains until cessation and resumption of ovulatory cycles (Tables 2, 3, 4). This finding was surprising, when considering the size of treatment differences. It is thus clear that differences in dietary levels need to be relatively large when studying ovarian responses at sub-maintenance nutritional levels. It is also clear from the results of this study that dry, non-pregnant, beef cows can be fed as little as 2,9 kg of hay per day for as long as 5 months during conditions of feed scarcity, providing physical activity is restricted. Furthermore, with restoration of nutritional levels the majority of such cows will resume normal reproductive ability, albeit at masses and conditions significantly greater than when sexual activity had previously come to a halt.

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References

ANDERSON, J., 1936. Studies on reproduction of cattle. The periodicity and duration of oestrus. *Emp. J. exp. agric.* 4, 186.

DONALDSON, L.E. & TAKKEN, A., 1968. Effects of nutritional changes on oestrus in heifers. *Proc. Austr. Soc. Anim. Prod.* 7, 180.

HALE, D.H., 1975. Nutrition, hormones and fertility. *Rhod. agric. J.* 72, 69.

HILL, J.R., LAMOND, D.R., HENRICKS, D.M., DICKEY, J.F. & NISWENDER, G.D., 1970. The effects of undernutrition on ovarian function and fertility in beef heifers. *Biol. Reprod.* 2, 78.

HUGHES, J.C., 1976. Short-term variation in animal live weight and reduction of its effect on weighing. *Anim. Breed. Abstr.* 44, 111.

MEAKER, H.J., COETSEE, T.P.N., SMITH, M. & LISHMAN, A.W., 1980. The relationship between body mass and fertility of beef cows of different ages. *S. Afr. J. Anim. Sci.* 10, 83.

OLDS, D. & COOPER, T., 1970. Effect of post-partum rest period in dairy cattle on the occurrence of breeding abnormalities and on calving intervals. *J. Amer. vet. med. Assoc.* 157, 92.

PLASSE, D., WARNICK, A.C. & KOGER, M., 1968. Reproductive behaviour of *Bos Indicus* females in a subtropical environment. 1 Puberty and ovulation frequency in Brahman and Brahman × British heifers. *J. Anim. Sci.* 27, 94.

RANDEL, R.D., 1976. LH and ovulation in Brahman, Brahman × Hereford and Hereford heifers. *J. Anim. Sci.* 43, 300 (Abstract).

REYNEKE, J., 1971. Wintering systems for cows in the eastern Highveld. *S. Afr. J. Anim. Sci.* 1, 39.

RHODES, R.C., RANDEL, R.D. & HARMS, P.G., 1978. Reproductive studies of Brahman cattle. IV. Luteinizing hormone levels in ovariectomized Brahman, Brahman × Hereford and Hereford cows following a 20 mg dose of oestradiol 17-β. *Theriogenology* 10, 429.

RICHARDS, M.W., WETTEMANN, R.P. & SCHOENEMANN, H.M., 1986. Effect of restricted intake on cyclic ovarian activity and estrus in Hereford cows. *Okl. Agric. Exp. Sta. Rept.* May, 1986.

SHORT, R.E., RANDEL, R.D. & BELLOWS, R.A., 1974. Factors affecting reproduction in the postpartum cow. *J. Anim. Sci.* 39, 226 (Abstract).

SPARKE, E.J. & LAMOND, D.R., 1968. The influence of supplementary feeding on growth and fertility of beef heifers grazing natural pastures. *Austr. J. Exp. Agric. and Anim. Husb.* 8, 425.

TAYLER, J.C., 1954. Techniques of weighing the grazing animal. *Proc. British Soc. Anim. Prod.* 1954, 3.

VAN NIEKERK, A. & LOUW, B.P., 1982. Condition scoring of beef cattle. Bull. N14/82. Dept. Agric., Natal Region.

WILTBANK, J.N., KASSON, C.W. & INGALLS, J.E., 1969. Puberty in crossbred and straightbred beef heifers on two levels of feed. *J. Anim Sci.* 29, 602.

TABLE 1 Duration of the post-partum anoestrous period among ewes on two planes of nutrition during lactation
TABEL 1 *Duur van die post-partum anestrusperiode by ooie op twee voedingspeile gedurende laktasie*

Year *Jaar*	Type of ewe *Tipe ooi*	Average Age (yr) *Gemiddelde ouderdom (jr)*	Nutritional plane during lactation *Voedingspeil gedurende laktasie*	Number of animals *Getal diere*	Parturition to first oestrus (days) *Partus tot eerste estrus (dae)*	Number of ewes anoestrus *Getal ooie anestrus*
1967............	Merino	5	High/*Hoog*	58	39,2± 9,5	2
			Low/*Laag*	57	42,6±13,0	4
1968............	Crossbred/*Kruis*	6	High/*Hoog*	110	42,9±11,9	8
			Low/*Laag*	109	46,8±13,1	11
1969............	Merino	7	High/*Hoog*	113	48,5±15,3	10
			Low/*Laag*	109	46,5±13,3	12
1970............	Merino	8	High/*Hoog*	69	52,8±12,9	13
			Low/*Laag*	67	49,8± 9,6	9
	Merino	Maiden/*Eersteling* 2–3	High/*Hoog*	30	47,5± 9,5	5
			Low/*Laag*	20	52,4±11,4	17
	Crossbred/*Kruis*	8	High/*Hoog*	42	51,7± 9,6	2
			Low/*Laag*	47	52,2±12,0	7
		Maiden/*Eersteling* 2–3	High/*Hoog*	28	46,7±12,0	1
			Low/*Laag*	24	48,4±12,2	8
1971............	Merino	3–5	High/*Hoog*	59	50,1±12,6	3
			Low/*Laag*	54	50,5±11,4	11
1972............	Merino	2–6	High/*Hoog*	49	50,0±11,0	5
			Low/*Laag*	76	51,6±10,3	3

Post-partum anoestrus

The plane of nutrition did not significantly influence the delay to first oestrus or the proportion of ewes anoestrus throughout lactation (Table 1).

Regression analyses were performed by considering the interval to oestrus as the dependent variable "Y" and using six measures of each ewe's body mass or change in body mass as the independent variables "X". These parameters were (i) body mass three days after parturition—initial body mass, (ii) body mass at weaning, (iii) the lowest body mass recorded during the lactation period—a measure of the maximum stress experienced during lactation. (iv) The accumulated difference between the post-partum body mass and the body mass measured each week until cessation of lactation—to assess the degree of stress and its duration. (v) The difference between the initial body mass and that recorded at weaning, expressed as a percentage of the initial mass. (vi) The difference between the initial body mass and the minimum live mass recorded, expressed as a percentage of the initial mass.

The only relationship to approach significance was the negative correlation between initial body mass and the duration of the post-partum anoestrous period. The "F" value obtained was 3,05 (3,84 required for significance) and this was achieved after pooling all the results, but omitting the data for which a positive correlation was obtained viz., 1968 and 1972.

Amongst the maiden ewes (1970) there was a negative correlation between the post-partum interval to oestrus and the maximum loss in body mass, expressed as a percentage of the post-partum mass (F=3,94, DF=98). During this study the earliest date of lambing was 28 March and no ewe lambed after 15 May. When all the data on the duration of anoestrus (Y) were combined, with the independent variable "X" expressed as days from 1 March to the date of parturition, the equation $Y=58,0-0,23X$ was obtained. The regression was highly significant ($P=0,001$) and the correlation between "Y" and "X" was negative for every year of this study. The instances in which the regression for a particular year was significant are illustrated in Fig. 3.

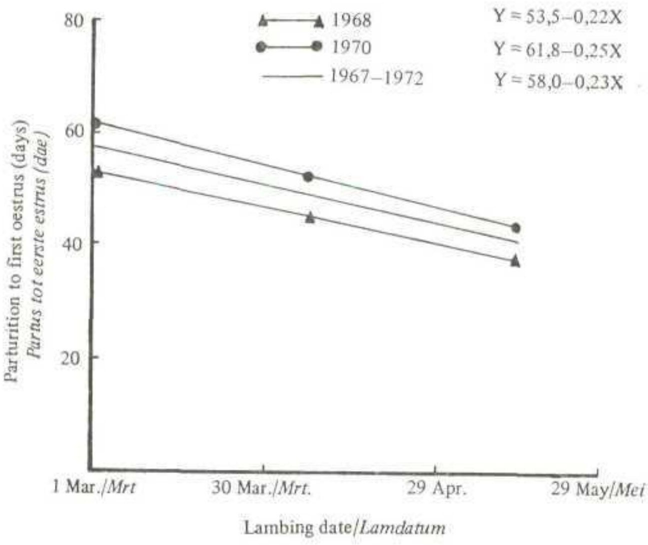

FIG. 3 Influence of date of lambing on the post-partum interval to first oestrus
FIG. 3 *Invloed van lamdatum op die post-partuminterval tot eerste estrus*

Incidence of oestrus

The difference in the incidence of oestrus between the ewes on the High and on the Low plane varied from year to year. Those differences which were significant, according to the Chi-square test, have been indicated in Fig. 4.

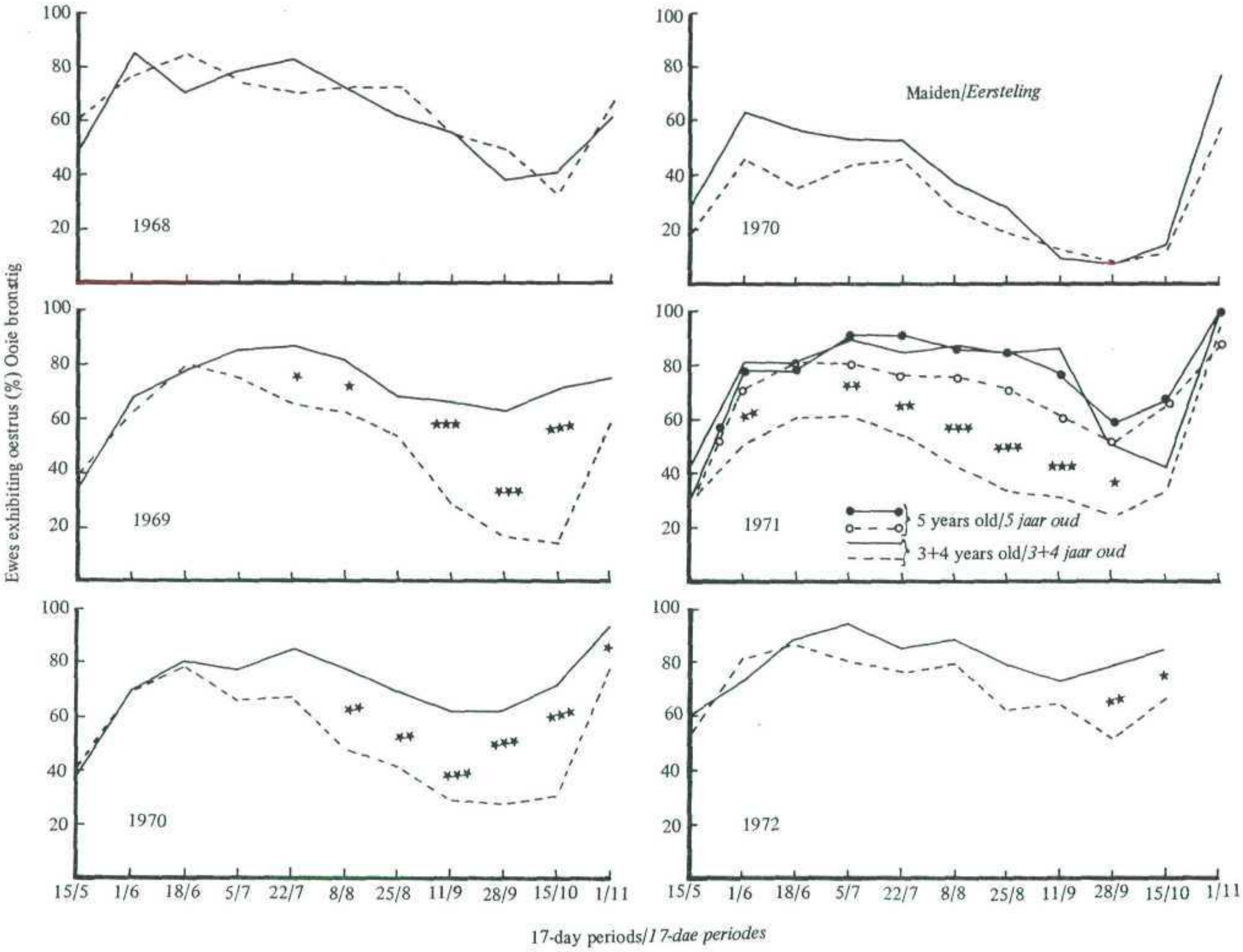

FIG. 4 Seasonal variations in the incidence of oestrus in groups of ewes subjected to either a high (———) or a low (- - - - -) plane of nutrition during the lactation period and continuously associated with rams after parturition. ***, **, * indicate $P<0{,}001$, 0,01 or 0,05 respectively but refer only to lines not interrupted by open or closed circles

FIG. 4 *Seisoensverskille in bronstigheid by groepe ooie onderworpe aan òf 'n hoë (———) òf 'n lae(- - - -)voedingspeil gedurende laktasie en voortdurend by ramme na partus.* ***, **, * *dui aan* $P<0{,}001$; 0,01 *of* 0,05 *onderskeidelik, maar verwys alleenlik na lyne wat nie deur oop of toe sirkels onderbreek word nie*

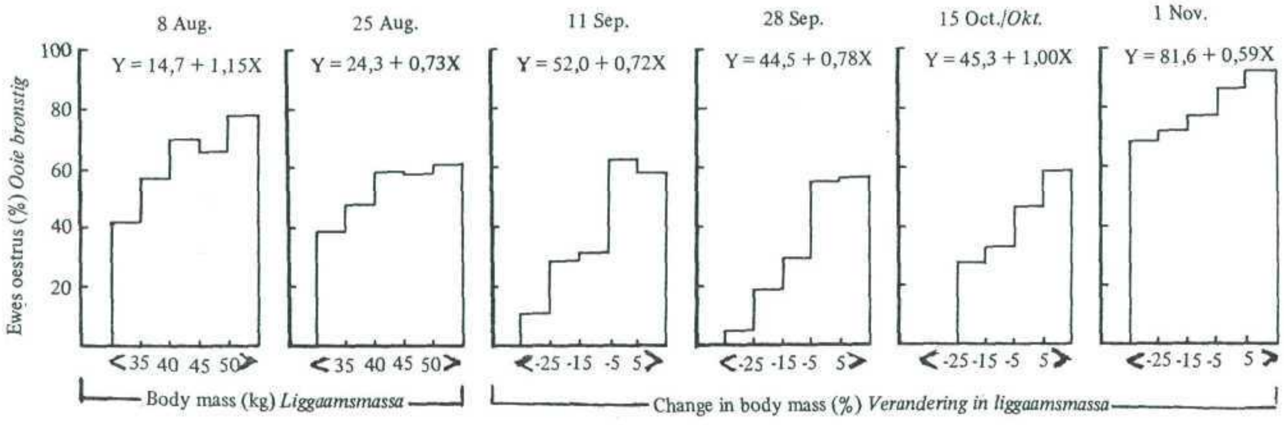

FIG. 5 Association between body mass or change in body mass (X) and the % of ewes showing oestrus (Y) during 17-day periods commencing on the dates shown

FIG. 5 *Verwantskap tussen liggaamsmassa of verandering in liggaamsmassa (X) en die % ooie bronstig (Y)gedurende 17-dae periodes wat begin het op die datums aangedui*

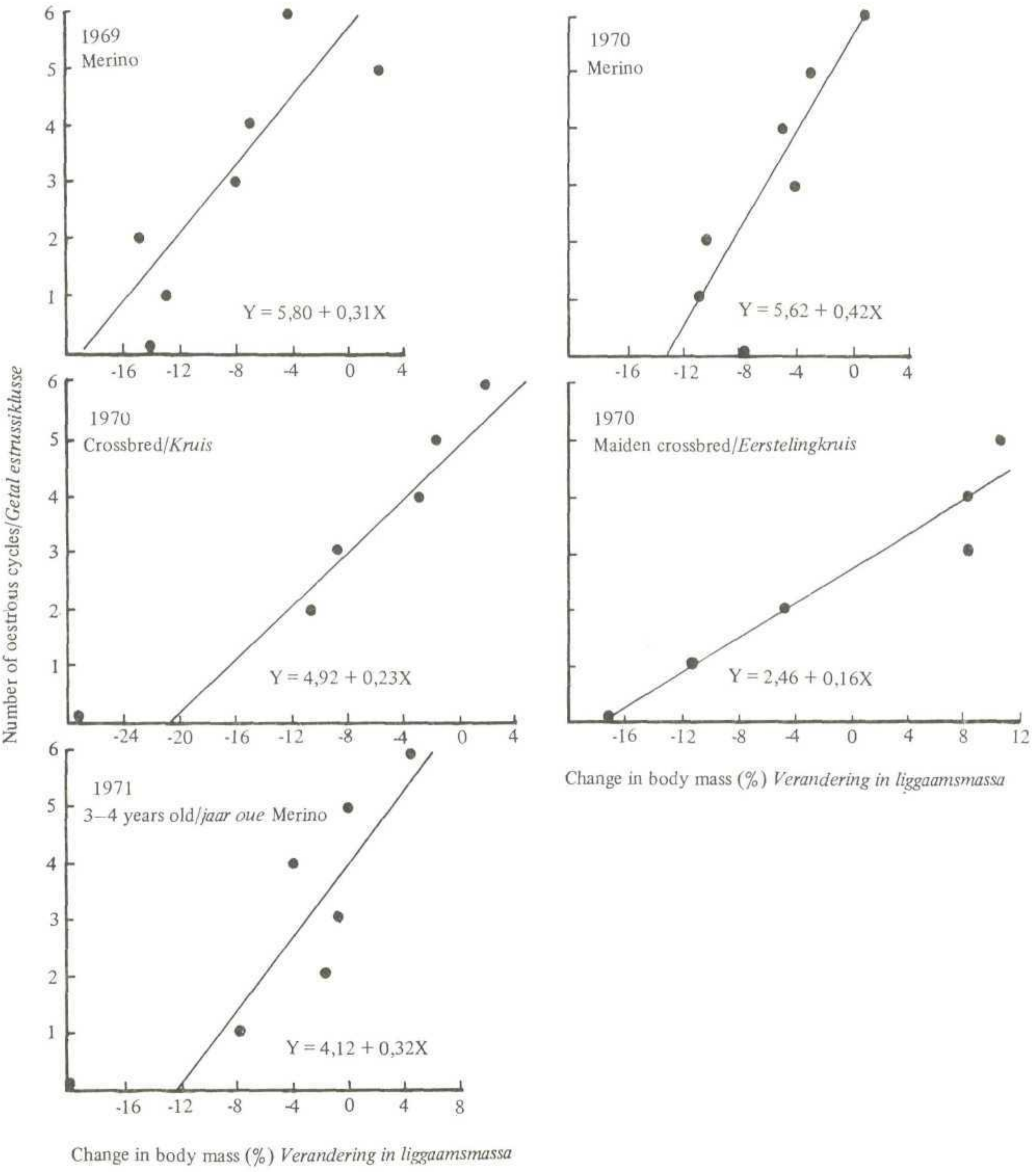

FIG. 6 Relationship between the incidence of oestrus and body mass change to weaning, expressed as a percentage of the body mass post-partum, amongst ewes classified according to breed, age and year of study

FIG. 6 *Verwantskap tussen die voorkoms van estrus en verandering in liggaamsmassa tot speen, uitgedruk as 'n persentasie van die liggaamsmassa post-partum by ooie wat volgens ras, ouderdom en jaar van studie geklassifiseer is*

Y = Number of oestrus cycles/*Getal estrussiklusse*
X = Change in body mass to weaning (%)/*Verandering in liggaamsmassa tot speen* (%)

During 1970 the difference in oestrous activity between maiden and mature ewes on the Low plane was significant from 1 June onwards, and from 18 June for ewes on the High plane. These differences persisted until joining with entire rams, the level of significance gradually increasing from $P < 0,05$ to $P < 0,001$. During 1971 the older ewes on the Low plane of nutrition also exhibited a higher level of oestrous activity than the 3 to 4-year-old females on the same plane. These differences were significant from 8 August up to and including 15 October.

TABLE 2 Oestrous activity of ewes maintained on two planes of nutrition during lactation and either continuously associated with or isolated from rams after weaning

TABEL 2 *Bronstigheid by ooie wat op twee voedingspeile onderhou is gedurende laktasie en na speen óf voortdurend by ramme geloop het óf van ramme afgesonder is*

Year/ *Jaar*	Type of ewe/ *Tipe ooi*	Average age (yr) *Gemiddelde ouderdom (jare)*	Nutritional plane during lactation/ *Voedingspeil gedurende laktasie*	Post-weaning association with rams *Assosiasie met ramme na speen*	Number of animals/ *Getal diere*	Number of ewes oestrus *Getal ooie bronstig* 15/10–31/10	Number of ewes marked by fertile rams *Getal ooie gemerk deur vrugbare ramme**		Number of ewes not marked by fertile rams *Getal ooie nie gemerk deur vrugbare ramme*
							1/11–17/11	18/11–3/12	
1967.....	Merino	5	High/*Hoog*	Isolated/ *Afgesonder*	58	—	16	42	0
			Low/*Laag*		56	—	10	46	0
1968.....	Merino + Crossbred/ *Kruis*	6	High/*Hoog*	Continuous/ *Voortdurend*	58	24	36	21	1
			High/*Hoog*	Isolated/ *Afgesonder*	58	26	45	11	1
			Low/*Laag*	Continuous/ *Voortdurend*	56	18	38	17	1
			Low/*Laag*	Isolated/ *Afgesonder*	58	14	43	15	0
1969.....	Merino	7	High/*Hoog*	Continuous/ *Voortdurend*	52	39	41	11	0
			High/*Hoog*	Isolated/ *Afgesonder*	57	11	46	11	0
			Low/*Laag*	Continuous/ *Voortdurend*	55	8	32	20	3
			Low/*Laag*	Isolated/ *Afgesonder*	55	4	30	23	1
1970.....	Merino + Crossbred/ *Kruis*	8	High/*Hoog*	Continuous/ *Voortdurend*	62	45	59	13	1
			High/*Hoog*	Isolated/ *Afgesonder*	65	29	53	12	0
			Low/*Laag*	Continuous/ *Voortdurend*	62	19	48†	13	0
			Low/*Laag*	Isolated/ *Afgesonder*	67	13	52	13	2
		Maiden/ *Eersteling* 2–3	High/*Hoog*	Continuous/ *Voortdurend*	32	5	25	7	0
			High/*Hoog*	Isolated/ *Afgesonder*	35	4	27	8	0
			Low/*Laag*	Continuous/ *Voortdurend*	37	5	21†	15	0
			Low/*Laag*	Isolated/ *Afgesonder*	33	2	13	19	1
1971.....	Merino	3–4	High/*Hoog*	Continuous/ *Voortdurend*	40	21	39†	0	0
			Low/*Laag*		44	15	39	5	0
		5	High/*Hoog*	Continuous/ *Voortdurend*	22	15	22	0	0
			Low/*Laag*		21	14	20	1	0
1972.....	Merino	2–6	High/*Hoog*	Continuous/ *Voortdurend*	53	45	—	—	—
			Low/*Laag*		78	52	—	—	—

* First oestrus only/*Alleenlik eerste estrus*

† Indicates one ewe less/*Dui aan een ooi minder*

Relationships between body mass and the incidence of oestrus

(*i*) *The period beginning* 8 *August*

The results presented in Fig. 4 indicate that by the 17-day period commencing 8 August the incidence of oestrus among the ewes on the two planes of nutrition began to differ significantly in three out of the five years reflected. In view of this finding attention was given to this and subsequent 17-day periods. Each ewe was categorized according to the parameters of body mass or change in body mass, described earlier, and the data for the years 1968, 1969, 1970 and 1971 were pooled. The body mass recorded just prior to joining with entire breeding rams (pre-mating body mass) was also considered. The significance of the observed relationship between the various parameters (independent variable, "X") and the proportion of ewes showing oestrus ("Y") was tested by the method of regression analysis.

No particular parameter was consistently more closely associated with the occurrence of oestrus than any other. During the first two 17-day periods the proportion of ewes exhibiting oestrus was most closely associated with their body mass at weaning. For the remaining periods, the % change in body mass to weaning, expressed as a percentage of the initial body mass gave a particularly close association with incidence of oestrus. (Fig. 5).

(ii) The number of oestrus cycles observed

The incidence of oestrus amongst the ewes during the period 8 August to 17 November can also be described by the number of occasions on which each ewe exhibited overt oestrus during the 17-day periods ("Y"). The variates previously described, were again used in a regression analysis and by limiting the analysis to the years or age groups where the ewes showed an effect of undernutrition during lactation (Fig. 4) it became clear that the % change in body mass to weaning produced the most consistent relationship with oestrus, and the instances where a significant association was attained are illustrated in Fig. 6.

Oestrus prior to and during the annual breeding period

Certain of the ewes which had been isolated from rams after weaning exhibited spontaneous oestrus when rejoined with rams on 15 October, while others mated only one or two cycles later (Table 2). Furthermore, among the ewes continuously associated with rams there was a notable increase in the proportion showing overt oestrus during the 17-day period commencing 1 November.

TABLE 3 Mean body mass prior to the annual breeding period and the mating of ewes during the 17-day period preceding and the two periods following introduction of entire rams. Figures which differ significantly are joined by a line and the level of significance is indicated by: *P=0,05; **P=0,01; ***P=0,001

TABEL 3 *Gemiddelde liggaamsmassa voor die jaarlikse paartydperk en die paring van ooie gedurende die 17-dae periode voor, en die twee 17-dae periodes na byvoeging van vrugbare ramme. Syfers wat betekenisvol verskil word deur 'n lyn verbind en die graad van betekenisvolheid word aangedui as:* *P=0,05; **P=0,01; ***P=0,001

Plane of nutrition during lactation *Voedingspeil gedurende laktasie*	Number of ewes mated[1] *Getal ooie gedek*[1]			Premating body mass (kg) of ewes mated: *Liggaamsmassa (kg) voor paring van ooie gedek:*		
	17-day periods beginning: *17-dae periodes wat begin:*					
	15/10	1/11	18/11	15/10	1/11	18/11
High/*Hoog*.....	205	205	79	48,3	46,5	43,2
				48,3–46,5: * 46,5–43,2: ** 48,3–43,2: *** SE^2/*SF*^{2}=±8,99		
Low/*Laag*......	111	222	136	46,7	43,7	40,7
				46,7–43,7: ** 43,7–40,7: *** 46,7–40,7: *** SE^2/*SF*^{2}=±8,17		

[1] Data for 1968–1971 pooled
Data vir 1968–71 *saamgestort*

[2] SE of a single observation
SF van 'n enkele obserwasie

Within each plane of nutrition, a grouping of the ewes (1968–1971) according to the 17-day periods during which mating first occurred, beginning on 15 October, shows that the ewes which mated early had either a greater body mass or had undergone a smaller reduction in mass during their preceding lactation than those which mated later. The differences between ewes which mated in the three 17-day periods shown (Table 3) were more often significant when the ewes were classified according to the pre-mating body mass than for any other variate studied. Association with rams did not influence the results.

Mating with entire breeding rams

The nutritional stress during lactation had little effect on the pattern of mating or on the total number of ewes mated during the first 17 days of the annual breeding period. The pooled Chi-square test for the 1968–1971 data indicated that amongst the ewes continuously associated with rams significantly more ewes on the high plane than on the low plane were mated during the first 17-day period after joining with entire rams ($P < 0,05$). Amongst the ewes isolated from rams the effect of the nutritional plane during their previous lactation was significant only during 1969. No other differences were significant. The effect of isolation from rams on the number of ewes mated was minor and varied with the year or age of the ewes considered.

DISCUSSION

According to data reviewed by Hunter (1968) ewes which lamb when the seasonal stimuli for the initiation of oestrus are strong, resume normal breeding within three to six weeks after parturition. The average interval reported here (39,2 to 52,8 days) agrees closely with that observed by Granger (1947), Smith (1964), Fletcher (1971) and Hunter & Van Aarde (1973), but is considerably shorter than that reported by Joubert (1962) for Merino ewes lambing in autumn.

The hypothesis that poor nutrition prolongs the period of post-parturient anoestrus is favoured by Kirillov (1944), Smith (1964) and Van Niekerk & Mulder (1965). However, Hunter & Van Aarde (1973) noted that the effect of plane of nutrition varied according to the season of lambing and the results reported here failed to show a consistent relationship between change in body mass during lactation and the delay to first oestrus. The tendency for ewes with the greatest body mass, at parturition, to exhibit oestrus (present experiments) and to ovulate sooner than lighter animals (Hunter & Lishman, 1967; Vosloo, Hunter & Carstens, 1969) suggests that this is either an inherent tendency of larger animals or that nutrition prior to parturition may play a role. The fairly high repeatability of stage of commencement of reproductive activity after parturition (Hafez, 1952; Lees, 1966) supports the former hypothesis.

The trends in the frequency of oestrus amongst the lactating ewes of the study reported here were similar to those of comparably treated, but non-lactating ewes studied by Hunter (1962). In our study the duration of anoestrus was considerably shorter than observed by Hunter (1962). Both Hunter (1962) and Smith (1962, 1965) observed that after adequate feeding had been restored the incidence of oestrus rose over a period of several months until the peak of the normal breeding season. However, the results in Table 2 show that by the end of the annual breeding period of six weeks the effect of restricted feeding during the previous lactation on the number of ewes mated, had disappeared.

Australian, American and South African workers are of the opinion that mating in spring results in fewer lambs than breeding during the autumn because of a reduced incidence of oestrus during spring (Dun *et al.*, 1960; Adler, 1964; Shelton & Morrow, 1965; Watson &

Radford, 1966). Our results suggest that although the incidence of oestrus may be low at the end of the winter, when ewes are regularly mated at a given time in spring very few animals will not be served by the breeding rams.

The results in Table 3 confirm the findings of Smith (1966) viz., that the level of nutrition, as well as the body mass independent of the level of nutrition, influence the incidence of oestrus in sheep during spring. Seasonal changes in the occurrence of oestrus are related to body mass (Fig. 5 and 6) but the overall effect on the onset of the breeding season is negligible (Table 2). This agrees with the findings of Smith (1965, 1966), but is somewhat contrary to the results obtained by Hunter (1962) and by Smith (1962).

Smith (1965) and Lishman & Hunter (1966, 1967) suggested that, when studying the factors influencing oestrus under practical farming conditions it is necessary to isolate the ewes for some time before they are rejoined with rams during the normal mating period. This opinion has been substantiated by the present results. Furthermore, rejoining with rams is probably the main stimulus which obliterates the deleterious effects of previous poor nutrition. A similar situation has been shown to exist in female mice subjected to undernutrition and stimulated by joining with males (Cooper & Haynes, 1967; McNeilly, Cooper & Crighton, 1970). The absence of the male stimulus) may account for the results obtained by Hunter (1962) and by Smith (1962) where the anoestrous period was markedly prolonged by undernutrition.

The data obtained in this study confirm earlier findings (Lishman & Hunter 1967, Lishman, 1969) that the presence of rams during the anoestrous season reduces the tendency of ewes to go into anoestrus. In addition, other factors may come into operation in spring so that ewes which have become anoestrous even when associated with rams will mate soon after the commencement of the annual breeding period on 1 November. It is possible that substitution of entire rams for the vasectomized rams used for six months constituted a new stimulus to the anoestrous ewes. Such a hypothesis is supported by the finding that in rats the influence of the male gradually wanes (McNeilly *et al.*, 1970; Cooper, Purvis & Haynes, 1972), but can be restored by removing a particular male and reintroducing it at a later stage (McNeilly *et al.*, 1970).

A different explanation of the results is that the ewes may have become synchronised with an annual mating-lambing rhythm, as suggested by Lyle & Hunter (1967). Accordingly, the new breeding season commences spontaneously during late October to early November of each year. In contrast, Lishman & De Lange (1967) have shown that anoestrus persisted in ewes until January, if the ewes were not joined with rams until this time. Lishman (1969) also noted that the new breeding season commenced only in late January when ewes were continuously associated with the same group of rams. Furthermore, the work of Joubert (1962) indicated that, in most of the breeds or crosses studied, the increase in sexual activity commenced gradually during October and continued to increase for six months. The data obtained by Hunter (1962, 1964) showed a similar tendency.

The demonstration of a significant functional relationship between body mass or change in body mass and the occurrence of oestrus, suggests that when ewes are to be mated more frequently than annually, attention should be given to selecting animals of above average size. In the same context, breeding ewes should be protected from excessive reduction in body mass during lactation.

Opsomming

VOORTPLANTING BY DIE OOI IN VERHOUDING TOT VOEDINGSPEIL, LIGGAAMSMASSA EN VERANDERING IN LIGGAAMSMASSA. I. VOORKOMS VAN ESTRUS TUSSEN PARTUS EN HERBESETTING

Ondersoek is ingestel na die mate waartoe ondervoeding en gevolglike verlies in liggaamsmassa die voorkoms van bronstigheid by ooie beïnvloed. Oor 'n tydperk van ses jaar wat in 1967 *begin het, is altesaam* 761 *Merino en* 360 *kruisooie wat in herfs gelam het òf* 100% *(Hoë peil) òf* 50% *(Lae peil) van hul beraamde daaglikse voedingsbenodigdhede gedurende die* 84 *dae van laktasie gevoer. Obserwasies vir estrus is twee keer per dag uitgevoer totdat die lammers gespeen is. By hierdie stadium is die helfte van die ooie van ramme afgesonder (alleenlik* 1968, 1969 *en* 1970*) tot een estrussiklus voor aanvang van die jaarlikse dekperiode.*

Die lae peil van voeding het nie die tydperk tussen partus en eerste estrus (gemiddeld 39,2 *tot* 52,8 *dae) asook die getal ooie wat geen estrus gedurende laktasie getoon het, beïnvloed nie. Regressie-ontleding van die effek van liggaamsmassa, of verandering in liggaamsmassa gedurende laktasie, op die aantal dae tot eerste estrus het geen konsekwente verwantskap tussen hierdie variante aangedui nie. 'n Hoogs betekenisvolle verwantskap* ($P=0{,}001$) *tussen die lamtyd na* 1 *Maart* (X) *en die duur van die post-partumanestrusperiode* (Y) *kon uitgedruk word as:* $Y=58{,}0-0{,}23X$. *Ondervoeding gedurende laktasie het die getal ooie wat na die eerste week in Augustus* 1969, 1970 *en* 1971 *bronstig was, betekenisvol verlaag. 'n Samevatting van die data verkry gedurende* 1968 *tot* 1971 *het aangedui dat vir die* 17*-dae periodes wat op* 8 *Augustus en* 25 *Augustus begin het, was daar 'n betekenisvolle verwantskap tussen die verhouding ooie bronstig en hul liggaamsmassa by speen. Gedurende die daaropvolgende vier* 17*-dae periodes was bronstigheid afhanklik van die verandering tussen partus- en speenmassa, uitgedruk as 'n persentasie van eersgenoemde. Verdere ontleding van die data het net die resultate waar ondervoeding wel 'n effek op voorkoms van estrus gehad het, ingesluit. Vervolgens is gevind dat die getal estrussiklusse wat elke ooi gedurende die tydperk* 8 *Augustus tot* 17 *November getoon het, afhanklik was van die* % *verandering in liggaamsmassa tot speen.*

Die ooie wat vroeg gedek is na 15 *Oktober het 'n hoër liggaamsmassa gehad voor die byvoeging van vrugbare ramme, as ooie wat later gedek is.*

Die voedingspeil gedurende laktasie en afsondering van ramme ná speen het geen noemenswaardige invloed op die getal ooie wat gedurende die jaarlikse paartyd van ses weke gedek is, gehad nie.

Résumé

REPRODUCTION DE LA BREBIS EN RELATION AVEC LE PLAN DE NUTRITION, LA MASSE CORPORELLE ET LE CHANGEMENT DE LA MASSE CORPORELLE. 1. *L'INCIDENCE DES CHALEURS ENTRE LA MISE BAS ET LA RE-CONCEPTION*

Durant une période de six ans commençant en 1967, *un total de* 761 *brebis Merino et de* 360 *brebis croisées qui avaient mis bas en automne furent alimentées soit à* 100% *(plan élevé) soit à* 50% *(plan réduit) des nécessités alimentaires journalières estimées durant la*

période de lactation de 84 jours. Des observations sur les chaleurs furent faites deux fois par jour jusqu'au sevrage des agneaux, stade auquel la moitié des brebis fut isolée des béliers (seulement en 1968, 1969 *et* 1970) *jusqu'à un cycle de chaleur antérieur à l'initiation de la période annuelle de reproduction. Les plans réduits d'alimentation ne prolongèrent pas l'interval entre la parturition et les premières chaleurs (moyenne de* 39,2 *à* 52,8 *jours) ou n'accrurent pas le nombre de brebis sans chaleur durant la lactation. La masse corporelle ou le changement dans la masse corporelle durant la lactation n'eut pas d'effet consistant sur l'interval jusqu'aux premières chaleurs. Une association significative élevée* ($P=0,001$) *entre la date de mise bas après le* 1 *er mars* (K) *et la durée de la période post-partum sans chaleur* (Y) *put être exprimée par:* $Y=58,0-0,23X$. *La sous alimentation durant la lactation réduisit significativement le nombre de brebis qui exhibèrent des chaleurs après la première semaine d'août* 1969, 1970 *et* 1971. *Pour les périodes de* 17 *jours commençant le* 8 *et* 25 *août il y eut une association significative entre le pourcentage des chaleurs des brebis et leur masse corporelle au sevrage (données pour* 1968–1971). *Durant les quatre périodes de* 17 *jours subséquentes l'activité des chaleurs fut en association étroite avec le pourcentage du changement de masse corporelle entre la parturition et le sevrage. Similairement, le nombre de cycles de chaleur montrés par chaque brebis du* 8 *août au* 17 *novembre fut en corrélation négative avec le pourcentage de masse corporelle perdu au sevrage. Les brebis qui furent accouplées tôt après le* 15 *octobre eurent une plus grande masse corporelle avant l'accouplement que celles qui furent accouplées plus tard. La nutrition ainsi que l'isolement des béliers n'eurent pas d'effet notable sur le nombre total des brebis servies durant la période annuelle de reproduction de six semaines.*

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REFERENCES

ADLER, E. D., 1964. Breeding practices, and the fertility of cattle, pigs and sheep in the Natal Region. *Proc. S. Afr. Soc. Anim. Prod.* 3, 13–23.

ALLDEN, W. G., 1956. Time of mating studies. Part I. Time of mating as a factor influencing prolificacy in cross-bred ewes. *J. Dep. Agric. S. Aust.* 59, 337–342.

COOPER, K. J. & HAYNES, N. B., 1967. Modification of the oestrous cycle of the underfed rat associated with presence of the male. *J. Reprod. Fert.* 14, 317–320.

COOPER, K. J., PURVIS, K. & HAYNES, N. B., 1972. Further observations on the ability of the male to influence the oestrous cycle of the underfed rat. *J. Reprod. Fert.* 28, 473–475.

DUN, R. B., AHMED, W. & MORRANT, A. J., 1960. Annual reproductive rhythm in Merino sheep related to the choice of a mating time at Trangie, central western New South Wales. *Aust. J. Agric. Res.* 11, 805–826.

FLETCHER, I. C., 1971. Relationships between frequency of suckling, lamb growth and post-partum oestrous behaviour in ewes. *Anim. Behav.* 19, 108–111.

GRANGER, W., 1947. Conception in lactating ewes. *Aust. vet. J.* 23, 143–145.

HAFEZ, E. S. E., 1952. Studies on the breeding season and reproduction of the ewe. Parts I–V. *J. agric. Sci. Camb.* 42, 189–265.

HUNTER, G. L., 1962. Observations on oestrus in Merinos. *Proc. S.Afr. Soc. Anim. Prod.* 1, 67–69.

HUNTER, G. L., 1964. The effects of season of mating on oestrus and fertility in the ewe. *Proc. S. Afr. Soc. Anim. Prod.* 3, 196–206.

HUNTER, G. L., 1968. Increasing the frequency of pregnancy in sheep. I. Some factors affecting rebreeding during the post-partum period. *Anim. Breed. Abstr.* 36, 347–378.

HUNTER, G. L. & LISHMAN, A. W., 1967. Post-partum ovulation and oestrus in spring-lambing ewes. *J. Reprod. Fert.* 14, 473–475.

HUNTER, G. L. & VAN AARDE, I. M. R., 1973. Influence of season of lambing on post-partum intervals to ovulation and oestrus in lactating and dry ewes at different nutritional levels. *J. Reprod. Fert.* 32, 1–8.

JOUBERT, D. M., 1962. Sex behaviour of purebred and crossbred Merino and Blackhead Persian ewes. *J. Reprod. Fert.* 3, 41–49.

KIRILLOV, V., 1944. Organisation of closely-spaced lamb crops. *Sovkhoz. Proizv.*, 1944 (12), 34–36. *Anim. Breed. Abstr.* 13, 148.

LEES, J. L., 1966. Variations in the time of onset of the breeding season in Clun ewes. *J. agric. Sci. Camb.* 67, 173–179.

LISHMAN, A. W., 1969. The seasonal pattern of oestrus amongst ewes as affected by isolation from and joining with rams. *Agroanimalia* 1, 95–102.

LISHMAN, A. W. & DE LANGE, G. M., 1967. The incidence of oestrus in Merino ewes following introduction of breeding rams in summer and spring. *Proc. S. Afr. Soc. Anim. Prod.* 6, 196–198.

LISHMAN, A. W. & HUNTER, G. L., 1966. Sexual activity in the ewe following isolation from the ram during the breeding season. *S. Afr. J. agric. Sci.* 9, 993–1004.

LISHMAN, A. W. & HUNTER, G. L., 1967. Oestrous activity in the ewe following isolation from the ram during anoestrus. *S. Afr. J. agric. Sci.* 10, 747–752.

LYLE, A. D. & HUNTER, G. L. 1967. The effects of ram introduction during spring, summer and autumn on the incidence of oestrus in three Merino breeding flocks in East Griqualand. *Proc. S. Afr. Soc. Anim. Prod.* 6, 202–207.

McNEILLY, A. S., COOPER, K. J. & CRICHTON, D. B., 1970. Modification of the oestrous cycle of the underfed rat induced by the proximity of a male. *J. Reprod. Fert.* 22, 359–361.

MORRISON, F. B., 1956. Feeds and Feeding. 22nd ed. Morrison Publishing Co., Ithaca. New York.

RADFORD, H. M., WATSON, R. H. & WOOD, G. F., 1960. A crayon and associated harness for the detection of mating under field conditions. *Aust. vet. J.* 36, 57–66.

REYNEKE, J., 1969. Autumn versus spring lambs. *Proc. S. Afr. Soc. Anim. Prod.* 8, 147–148.

ROUX, L. L., 1936. Sex physiology of the sheep. *Onderstepoort J. vet. Sci. Anim. Ind.* 6, 465–717.

SHELTON, M. & MORROW, J. T., 1965. Effect of season on reproduction of Rambouillet ewes. *J. Anim. Sci.* 24, 795–799.

SHELTON & RADFORD, 1966.

SMITH, I. D., 1962. The effect of plane of nutrition on the incidence of oestrus in the Merino ewe in Queensland. *Aust. vet. J.* 38, 338–340.

SMITH, I. D., 1964. Postparturient anoestrus in the Peppin Merino in western Queensland. *Aust. vet. J.* 40, 199–201.

SMITH, I. D., 1965. The influence of level of nutrition during winter and spring upon oestrous activity in the ewe. *Wld. Rev. Anim. Prod.* 1, 95–102.

SMITH, I. D., 1966. Oestrous activity in Merino ewes in western Queensland. *Proc. Aust. Soc. Anim. Prod.* 6, 69–79.

THOMAS, W. & AITKEN, F. C., 1959 Diet in relation to reproduction and the viability of the young. Pt II Sheep: World survey of reproduction and review of feeding requirements *Tech. Commun. Bur. Anim. Nutr.* 20, Comw. Agric. Bur. 93 p.

VAN NIEKERK, B. D. H. & MULDER, A. M., 1965. Duration of pregnancy and post-partum anoestrus in autumn-mated Dorper, Döhne Merino and Merino ewes. *Proc. S. Afr. Soc. Anim. Prod.* 4, 205–212.

VOSLOO, L. P., HUNTER, G. L. & CARSTENS, J. de W., 1969. Influence of level of nutrition during gestation and lactation on post-partum interval to ovulation and rebreeding of ewes. *Proc. S. Afr. Soc. Anim. Prod.* 8, 145–146.

WATSON, R. H. & RADFORD, H. M., 1966. Seasonal variation in fertility in Merino ewes: The reproductive wastage associated with mating in winter, spring, summer or autumn. *Aust. J. agric. Res.* 17, 335–345.

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REPRODUCTION IN THE EWE IN RELATION TO PLANE OF NUTRITION, BODY MASS AND CHANGE OF BODY MASS. II. LAMBING PERFORMANCE

(*Met opsomming in Afrikaans*)
(*Avec résumé en français*)

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ABSTRACT

During an 84-day autumn lactation Merino and crossbred ewes were fed rations which supplied either 100% or 50% of the estimated daily nutrient requirements. The effect of flushing (140–147% of daily nutrient requirements) prior to mating and of isolation from rams after lactation on the lambing performance of the ewes was also investigated. The number of lambs (0, 1 or 2) produced during the subsequent lambing season was correlated positively with the body mass and negatively with the loss in body mass during lactation of each ewe. The size of the litter was not significantly influenced by flushing or isolation from rams.

INTRODUCTION

It is generally recognised that the level of nutrition and attendant changes in body mass play an important role in determining ovulation rate (number of ovulations per oestrus) in the ewe (Clark, 1934; McKenzie & Terrill, 1937; Allen & Lamming, 1961; Wallace, 1961; Hill, Lamond & Godley, 1969; Lamond, Gaddy & Kennedy, 1972). The practice of flushing during and/or prior to the breeding period is usually found to increase the ovulation rate (Allen & Lamming, 1961; Wallace, 1961; Killeen, 1967). The improved lambing percentage noted when ewes are mated in autumn has has been demonstrated to be due largely to the high incidence of multiple ovulations at this time (Dun, Ahmed, & Morrant, 1960).

Studies by Coop (1966) have shown very clearly that ovulation rate is partly dependent on the body mass of a ewe at mating (the static effect) and partly upon the rising plane of nutrition at this time (the dynamic effect). The possibility that lambing rates may be reduced by inadequate nutrition during the previous lactation appears to be a distinct possibility and according to Coop (1966) flushing prior to mating cannot entirely overcome the effect on ovulation rate of poor nutrition at some earlier stage.

These considerations formed the basis of the study to be reported here and attention was also given to the effect of nutrition on barrenness.

PROCEDURE

Three days after parturition ewes drawn from a flock of Merino and a flock of crossbred ewes (South African Mutton Merino × Merino) were randomly divided into groups which received either 100% (High plane) or 50% (Low plane) of their estimated daily feed requirements during the 12-week lactation period. After weaning of the lambs all the ewes received 100% of their estimated daily requirements. On 11 October 1967, 1968 and 1969 the two groups were each further sub-divided so that about half the ewes could be fed 140–147% of the estimated daily feed requirements of non-lactating ewes for the following 21 days (flushing period). The remaining sheep continued at the post-weaning level of nutrition until 1 November.

Twice-daily observations for oestrus were conducted from parturition until initiation of the annual breeding period (1 November), but in 1967 (all the ewes) and 1968, 1969 and 1970 (half of each sub-group) the ewes were isolated from rams when lactation ceased. On 15 October these ewes were rejoined with vasectomized rams. This date was chosen in order to synchronise the expected period of maximum incidence of oestrus with the end of the flushing period.

The rations fed and general conduct of the experiment have been described earlier (Lishman, Stielau & Botha, 1974).

RESULTS

Changes in body mass

The overall response to flushing was small, being generally less than a 10% gain in body mass (Table 1).

Number of lambs

The number of lambs produced by the ewes during each yearly replication of the experiment, and the proportion of barren ewes, are presented in Table 1. Neither the Low plane of nutrition during lactation, nor flushing prior to mating significantly influenced the lambing performance of the ewes.

Relationships between body mass and the number of lambs produced

Classification of the ewes according to the number of lambs produced during the lambing season and the various measures of stress described earlier (Lishman *et al.*, 1974) showed (Table 2, Fig. 1) that the individuals with the greatest body mass, or those undergoing the smallest change in body mass during the previous lactation, were likely to produce the most lambs subsequently. Isolation of the ewes from rams after weaning of their lambs had no consistent effect on the lambing performance.

The results in Table 2 suggest that the number of lambs born per ewe was more closely associated with the change in body mass of the ewe prior to the cessation of lactation than with her body mass. Virtually all the results in Table 2, suggest curvilinear relationships.

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TABLE 1 Average change in body mass during the flushing period and the lambing performance of groups of ewes maintained on two levels of nutrition during the preceding lactation
TABEL 1 *Gemiddelde verandering in liggaamsmassa gedurende die prikkelvoedingstydperk en die lamprestasie van groepe ooie op twee voedingspeile gedurende die vorige laktasie*

Year/ *Jaar*	Type of ewe/ *Tipe ooi*	Average age (yr) *Gemiddelde ouderdom (jr)*	Nutritional plane/ *Voedingspeil*		Number of animals/ *Getal diere*	Flushing gain (%)/ *Toename a.g.v. prikkelvoeding (%)*	Number of ewes/ *Getal ooie*						Lambs/100 ewes mated/ *Lammers/100 ooie gedek*
			Lactation/ *Laktasie*	Pre-mating/ *Voor paring*			Served and not lambing/ *Gedek en nie gelam*		Producing singles/ *Enkelinge voortgebring*		Producing twins/ *Tweelinge voortgebring*		
							No./*Getal*	%	No./*Getal*	%	No./*Getal*	%	
1967	Merino........................	5	High/*Hoog*	High/*Hoog*	28	9,7	1	3,6	23	82,0	4	14,3	110,7
			High/*Hoog*	Low/*Laag*	29	0	2	10,7	23	79,3	3	10,3	100,0
			Low/*Laag*	High/*Hoog*	28	10,9	3	10,7	24	85,7	1	3,6	92,8
			Low/*Laag*	Low/*Laag*	24	−2,0	5	20,8	18	75,0	1	4,2	83,3
1968	Crossbred/*Kruis*................	6	High/*Hoog*	High/*Hoog*	58	6,7	9	15,5	39	67,2	10	17,2	101,7
			High/*Hoog*	Low/*Laag*	57	0,2	8	14,6	41	71,9	8	14,0	100,0
			Low/*Laag*	High/*Hoog*	58	10,2	8	13,8	38	65,5	12	20,7	106,9
			Low/*Laag*	Low/*Laag*	55	−3,3	9	16,4	36	65,4	10	18,2	101,8
1969	Merino........................	7	High/*Hoog*	High/*Hoog*	56	6,7	3	5,3	50	89,3	3	5,3	100,8
			High/*Hoog*	Low/*Laag*	52	0,7	5	9,6	40	76,9	7	13,5	103,8
			Low/*Laag*	High/*Hoog*	51	9,5	9	17,6	36	70,6	6	11,8	94,1
			Low/*Laag*	High/*Hoog*	54	1,3	5	9,2	44	81,5	5	9,2	100,0
1970	Merino........................	8	High/*Hoog*	Low/*Laag*	89	—	15	16,8	65	73,0	9	10,1	93,2
			Low/*Laag*	Low/*Laag*	82	—	13	15,8	65	79,3	4	4,9	89,0
		Maiden/ *Eersteling* 2–3	High/*Hoog*	Low/*Laag*	35	—	4	11,4	27	77,1	4	11,4	100,0
			Low/*Laag*	Low/*Laag*	37	—	7	18,9	29	78,4	1	2,7	83,7
	Crossbred/*Kruis*................	8	High/*Hoog*	Low/*Laag*	38	—	6	15,8	22	57,9	10	26,3	115,8
			Low/*Laag*	Low/*Laag*	36	—	8	22,2	21	58,3	7	19,4	97,2
		Maiden/ *Eersteling* 2–3	High/*Hoog*	Low/*Laag*	32	—	1	3,1	24	75,0	7	21,9	118,7
			Low/*Laag*	Low/*Laag*	31	—	3	9,7	25	80,6	3	9,7	100,0
1971	Merino........................	3–5	High/*Hoog*	Low/*Laag*	60	—	8	13,3	42	70,0	10	16,7	103,3
			Low/*Laag*	Low/*Laag*	64	—	12	18,7	44	68,7	8	12,5	93,7

TABLE 2 Relationship between number of lambs born (Y) and body mass or change in body mass (X) of ewes. Results which differ significantly are joined by a solid line and the level of significance is designated as: *P = 0,05; **P = 0,01; ***P = 0,001

TABEL 2 *Verwantskap tussen getal lammers gebore (Y) en liggaamsmassa of verandering in liggaamsmassa (X) van ooie. Resultate wat betekenisvol verskil word verbind met 'n soliede lyn en die peil van betekenisvolheid word aangedui as: *P = 0,05; **P = 0,01; ***P = 0,001*

Number of ewes in each category/ *Getal ooie in in elke kategorie*			Classification of ewes according to body mass or change in body mass during lactation and subsequent lamb production/ *Klassifikasie van ooie volgens liggaamsmassa of verandering in liggaamsmassa gedurende laktasie en daaropvolgende lamproduksie*																				
			Post-partum (kg)			Weaning/*Speen* (kg)			Minimum (kg)			Accumulative difference (kg)/ *Kumulatiewe verskil (kg)*			Per cent change to weaning/ *Persentasie verandering tot speen*			Per cent change to minimum/ *Persentasie verandering tot minimum*			Pre-mating (kg)/ *Voor paring* (kg)		
Lambs/*Lammers*			Lambs/*Lammers*			Lambs/*Lammers*			Lambs/*Lammers*			Lambs/*Lammers*			Lambs/*Lammers*			Lambs/*Lammers*			Lambs/*Lammers*		
0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
					***			***			***		*	*			**		*	**			***
148	770	136	44,3	44,6	49,2	40,1	41,3	47,0	37,4	38,4	43,6	−52,7	−43,7	−34,6	−9,1	−7,1	−4,2	−15,4	−13,8	−11,2	42,9	44,2	51,1
				***			***			***			***			**			**			***	
SE^1/*SF*^{1}			±6,28			±7,49			±6,56			±47,64			±12,59			±8,93			±8,38		

(1) SE of a single observation/(1) *SF van 'n enkele obserwasie*

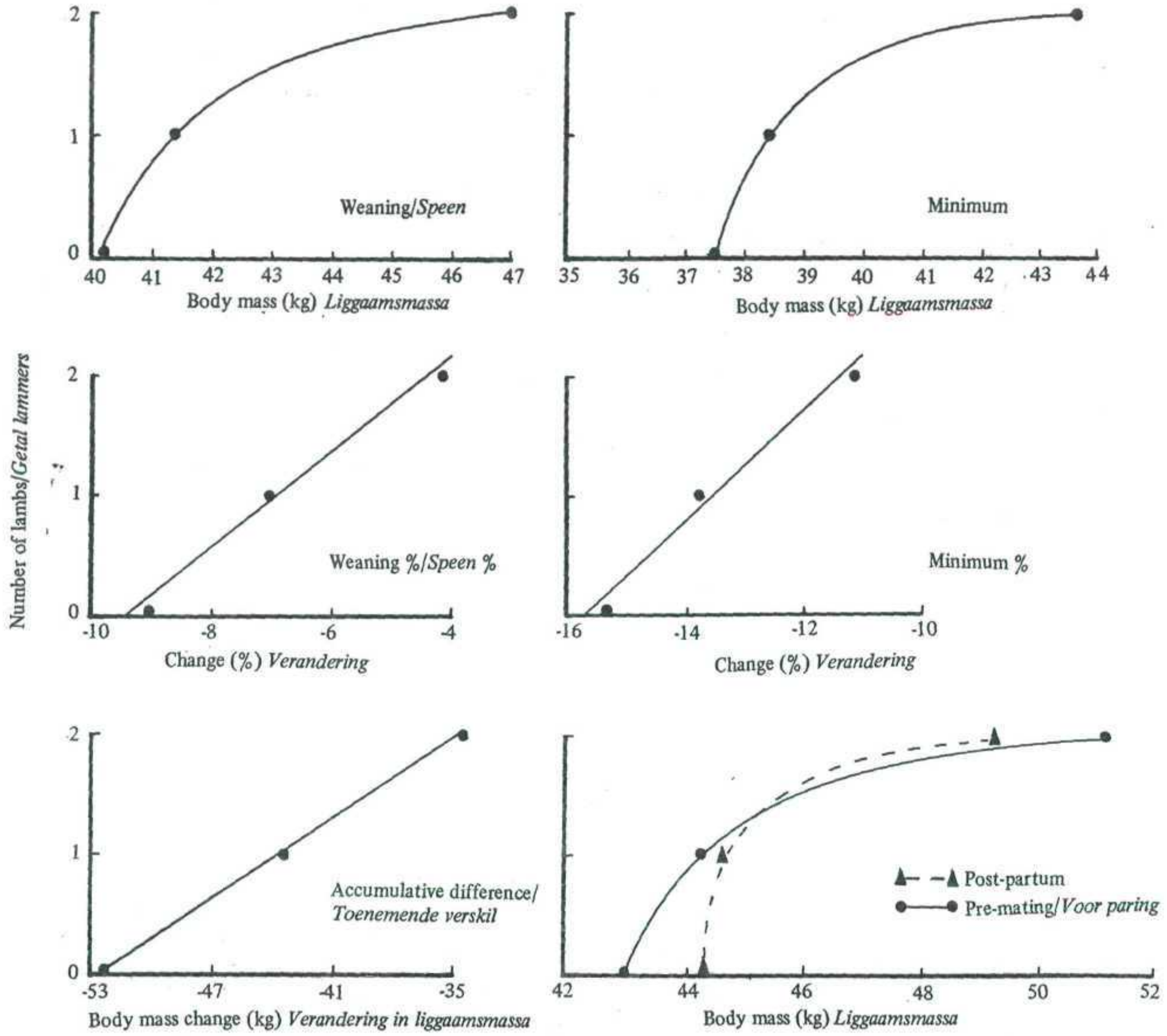

FIG. 1 Relationship between body mass or change in body mass and number of lambs born
FIG. 1 *Verwantskap tussen liggaamsmassa of verandering in liggaamsmassa en getal lammers gebore*

DISCUSSION

The number of lambs born to a ewe is determined not only by the ovulation rate but also by the success of both conception and gestation. Information now available shows that the physical condition of the ewe at or prior to the onset of a new breeding season can have a marked effect on her breeding performance. The finding that ewes with a high body mass, or those suffering the smallest loss in body mass during their previous lactation, gave the best lambing performance is of considerable interest and practical importance. Coop (1962) and others (Wallace, 1961; Tribe & Seebeck, 1962; Coop, 1966; Lino & Braden, 1968; Cumming & Blockey, 1971) have shown that a relationship exists between the body mass of a ewe and litter size. The present data suggest that the body mass of the ewe at mating may be less closely related to the subsequent lambing rate than changes in body mass occurring at an earlier stage. This is in agreement with the conclusions of Coop (1966), and Guerra, Thwaites & Edey (1972) believe that the body mass of the ewe is more closely associated with ovulation rate than either size or condition of the ewe taken separately. The apparent curvilinear relationship between the parameters relating to body mass and lambing rate (Table 2, Fig. 1) agrees with the findings concerning ovulation rate (Edey, 1968) and barrenness (Coop, 1966; Gunn, Doney & Russell, 1969). Clearly, undernutrition of the lactating ewe is undesirable and this factor may play a major role where the frequency of lambing is increased to more than once every 12 months. The findings also confirm the contention (Guerra *et al.*, 1972) that when the plane of nutrition is high, body size may limit the reproductive rate. Large animals do not only cycle more regularly, but they are also more likely to be served early in the breeding season and to produce more lambs per parturition than small individuals. For example, a ewe with a body mass exceeding 45 kg at the conclusion of the lactation period is more likely to exhibit continued oestrous activity during the accepted non-breeding period (July-September), to be mated within one oestrous cycle after exposure to breeding rams and to produce twin lambs, than a ewe with a lesser body mass. The evidence is therefore in favour of culling ewes of sub-standard body size. It has been suggested that the critical body mass at joining for Corriedale, Romney Marsh (Coop, 1962) and Border Leicester

× Merino ewes (Killeen, 1967) is between 41 to 45 kg. A somewhat lower body mass of below 38,7 kg has been proposed for Merino ewes (McInnes & Smith, 1966). The commonly accepted figure of 36 kg (80 lb) for maiden Merino ewes at first mating therefore appears acceptable. The additional advantage of higher wool yield and heavier lambs (Ray & Smith, 1966) further amplifies the advantage to be gained by selecting heavy breeding females.

In this investigation no attention has been given to the basic reasons for the failure of conception or gestation. Moule (1966), Quinlivan, Martin, Taylor & Cairney (1966 a, b) and Chopping & Lindsay (1970) have concluded that ewes which are mated, but do not lamb, contribute greatly to the wastage of potential lambs. Our data show that this figure varied from 3,1 to 33,3%, which agrees closely with the results obtained by Mullaney (1966).

It is generally agreed that flushing for three weeks or less prior to mating can increase the twinning rate (Hulet, Blackwell, Ercanbrack, Price & Humphrey, 1962; Tribe & Seebeck, 1962; Coetzee, 1964; Coop, 1966). In the present experiment flushing had little effect on either the body mass of the ewe or on the proportion of multiple births recorded. The low gains in body mass were probably due to the fact that the ewes had been well fed for some 11 weeks prior to the onset of the flushing period. According to Coop (1966) such conditions are conducive to a high incidence of twinning. Since this was not achieved it is possible that selection, either natural or artificial, against twinning has taken place in the parental flocks. Darlow (1942) has suggested that in ewes having a low genetic potential for increased ova production the response to flushing will be small. The findings of Bellows, Pope, Chapman & Casida (1963), Bellows, Pope, Meyer, Chapman & Casida (1963) and Lamond (1963) support this contention.

Opsomming

VOORTPLANTING BY DIE OOI IN VERHOUDING TOT VOEDINGSPEIL, LIGGAAMSMASSA EN VERANDERING IN LIGGAAMSMASSA. II. LAMPRESTASIE

Die moontlikheid dat lampersentasies deur ondervoeding verlaag kan word is beproef deur aan Merino en kruisgeteelde ooie wat 84 dae lank gedurende herfs in melk was, òf 100% òf 50% van hul beraamde daaglikse voedingsbenodigdhede te voer. Die invloed van prikkelvoeding (140 *tot* 147% *van daaglikse voedingsbenodigdhede) voor paring en van afsondering van ramme na beëindiging van laktasie op die lamprestasie is ook ondersoek. Die getal lammers* (0,1 *of* 2) *wat gedurende die daaropvolgende lamseisoen gebore is, was positief gekorreleerd met liggaamsmassa en negatief gekorreleerd met veranderings in liggaamsmassa gedurende laktasie. Die grootte van die werpsel is nie betekenisvol beïnvloed deur prikkelvoeding of die afsondering van ramme nie.*

Résumé

LA REPRODUCTION DE LA BREBIS PAR RAPPORT AU PLAN DE NUTRITION, À LA MASSE CORPORELLE ET AU CHANGEMENT INTERVENENT DANS LA MASSE CORPORELLE

Durant une lactation automnale de 84 *jours, des brebis Merino et des brebis croisées furent alimentées sur des rations qui suppléaient soit* 100 *ou* 50% *des nécessités nutritives journalières estimées. L'effet de la douche* (140-147% *des nécessités nutritives journalières) préalable à l'accouplement ainsi que l'isolation des béliers après la lactation, sur la performance de mise bas brebis ont également été observés. Le nombre d'agneaux* (0, 1 *ou* 2) *produits pendant la saison d'agnelage subséquente fut en corrélation positive avec la masse corporelle et négativement avec la perte de masse corporelle survenant durant la lactation de chaque brebis. La taille du nouveau né ne fut pas significativement influencée par la douche ou l'isolation des béliers.*

REFERENCES

ALLEN, D. M. & LAMMING, G. E., 1961. Nutrition and reproduction in the ewe. *J. agric. Sci. Camb.* 56, 69–79.

BELLOWS, R. A., POPE, A. L., CHAPMAN, A. B. & CASIDA, L. E., 1963. Effect of level and sequence of feeding and breed on ovulation rate, embryo survival and foetal growth in the mature ewe. *J. Anim. Sci.* 22, 101–108.

BELLOWS, R. A., POPE, A. L., MEYER, R. K., CHAPMAN, A. B. & CASIDA, L. E., 1963. Physiological mechanisms in nutritionally-induced differences in ovarian activity of mature ewes. *J. Anim. Sci.* 22, 93–100.

CHOPPING, M. H. & LINDSAY, D. R., 1970. An approach to the study of reproductive wastage in sheep. *Proc. Aust. Soc. Anim. Prod.* 8, 312–316.

CLARK, R. T., 1934. Studies on the physiology of reproduction in the sheep. I. The ovulation rate of the ewe as affected by plane of nutrition. *Anat. Rec.* 60, 125–135.

COETZEE, C. G., 1964. Invloed van kragvoerprikkelvoeding op aanteelvermoë van ooie. *Proc. S. Afr. Soc. Anim. Prod.* 3, 63–65.

COOP, I. E., 1962. Liveweight-productivity relationships in sheep. I. Liveweight and reproduction. *N.Z. Jl agric. Res.* 5, 249–264.

COOP, I. E., 1966. Effect of flushing on reproductive performance of ewes. *J. agric. Sci. Camb.* 67, 305–323.

CUMMING, J. A. & BLOCKEY, M. A. de B., 1971. The effect of liveweight and plane of nutrition on ovulation, plasma progesterone and luteinizing hormone in the ewe. 4th Asia & Oceania Congr. Endocr., Auckland, No. 42.

DARLOW, A. E., 1942. Effect of plane of nutrition on reproductive processes in the ewe. Ph. D. Thesis, Univ. Wisconsin, Madison, Wisconsin.

DUN, R. B., AHMED, W., & MORRANT, A. J., 1960. Annual reproductive rhythm in Merino sheep related to the choice of a mating time at Trangie, central western New South Wales. *Aust. J. agric. Res.* 11, 805–826.

EDEY, T. N., 1968. Bodyweight and ovulation rate in sheep. *Proc. Aust. Soc. Anim. Prod.* 7, 188–191.

GUERRA, J. C., THWAITES, C. J., & EDEY, T. N., 1972. The effects of components of bodyweight on reproductive efficiency in the Merino ewe. *J. agric. Sci., Camb.* 78, 245–249.

GUNN, R. G., DONEY, J. M. & RUSSEL, A. J. F., 1969. Fertility in Scottish Blackface ewes as influenced by nutrition and body condition at mating. *J. agric. Sci., Camb.* 73, 289–294.

HILL, J. R., LAMOND, D. R. & GODLEY, W. C., 1969. Nutritional effects on fertilization in the ewe. *J. Anim. Sci.* 28, 148–149.

HULET, C. V., BLACKWELL, R. L., ERCANBRACK, S. K., PRICE, D. A. & HUMPHREY, R. D., 1962. Effects of feed and length of flushing period on lamb production in range ewes. *J. Anim. Sci.* 21, 505–510.

KILLEEN, I. D., 1967. The effects of body weight and level of nutrition before, during, and after joining on ewe fertility. *Aust. J. exp. Agric. Anim. Husb.* 7, 126–136.

LAMOND, D. R., 1963. Effect of nutritive status on oestrus, ovulation and graafian follicles in Merino ewes. *Aust. J. agric. Res.* 14, 483–491.

LAMOND, D. R., GADDY, R. G. & KENNEDY, S. W., 1972. Influence of season and nutrition on luteal plasma progesterone in Rambouillet ewes. *J. Anim. Sci.* 34, 626–629.

LINO, B. F. & BRADEN, A. W. H., 1968. An investigation of early prenatal loss in a commercial sheep flock. *Aust. J. exp. Agric. Anim. Husb.* 8, 505–509.

LISHMAN, A. W., STIELAU, W. J. & BOTHA, W. A., 1974. Reproduction in the ewe in relation to plane of nutrition, body mass and change of body mass. I. The incidence of oestrus between lambing and reconception. *Agroanimalia* 6 (in press).

McINNES, P. & SMITH, M. D., 1966. The effect of nutrition before mating on the reproductive performance of Merino ewes. *Aust. J. exp. Agric. Anim. Husb.* 6, 455–459.

McKENZIE, F. F. & TERRILL, G. E., 1937. Oestrus, ovulation, and related phenomena in the ewe. *Univ. Miss. agric. exp. Stn. Bull.* 264, 3–88.

MOULE, G. R., 1966. Ovine reproduction in tropical Australia. *Aust. vet. J.* 42, 13–18.

MULLANEY, P. D., 1966. Pre-natal losses in sheep in western Victoria, *Proc. Aust. Soc. Anim. Prod.* 6, 56–59.

QUINLIVAN, T. D., MARTIN, C. A., TAYLOR, W. B. & CAIRNEY, I. M., 1966a. Estimates of pre- and perinatal mortality in the New Zealand Rommey Marsh ewe. I. Pre- and perinatal mortality in those ewes that conceived to one service. *J. Reprod. Fert.* 11, 379–390.

QUINLIVAN, T. D., MARTIN, C. A., TAYLOR, W. B. & CAIRNEY, I. M., 1966b. Estimates of pre- and perinatal mortality in the New Zealand Romney Marsh ewe. II. Pre- and perinatal loss in those ewes that conceived to second service and those that returned to second service and were mated a third time. *J. Reprod. Fert.* 11, 391–398.

RAY, E. E. & SMITH, S. L., 1966. Effect of body weight of ewes on subsequent lamb production. *J. Anim. Sci.* 25, 1172–1175.

TRIBE, D. E. & SEEBECK, R. M., 1962. Effect of live-weight and live-weight change on the lambing performance of ewes. *J. agric. Sci. Camb.* 59, 105–110.

WALLACE, L. R., 1961. Influence of liveweight and condition on ewe fertility. *Proc. Ruakura Fmrs Conf. Wk.* 14–23.

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UNDERNUTRITION DURING THE LACTATION AND/OR POST-WEANING PERIODS AND THE REPRODUCTIVE PERFORMANCE OF MERINO EWES

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The lower lambing rate, where ewes are mated in spring and early summer, compared to autumn, might be due to the fact that anoestrus can continue even into the summer months. (Dun, Ahmed & Morrant, 1960; Adler, 1964; Shelton & Morrow, 1965; Watson & Radford, 1966). The possibility also exists that inadequate nutrition during the preceding winter will further reduce the proportion of ewes mating during spring (Hunter, 1962; Smith, 1965).

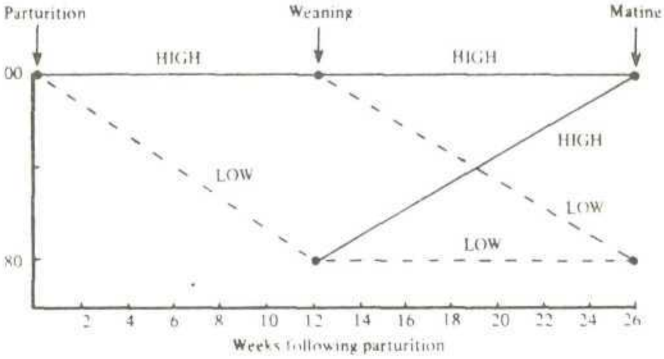

Fig. 1 *Planned changes in bodymass of ewes on high (–) or low (– –) pre- and or post-weaning planes of feeding*

Lishman, Stielau & Botha (1974 a, b) set out to test whether these factors would affect the lambing rate of ewes subjected to a regular annual mating-lambing regime. Contrary to expectation, they found that even where a 20% loss in bodymass occurred during the autumn lactation period, this had only a minor depressing effect on the lambing rate of the ewes. However, they emphasised that their results provided no indication of what the consequences would be if feeding conditions did not improve during the post-weaning period. Their caution was justified since Smith (1965) had found that the oestrous activity during the mating period was influenced by both the current level of nutrition and that which prevailed several months earlier. Accordingly, the object of this study was to examine the effect of periods of undernutrition which did not occur only during the lactation period.

One day after parturition, 113 Merino ewes (2–7 years old) which had been grazed on kikuyu pasture and which lambed 28 March to 21 April were weighed and randomly allocated into 4 treatment groups (Fig. 1) Each ewe sucked only a single lamb and during the 12-week lactation period 2 groups of ewes (HH and HL) were fed on dry-lot so as to maintain the bodymass

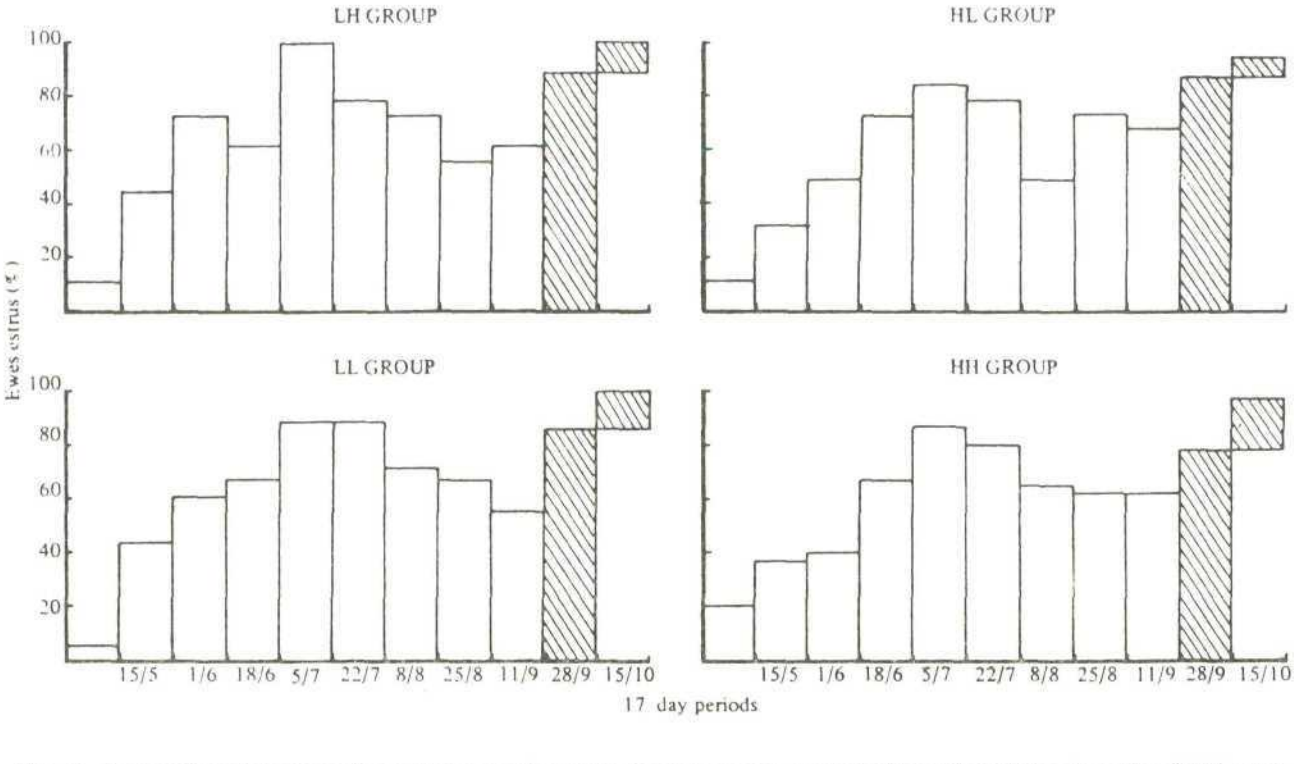

Fig. 2 *Seasonal variations in the occurrence of oestrus in groups of ewes underfed either during lactation (LH), post-weaning (HL), both during lactation and postweaning (LL) or not underfed (HH). The cross-hatched section refers to mating with fertile rams.*

(wool growth excluded) recorded at parturition (PBM). The ration was compiled so as to meet the requirements of a 45 kg lactating ewe (Morrison, 1956; NRC 1968) and consisted of 1,6 kg maize silage, 0,9 kg milled lucerne hay and 0,7 kg of a maizemeal-fishmeal concentrate. The remaining 2 groups (LL and LH) were fed so that at weaning they would weigh approximately 20% less than at lambing. Their daily feed allowance was 50% of that fed to the HH and LH groups, except for maize silage which was reduced to 42%. After weaning of the lambs, the plane of feeding was reversed in two groups (HL and LH) and during the post-weaning period the ewes were fed the same ration as during the suckling period, except that the quantities were reduced.

The body mass of the experimental ewes was measured weekly until commencement of the annual breeding period. Observations for oestrus were made twice daily using sexually active vasectomized rams. On 15 October the ewes were returned to kikuyu grazing and joined with vasectomized rams bearing raddle crayons (Radford, Watson & Wood, 1960). The ewes marked were recorded daily during the 42-day breeding period and service was by hand mating to fertile rams. Dosing, inoculation, shearing and mineral supplementation were according to standard procedures.

The changes in bodymass were such that at weanning the average bodymass of the high plane (HH + HL) ewes was 99,2% of the PBM (range 92,6–116,4%). In contrast, the low plane (LL + LH) groups very nearly attained the desired 20% loss, with the average bodymass after 12 weeks of lactation being 83,1% of the PBM (range 65,4–89,4%). During the post-weaning period the change from the low to the high plane of feeding did not result in complete compensation in bodymass (LH = 107,1%; HH = 116,9% of PBM), whereas by 15 October the HL group had achieved a 12,9% loss in bodymass. At this time the LL group averaged 86,0% of the PBM. The change in bodymass of the underfed ewes is similar to that reported for ewes on poor quality pasture during lactation (Allden, 1970).

The nutritional treatments did not significantly influence the occurrence of oestrus until 1 November (Fig. 2) and except for 2 ewes (Table 1) breeding was completed within 30 days after mating with fertile rams had commenced. The failure of underfeeding to depress the oestrous activity of the ewes was surprising in view of several studies which indicated that undernutrition reduced the incidence of oestrus (Allen & Lamming, 1961; Hunter, 1962; Smith, 1962, 1965; McKenzie & Edey, 1975). However, Lishman *et al.* (1974a) showed that the effect of poor nutrition was not consistent from year to year. Furthermore, even where the incidence of oestrus during late winter was reduced by earlier poor feeding, very few ewes failed to be mated during the spring breeding period (Lishman *et al.* 1974a).

The data in Table 1 show that although the proportion of barren ewes (served but not lambing) was considerably higher (35,7%) in the LL than in the HH group (11,4%) this difference was not significant ($\chi^2 = 2,481$; $P < 0,10$). Similarly, when underfeeding during lactation was considered (LH + LL) the percentage of barren ewes (26,6) was not significantly greater ($\chi^2 = 2,557$; $P < 0,10$) than among ewes on the HP at this time (HH + HL = 11,6%). When the nutritional treatments were disregarded, more ewes ($\chi^2 = 2,58$; $P < 0,10$) that had a bodymass of less than 45 kg at mating (28,5%) did not lamb, than ewes weighing in excess of 45 kg (11,1%). Although not statistically significant, the marked increase in the occurrence of barrenness, where ewes were underfed throughout the period from parturition to mating (Table 1), supports the findings of Coop (1966) and Gunn, Doney & Russell (1969). Both Mullaney (1966) and Lishman *et al.* (1974b) have reported that, at times, more than 30% of the ewes which were mated, failed to lamb. The figure of 35,7% for the LL treatment is similar to that recorded for ewes on restricted feeding during lactation and not flushed prior to mating (Lishman *et al.*, 1974b).

The results presented here suggest that if ewes suffer a severe loss in bodymass during an autumn lactation and are unable to compensate for this loss during the post-weaning period then their subsequent lambing rate is likely to be reduced. Contrary to expectation, the ability of such ewes to exhibit oestrus during the spring mating period may not be deleteriously affected. Consequently, the reduced lambing rate observed must be the result of a failure of some mechan-

Table 1

Reproductive performance of ewes subjected to different pre- and post-weaning planes of nutrition

Level of feeding:		Number of ewes					Lambs as a %of ewes mated
Pre-weaning	Post-weaning	Mated	Not mated	Culled+	Lambing	Not lambing	
High	High	36	1	4	31	4	112,9
	Low	35	1	2	30	4	103,3
Low	High	17	0	2	13	3	107,7
	Low	14	0	4	9	5	100,0

\+ Old age

ism other than that which controls the occurrence of oestrus. this hypothesis is supported by the observation of a reduced conception rate in beef cows that received inadequate feeding during lactation (Wiltbank, Rowden, Ingalls, Gregory & Koch 1962). Although these workers did not determine whether ovulation, fertilization or gestation failed, Spitzer, Niswender, Seidel & Wiltbank (1975) concluded that the sub-normal pregnancy rate of heifers on a low energy diet was the consequence of early embyonic loss and not due to a failure of fertilization. Supporting evidence can be found in the observed association between the occurrence of abnormal ova and diet restriction in ewes (Hill *et al.*, 1969). Spitzer *et al.* (1975) could not implicate changes in the secretion of luteinizing hormone and of progesterone in the early death of embryos and further attention needs to be given to the basic mechanisms whereby undernutrition reduces the birth rate in cattle and sheep.

References

ADLER, E.D., 1964. Breeding practices, and the fertility of cattle, pigs and sheep in the Natal Region. *Proc. S. Afr. Soc. Anim. Prod.* 3, 13.

ALLDEN, W.G., 1970. The effects of nutritional deprivation on the subsequent productivity of sheep and cattle. *Nutr. Abstr. Rev.* 40, 1167.

ALLEN, D.M. & LAMMING, G.E., 1961. Nutrition and reproduction in the ewe. *J. agric. Sci. Camb.* 56, 69.

COOP, I.E., 1966. Effect of flushing on reproductive performance of ewes. *J. agric. Sci. Camb.* 56, 305.

DUN, R.B., AHMED, W. & MORRANT, A.J., 1960. Annual reproductive rhythm in Merino sheep related to the choice of a mating time at Trangie, central western New South Wales. *Aust. J. agric. Res.* 11, 805.

GUNN, R.G., DONEY, J.M. & RUSSELL, A.J.F., 1969. Fertility in Scottish Blackface ewes as influenced by nutrition and body condition at mating. *J. agric. Sci. Camb.* 73, 289.

HILL, J.R., LAMOND, D.R. & GODLEY, W.C., 1969. Nutritional effects on fertilization in the ewes. *J. Anim. Sci.* 28, 148.

HUNTER, G.L., 1962. Observations on oestrus in Merinos. *Proc. S. Afr. Soc. Anim. Prod.* 1, 67.

LISHMAN, A.W., STIELAU, W.J. & BOTHA, W.A., 1974a. Reproduction in the ewe in relation to plane of nutrition, bodymass and change in bodymass. I. The incidence of oestrus between lambing and reconception. *Agroanimalia* 6, 25.

LISHMAN, A.W., STIELAU, W.J. & BOTHA, W.A., 1974b. Reproduction in the ewe in relation to plane of nutrition, bodymass and change in bodymass. II. Lambing performance. *Agroanimalia* 6, 75.

McKENZIE, A.J. & EDEY, T.N., 1975. Effects of pre-mating undernutrition on oestrus, ovulation and pre-natal mortality in Merino ewes. *J. agric. Sci. Camb.* 84, 19.

MORRISON, F.B., 1965. Feeds and feeding. 22nd Ed. Morrison Publishing Co., Ithaca, N.Y.

MULLANEY, P.D., 1966. Pre-natal losses in sheep in western Victoria. *Proc. Aust. Soc. Anim. Prod.* 6, 56.

N.R.C., 1968. Nutrient requirements of domestic animals, No. 5. Nutrient requirements of sheep. National Research Council. Washington D.C.

RADFORD, H.M., WATSON, R.H. & WOOD, G.F., 1960. A crayon and harness for the detection of mating under field conditions. *Aust. vet. J.* 36, 57.

SHELTON, M. & MORROW, J.T., 1965. Effect of season on reproduction in Rambouillet ewes. *J. Anim. Sci.* 24, 795.

SMITH, I.D., 1962. The effect of plane of nutrition on the incidence of oestrus in the Merino ewe in Queensland. *Aust. vet. J. 38, 338.*

SMITH, I.D., 1965. The influence of level of nutrition during winter and spring upon oestrous activity in the ewe. *Wld. Rev. Anim. Prod.* 3, 95.

SPITZER, J.C., NISWENDER, G.D., SEIDEL, G.E. & WILTBANK, J.N., 1975. Fertilization and endocrinology in underfed heifers. *J. Anim. Sci.* 41, 380.

WATSON, R.H. & RADFORD, R.M., 1966. Seasonal variation in fertility in Merino ewes: The reproductive wastage associated with mating in winter, spring, summer or autumn. *Aust. J. agric. Res.* 17, 335.

WILTBANK, J.N., ROWDEN, W.W., INGALLS, J.E. GREGORY, K.E. & KOCH, R.M., 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. *J. Anim. Sci.* 21, 219.

PAPER 14

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BRIEF COMMUNICATION

POST-PARTUM OVULATION AND OESTRUS IN SPRING-LAMBING EWES

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Summary. By means of ovarian examination and teasing with vasectomized rams, the intervals to first post-partum ovulation and oestrus were determined in a 2^3-factorial experiment with twenty-four German Merino ewes which lambed between 28th October and 15th November 1966. First ovulation occurred 32·1 ± 1·9 (8 to 45) days after lambing. Three, fifteen and six ewes had none, one or more than two silent ovulations respectively, before showing heat after a mean interval from lambing for all ewes of 57·1 ± 4·5 days. The regression of post-partum interval to ovulation (Y) on ewe's weight 3 days *post partum* (X) was significant ($P<0{\cdot}01$); for ewes weighing 83 to 162 lb, $Y = 55{\cdot}15 - 0{\cdot}21X$. The regression of post-partum interval to oestrus on ewe's weight was not significant, and neither were the effects of treatments, namely, weaning at 3 or 20 days *post partum*, supplementary feeding of 0 or 1 lb maize grain daily from 3 to 28 days *post partum*, and joining with vasectomized rams at 3 or 14 days *post partum.*

With a view to increasing the frequency of lambing without resorting to the use of exogenous hormones, some attention has recently been paid to the natural recommencement of ovarian and oestrous cycles during the puerperium of the ewe (Barker & Wiggins, 1964; Smith, 1964; Mauléon & Dauzier, 1965; van Niekerk & Mulder, 1965). It appears that suckling a lamb, the level of nutrition and the stimulus due to the presence of rams may under certain conditions be three important factors which influence the post-partum intervals to ovulation and oestrus.

Twenty-four German Merino ewes, which were being kept continuously in pens and group-fed a maintenance ration of *Eragrostis curvula* hay, maize silage, salt and bonemeal, were mated late in the breeding season in June 1966. During approximately the last month of pregnancy a supplementary ration of uncrushed maize grain was fed at the rate of ¼ lb/ewe daily. The ewes lambed between 28th October and 15th November, which is the time of year

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in South Africa when non-pregnant ewes of this breed are about to start their new breeding season (Hunter, 1964). All ewes suckled their lambs until Day 3 (day of lambing taken as Day 0) and were then randomly allocated in three replications to the eight treatment groups of a 2^3-factorial experiment. The three factors were as follows: (1) weaning: the ewes either suckled their lambs only until Day 3 or continued to suckle one lamb until Day 20, when these lambs were also removed; (2) feeding: on Days 3 to 28 the ewes either received a substantial supplementary ration of 1 lb uncrushed maize grain, or received no supplementary ration; (3) teasing: the ewes were teased with vasectomized rams either from Day 3 or from Day 14, until the first post-partum oestrous periods of all ewes had been recorded. From the start of teasing in each case, vigorous vasectomized rams (which were regularly changed) remained continually with the ewes and, in addition, at 8-hr intervals fresh teasers were introduced to the groups of ewes for periods of 15 min, and mating behaviour observed. The day of first post-partum ovulation was determined at laparotomies performed on all ewes at 20 days *post partum* and, if necessary, at 20-day intervals thereafter, using local anaesthesia and a laparotomy cradle (Lamond & Urquhart, 1961). The day of first post-partum ovulation was estimated by inspection of the ovaries and reference to the illustrations of Quinlan & Maré (1931), showing the appearance of ovaries and corpora lutea of the ewe at different stages of the oestrous cycle.

Apart from one ewe which ovulated 8 days *post partum*, the animals in this experiment ovulated 18 to 45 days after lambing (mean $32{\cdot}1 \pm 1{\cdot}9$ days), sixteen of the twenty-four ewes ovulating after 26 to 40 days. Following lambing, three of the ewes showed oestrus at the time of their first ovulation, fifteen first ovulated without heat, i.e. underwent a silent ovulation, and then showed oestrus one cycle later, while the remaining six ewes had three or more silent ovulations before showing heat. The first oestrous period of the season in the experimental ewes was recorded on 7th December. Thereafter the incidence of oestrus was quite rapid until 12th January, when nineteen of the ewes (79%) had shown heat. An interval of at least a month then passed before the remaining five showed heat. For all ewes, the mean interval between lambing and first heat was $57{\cdot}1 \pm 4{\cdot}5$ days. For the nineteen ewes on heat by 12th January, the mean interval from lambing to first heat was 47·5 (36 to 61) days.

Although the ewes were uniformly fed throughout pregnancy and all lambed in fairly good condition, there was considerable variation in the size of the ewes and a significant linear regression ($P < 0{\cdot}01$) of the post-partum interval to first ovulation (Y) on the ewe's weight 3 days *post partum* (X). For the range in X in this experiment between 83 and 162 lb, $Y = 55{\cdot}15 - 0{\cdot}21X$. Thus, those ewes which weighed about 85 lb shortly after lambing ovulated after a mean of almost 40 days, while heavy ewes, weighing about 160 lb, ovulated about 20 days *post partum*. Due to the greater variation in the post-partum interval to first heat, the regression of this interval on weight of ewe was not significant.

Between 3 and 28 days *post partum* the ewes lost a mean of $4{\cdot}6 \pm 1{\cdot}3$ lb in weight. This loss was significantly increased ($P < 0{\cdot}05$) by suckling a lamb for

20 as opposed to 3 days and significantly decreased ($P < 0.05$) by the supplementary maize ration. However, neither of these treatments, nor the commencement of teasing at different intervals *post partum*, nor any of the interactions, significantly affected the post-partum intervals to ovulation or to oestrus. No ewe ovulated while suckling a lamb. Although two ewes ovulated within 20 days of lambing, both had had their lambs removed 3 days after lambing.

The post-partum intervals to first ovulation and to first oestrus in ewes lambing later in the breeding season may differ markedly from these findings.

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REFERENCES

BARKER, H. B. & WIGGINS, E. L. (1964) Estrual activity in lactating ewes. *J. Anim. Sci.* **23**, 973.

HUNTER, G. L. (1964) The effects of season and mating on oestrus and fertility in the ewe. *Proc. S. Afr. Soc. Anim. Prod.* **3**, 196.

LAMOND, D. R. & URQUHART, E. J. (1961) Sheep laparotomy cradle. *Aust. vet. J.* **37**, 430.

MAULÉON, P. & DAUZIER, L. (1965) Variations de durée de l'anoestrus de lactation chez les brebis de race Ile-de-France. *Annls Biol. anim. Biochim. Biophys.* **5**, 131.

QUINLAN, J. & MARÉ, G. S. (1931) *The physiological changes in the ovary of the Merino sheep in South Africa, and their practical application in breeding.* 17th Rep. Div. Vet. Serv. Anim. Ind., Onderstepoort, p. 663.

SMITH, I. D. (1964) Postparturient anoestrus in the Peppin Merino in western Queensland. *Aust. vet. J.* **40**, 199.

VAN NIEKERK, B. D. H. & MULDER, A. M. (1965) Duration of pregnancy and of post-partum anoestrus in autumn-mated Dorper, Döhne Merino and Merino ewes. *Proc. S. Afr. Soc. Anim. Prod.* **4**, 205.

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NUTRITION OF THE EWE AND THE OVARIAN SENSITIVITY TO GONADOTROPHIN

(*Met opsomming in Afrikaans*)
(*Avec résumé en français*)

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ABSTRACT

The sensitivity of the ovary to gonadotrophic stimulation was tested in Merino and crossbred ewes which had been fed so as to either gain (high plane) or lose (low plane) body mass over a five-month period. The release of endogenous luteinizing hormone (LH) was blocked by daily injections of progesterone and the ovaries were stimulated by injecting the ewes with various doses of human chorionic gonadotrophin (HCG). Undernutrition had no marked influence on ovulation, but the ovaries of the ewes on the high plane of nutrition tended to contain more ovarian fluid than on the low plane of feeding. There was also a tendency for ewes which gained body mass to exhibit a greater ovarian mass than ewes which were underfed. The crossbred ewes showed a greater response to HCG than the Merino ewes.

INTRODUCTION

The ewe has been shown to experience seasonal variations in ovulation rate (Dermody, Foote & Hulet, 1970), ovarian response to exogenous gonadotrophins (Braden, Lamond & Radford, 1960), sensitivity to progesterone (Lamond & Bindon, 1962; Lamond, 1964) and in its response to gonadal steroids (Raeside & McDonald, 1959; Reardon & Robinson, 1960). The finding that the sensitivity of the reproductive mechanisms is usually greatest at the peak of the breeding season suggests that these factors may be involved in the seasonality of breeding. Long-term variations in the level of nutrition of ewes may also affect reproduction via an alteration of one or more of these ovarian mechanisms.

It has been suggested that under conditions of poor nutrition the gonads and accessory sex structures atrophy due to a relative lack of gonadotrophins reaching the target organs, and not because of the development of a refractory state in the gonads (Ratner & Meites, 1963). In order to study variations in ovarian sensitivity to gonadotrophins, a possible approach would be to subject test animals to equal stimuli, for example, equal doses of a gonadotrophin. The administration of exogenous gonadotrophins usually results in a concomitant release of endogenous gonadotrophins (Sugawara, Umezu & Takeuchi, 1969; Cumming, Brown, Blockey & Goding, 1971; Christenson & Eleftheriou, 1972) and the ovarian response then represents the effect of both exogenous and endogenous stimuli acting in concert. The obvious method for excluding the unknown quantities of endogenous gonadotrophins is to remove the pituitary gland, but the problems associated with hypophysectomy on a large scale, and the low survival rate of operated animals, serve as major deterrents to work of this nature.

It is generally believed that progesterone suppresses ovarian cycles by blocking the release of LH from the pituitary gland (Hilliard, Schally & Sawyer, 1971). This principle was used to develop a technique whereby ovulation in ewes could be induced with physiological levels of HCG after prior treatment with progesterone to suppress pituitary release of endogenous gonadotrophin. The ovarian response of ewes which had been subjected to undernutrition could then be tested to determine whether or not the sensitivity of the ovary was lowered by feed restriction.

PROCEDURE

Experiment 1

On May 27, 1970, Merino ewes (two years of age) were randomly divided into two equal groups of 30. Commencing on this date they were placed on high and low levels of nutrition respectively, such that a body mass difference of at least 20% would be produced at the end of a five-month period. The high plane ration consisted of 0,45 kg milled lucerne hay, 0,1 kg maize meal and maize silage *ad lib.* and supplied approximately 0,07 kg digestible protein and 0,56 kg total digestible nutrients. The animals on the low plane were restricted to half the silage and lucerne hay intake of those on the high plane, and the maize meal was replaced by *Eragrostis curvula* hay fed *ad lib*. The body mass of the ewes was recorded at weekly intervals. The ewes were housed in partly-roofed pens.

Commencing on October 27 (day 1) each ewe received a daily intramuscular injection of 10 mg progesterone in 1,0 cm^3 arachis oil. Administration continued at this rate for a further nine days and the dose was then doubled for a further three days. On day 11 the ewes on each plane of nutrition were randomly subdivided into sub-groups and either 350 or 500 IU HCG in 1,0 cm^3 aqueous solution was injected subcutaneously at 08h00. At laparotomy, three days later, the ovaries were excised, trimmed and placed in 0,9% saline. Examinations were made for corpora lutea, indicating recent ovulations, and also for follicular development. The ovaries were then dried on filter paper and the mass measured prior to being sliced into small sections. The ovarian fluid was absorbed onto filter paper and the mass of the remaining ovarian tissue again determined. The quantity of ovarian fluid was taken to equal the total mass of each ovary minus the mass after release of the follicular fluid.

Experiment 2

The results obtained in Expt. 1 suggested that the quantity of ovarian fluid could be used as a sensitive measure of ovarian response. Therefore, the experiment was repeated, but the interval between administration of gonadotrophin and removal of the ovaries was reduced to not more than 24 h. By this means it was hoped to attain maximum ovarian stimulation with a minimum of ovulations occurring. The dose of HCG was also reduced to avoid masking possible differences in ovarian sensitivity.

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A total of 38 Merino and 44 Crossbred (SAMM X Merino) ewes (18 months of age) was randomly allocated to two groups and, commencing on 25 November 1970, they were placed on high and low planes of nutrition. The rations fed were identical to those used in Expt. 1. Each group of ewes was sub-divided prior to the commencement of progesterone administration. The date on which the latter commenced was staggered so as to limit the number of ovariectomies to be performed on any one day. Daily injections of progesterone (10 mg/day) commenced in the first sub-group on 15 April 1971 and continued for 10 days in all animals. On day 11, the daily dose was doubled, as in Expt. 1, and either 0, 250 or 350 IU HCG was injected on this day. The gonadotrophin was administered at 08h00 and ovariectomy took place within 24 hours.

In order to avoid stimulation due to the presence of rams no observations for oestrus were performed.

RESULTS

Change in body mass

The change in body mass of the ewes on the two planes of nutrition is illustrated in Fig. 1. During the 22-week period prior to commencement of progesterone therapy in Expt. 1 the difference in body mass between the ewes on the low and those on the high plane gradually increased until the difference was 39,5%. Most of this difference was due to increases in the mass of the well-fed ewes; the low-plane ewes lost relatively less mass than was gained by the high-plane animals.

A similar situation applied in Expt. 2 where the average body mass of the ewes on the high plane of nutrition exceeded that of the underfed animals by 26,7% in the case of the crossbreds and by 27,0% amongst the Merinos.

Ovarian response

Experiment 1

As may be expected the presence of a corpus luteum markedly increased the mass of the ovary after removal of the ovarian fluid. The quantity of ovarian fluid was also greatly reduced when ovulation occurred. Therefore, the data in Table 1, reflecting ovarian mass and quantity of fluid, represent only ovaries in which no recent ovulation had occurred.

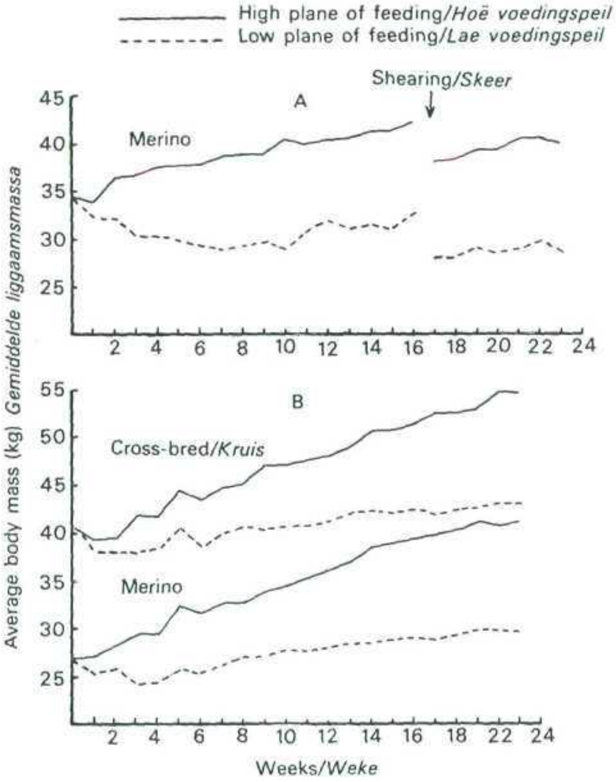

FIG. 1 Changes in body mass of groups of ewes on high and low planes of nutrition
A—commenced 27 May 1970
B—commenced 25 Nov 1970

FIG. 1 *Veranderinge in liggaamsmassa by groepe ooie op hoë en lae voedingspeile*
A—begin 27 Mei 1970
B—begin 25 Nov 1970

TABLE 1 The ovarian response to exogenous gonadotrophin (HCG) in ewes maintained on two planes of nutrition

TABEL 1 *Die reaksie van die eierstokke tot eksogene gonadotrofien (MKH) by ooie wat op twee voedingspeile gehandhaaf is*

Plane of Nutrition *Voedingspeil*	Dose of HCG (IU) *Dosis MKH (IE)*	Number of ewes *Getal ooie*		Total number of *Totaal getal*		Ovaries in which no ovulation occurred Eierstokke met geen ovulasie		
							Left ovary *Linker eierstok*	Right ovary *Regter eierstok*
		Per group *Per groep*	Ovulating *Ovuleer*	Ovulations *Ovulasies*	Large follicles *Groot follikels*	Mean ovarian fluid *Gemiddelde ovarium-vloeistof* (g)	Mean mass *Gemiddelde massa* (g)	Mean mass *Gemiddelde massa* (g)
High/*Hoog*..........	350	15	13	20	16	0,6162	0,3739- SE = ±0,0327	—*—0,5371 ±0,0288
	500	15	13	17	20	0,5961	0,4562 SE = ±0,0327	0,5147 ±0,0306
Low/*Laag*...........	350	15	11	16	20	0,4665	0,4413 SE = ±0,0274	0,4474 ±0,0274
	500	15	12	15	18	0,5480 SE = ±0,0751	0,4926 SE = ±0,0274	0,4908 ±0,0274

* Indicates difference significant, P = 0,05
Dui aan verskil betekenisvol, P = 0,05

TABLE 2 Ovarian response in ewes subjected to two planes of nutrition prior to HCG treatment. Means which differ significantly have been joined by a line. *P = 0,05; **P = 0,01
TABEL 2 *Reaksie van die eierstokke by ooie wat op twee voedingspeile gehandhaaf is voor behandeling met MKH. Gemiddeldes wat betekenisvol verskil word deur 'n lyn verbind.* *P = 0,05; **P = 0,01

Breed *Ras*	Plane of nutrition *Voedingspeil*	Ewes ovulating *Ooie geovuleer*			Ovaries in which no ovulation occurred: *Eierstokke met geen ovulasie:*					
					Mean ovarian fluid (g) *Gemiddelde ovariumvloeistof (g)*			Mean ovarian mass (g) *Gemiddelde ovariummassa (g)*		
		HCG/*MKH*			HCG/*MKH*			HCG/*MKH*		
		0 IU/*IE*	250 IU/*IE*	350 IU/*IE*	0 IU/*IE*	250 IU/*IE*	350 IU/*IE*	0 IU/*IE*	250 IU/*IE*	350 IU/*IE*
Merino	High/*Hoog*	0/6	2/6	2/6	0,2914 SE = ±0,0579	0,2909 ±0,0579	0,4777 ±0,0579	0,5012 SE = ±0,0441	0,4966 ±0,0483	0,4745 ±0,0597
	Low/*Laag*	0/6	1/8	0/6	0,2646 SE = ±0,0579	0,2554 ±0,0458	0,2839 ±0,0579	0,3796 SE = ±0,0441	0,3157 ±0,0408	0,3709 ±0,0441
Crossbred/*Kruis*	High/*Hoog*	0/6	5/8	2/8	0,4615 SE = ±0,0514	0,6018 ±0,0476	0,4575 ±0,0476	0,7729 SE = ±0,0634	0,7078 ±0,0607	0,6005 ±0,0562
	Low/*Laag*	0/6	5/8	6/8	0,3090 SE = ±0,0514	0,4669 ±0,0476	0,5147 ±0,0476	0,5645 SE = ±0,0607	0,5980 ±0,0634	0,6599 ±0,0634

The data in Table 1 show that there was some tendency for the ovaries of the well-fed ewes to contain more ovarian fluid than was present in the ovaries of the ewes subjected to nutritional restriction. This was particularly evident when 350 IU HCG was administered. The left ovary in the underfed group had a significantly lower (P=0,05) mass than the right ovary. No other differences were significant and it is clear that undernutrition had no marked influence upon the ovulatory response of the ovary.

Experiment 2

Even though ovariectomy was performed not more than 24 h after administration of HCG a large proportion of the crossbred ewes had already ovulated by this time (Table 2). Consequently, the mass of the ovary and mass of ovarian fluid are again presented only where ovulation had not occurred at the time of ovariectomy. Since the left and right ovaries showed very little difference in mass, the results in Table 2 represent the average of all ovaries in which no ovulation had occurred.

When the ovarian response to HCG is measured in terms of ovarian fluid, the ewes on the high plane of nutrition tended to show a greater reaction to increasing levels of HCG than animals maintained on a low plane of feeding (Fig. 2). The gonadotrophin treatment had no notable effect on the mass of the ovary (Table 2). Where no gonadotrophin was administered, the high plane of nutrition increased the ovarian mass without markedly influencing the fluid content of such ovaries.

The crossbred ewes appeared to show a greater response to HCG stimulation, both in terms of ovulation and ovarian fluid, than the Merinos. This difference may be influenced to a certain extent by the greater ovarian mass of the former animals. This, in turn, appears to be correlated with the body mass of the ewes.

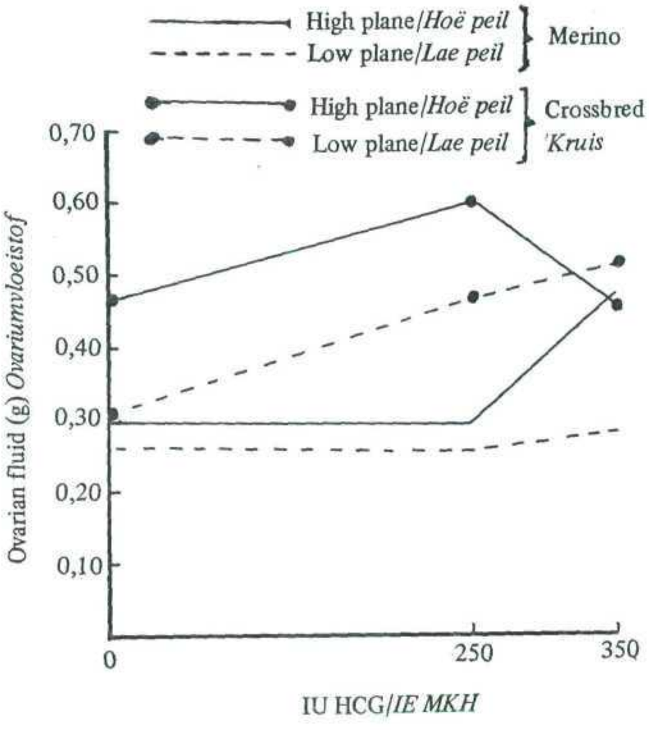

FIG. 2 Ovarian response to HCG in ewes on two planes of nutrition

FIG. 2 *Reaksie van die eierstokke tot MKH by ooie op twee voedingspeile*

DISCUSSION

The hypothesis that the sensitivity of the ovary can vary is supported by an increasing number of reports. Genetic differences in the ovarian response of ewes to exogenous gonadotrophin (PMS) have been reported by Larson, Banbury & Spaeth (1970) and Bindon, Chang & Turner (1971). It is possible that the greater response of animals with a high inherent fecundity could be ascribed to either high levels of endogenous gonadotrophin or to sensitive ovaries. However, comparable studies in rats suggest that the latter mechanism accounts for the genetic differences observed (McLaren, 1962; Mauleón & Rao, 1963; Land & Falconer, 1969). A reduced ovarian sensitivity in rats has also been reported by Liu, Lin & Johnson (1972) and Uilenbrock & van der Werff ten Bosch (1972).

Investigations using hypophysectomized mice have demonstrated unequivocally that under conditions of restricted feeding both the proportion of mice ovulating and the number of ova per ovulating female are lower following gonadotrophin treatment than in similarly treated animals fed *ad lib.* (Fielden & Brumby, 1962). An association between body mass and ovulation rate following treatment with PMSG has been observed in ewes (Guerra, Thwaites & Edey, 1971). The results presented in Fig. 2 suggest that the ovaries of the crossbred ewes were more sensitive to gonadotrophic stimulation, in terms of both ovulation and mass of ovarian fluid, than those of the Merino ewes. This may, perhaps, be correlated with the greater ovarian mass of the former animals rather than with genetic differences, but Land & Falconer (1969) have concluded that the size of the ovary has little or no effect on the ovulation rate of mice.

Howland (1972) reported that underfed rats experience a progressive decline in ovarian mass during a 34-day experimental period and good nutrition showed a tendency to increase the ovarian mass of the ewes in Expt. 2 (Table 2), but the results in Expt. 1 (Table 1) were less conclusive. Varying the dose of gonadotrophin appeared to have little effect on the ovarian mass.

The data obtained in Expt. 2 confirm the finding that ewes ovulate within 20 to 28 h after HCG treatment (Braden, Lamond & Radford, 1960; Dziuk, Hinds, Mansfield & Baker, 1964), but it was considered probable that under conditions of continued pituitary suppression ovulation would be further delayed. The involvement of the pituitary in the response to HCG has also been questioned by Callantine & Humphrey (1965) and these workers concluded that HCG acts only at the ovarian level.

It is obviously necessary to delay the examination of the ovary as much as possible in order to obtain the maximum response to administered gonadotrophins. The apparent shortening of the interval to ovulation, which was observed in the crossbred ewes in Expt. 2, suggests that his latent period could be a useful measure of the degree of stimulation by gonadotrophin.

Available evidence suggests that undernutrition could lead to an early onset or prolonged duration of anoestrus through an interaction of at least three separate mechanisms viz., (i) inadequate synthesis of hypothalamic release factors (Piacsek & Meites, 1967) (ii) a reduced response of the pituitary gland to gonadotrophin release factors and (iii) lowered sensitivity of the hypothalamic centres controlling oestrous behaviour (Gibson & Robinson, 1971).

If the quantity of ovarian fluid is an acceptable measure of ovarian oestrogen production then our results suggest that both the hypothalamic nucleus controlling oestrous behaviour and the pituitary gland may be inadequately sensitized by oestrogens.

This subject requires further study and the use of a progesterone-oestrogen block to gonadotrophin release or the inactivation of endogenous gonadotrophin by appropriate antisera may prove to be useful techniques for this purpose.

Opsomming

VOEDING VAN DIE OOI EN DIE EIERSTOK-SENSITIWITEIT VIR GONADOTROFIEN

Merino- en kruisgeteelde ooie is gevoer om òf 'n toename (hoë peil) òf 'n verlies (lae peil) in liggaamsmassa oor 'n tydperk van vyf maande te ondergaan, met die doel om vas te stel of voeding die sensitiwiteit van die eierstokke kon beïnvloed. Die afskeiding van endogene luteïeniserende hormoon is verhoed deur daaglikse inspuiting met progesteroon en die eierstokke is met verskillende hoeveelhede menslike korioniese hormoon (MKH) gestimuleer. Ondervoeding het geen noemenswaardige invloed op ovulasie gehad nie, maar daar was 'n neiging tot 'n groter hoeveelheid ovariumvloeistof by die ooie op die hoë as op die lae voedingspeil. Die eierstokke van die ooie wat in liggaamsmassa toegeneem het, het 'n hoër massa getoon as dié van ooie op die lae voedingspeil. Die kruisgeteelde ooie het meer gereageer op stimulering met MKH as die Merino-ooie.

Résumé

NUTRITION DE LA BREBIS ET SENSIBILITÉ OVARIENNE A LA STIMULATION PAR GONADOTROPHINE

La sensibilité de l'ovaire à la stimulation gonadotrophique a été testée chez des brebis Merino ainsi que des brebis hybrides qui avaient été alimentées sur des rations à gain et des rations à perte de masse corporelle pour une période de plus de cinq mois.

Le dégagement d'hormone lutéinisante endogène (LH) fut bloqué par des injections quotidiennes de progesterone et les ovaires furent stimulés en injectant les brebis avec des doses variées de gonadotrophine chorionique humaine (HGG). La sous alimentation n'eut pas d'influence marquée sur l'ovulation mais les ovaires des brebis sur un niveau alimentaire plus élevé révélèrent une tendance á contenir plus de fluide ovarien que celles vivant sur une alimentation de bas niveau. Il y eut aussi une tendance pour les brebis qui gagnèrent du poids en masse corporelle à révéler une masse ovarienne plus grande que les brebis sous alimentées. Les brebis hybrides montrèrent une réponse plus marquée au HGG que les brebis Merino.

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REFERENCES

BINDON, B. M., CH'ANG, T. S. & TURNER, H. N., 1971. Ovarian response to gonadotrophin by Merino ewes selected for fecundity. *Aust. J. agric. Res.* 22, 809–820.

BRADEN, A. W. H., LAMOND, D. R. & RADFORD, H. M., 1960. The control of the time of ovulation in sheep. *Aust. J. agric. Res.* 11, 389–401.

CALLANTINE, M. R. & HUMPHREY, R. R., 1965. Effect of progesterone on pituitary and ovarian responsiveness to placental gonadotrophins. *Endocrinology* 77, 921–931.

CHRISTENSON, C. M. & ELEFTHERIOU, B. E., 1972. Dose-dependence of superovulation response in mice to two injections of PMSG. *J. Reprod. Fert.* 29, 287–289.

CUMMING, I. A., BROWN, J. M., BLOCKEY, M. A. de B. & GODING, J. R., 1971. Regulation of the oestrous cycle in the ewe. *J. Reprod. Fert.* 24, 148–149.

DERMODY, W. C., FOOTE, W. C. & HULET, C. V., 1970. Effects of season and progesterone synchronization on ovulation rate in mature western range ewes. *J. Anim. Sci.* 30, 214–218.

DZIUK, P. J., HINDS, F. C., MANSFIELD, M. E. & BAKER, R. D., 1964. Follicle growth and control of ovulation in the ewe following treatment with 6-methyl-17-acetoxyprogesterone. *J. Anim. Sci.* 23, 787–790.

FIELDEN, E. D. & BRUMBY, P. J., 1962. The effect of reduced food intake on the response to induced ovulation in hypophysectomized mice. *Aust. J. biol. Sci.* 15, 213–217.

GIBSON, W. R. & ROBINSON, T. J., 1971. The seasonal nature of reproductive phenomena in the sheep I. Variation in sensitivity to oestrogen. *J. Reprod. Fert.* 24, 9–18.

GUERRA, J. C., THWAITES, C. J. & EDEY, T. N., 1971. The effects of liveweight on the ovarian response to pregnant mare serum gonadotrophin and on embryo mortality in the ewe. *J. agric. Sci. Camb.* 76, 177–178.

HILLIARD, J., SCHALLY, A. V. & SAWYER, C. H., 1971. Progesterone blockade of the ovulatory response to intrapituitary infusion of LH-RH in rabbits. *Endocrinology* 88, 730–736.

HOWLAND, B. E., 1972. Ovarian weight and ovarian compensatory hypertrophy in the rat as affected by duration of underfeeding. *J. Reprod. Fert.* 28, 321–323.

LAMOND, D. R., 1964. Seasonal changes in the occurrence of oestrus following progesterone suppression of ovarian function in the Merino ewe. *J. Reprod. Fert.* 8, 101–114.

LAMOND, D. R. & BINDON, B. M., 1962. Oestrus, ovulation and fertility following suppression of ovarian cycles in Merino ewes by progesterone. *J. Reprod. Fert.* 4, 57–66.

LAND, R. B. & FALCONER, D. S., 1969. Genetic studies of ovulation rate in the mouse. *Genet. Res.* 13, 25–46.

LARSON, W. M., BANBURY, E. E. & SPAETH, C. W., 1970. Effect of previous lambing rate on response to PMS. *J. Anim. Sci.* 31, 225.

LIU, F.T.Y., LIN, H. S. & JOHNSON, D. C., 1972. Serum FSH, LH and the ovarian response to exogenous gonadotrophins in alloxan diabetic immature female rats. *Endocrinology* 91, 1172–1179.

MCLAREN, A., 1962. The relation between natural fecundity and response to follicle stimulating hormone. *J. Endocr.* 25, 137–144.

MAULÉON, P. & RAO, K. H., 1963. Variations génétiques des populations folliculaires dans les ovaires de rattes impubères. *Annls Biol. anim. Biochim. Biophys.* 3, 21–31.

PIACKSEK, B. E. & MEITES, J., 1967. Reinitiation of gonadotrophin release in underfed rats by constant light or epinephrine. *Endocrinology* 81, 535–541.

RAESIDE, J. I. & McDONALD, M. F., 1959. Seasonal changes in the oestrous response by the ovariectomized ewe to progesterone and oestrogen. *Nature* 184, 458–459.

RATNER, A. & MEITES, J., 1963. Effects of hormone administration on milk production of underfed rats. *Am. J. Physiol.* 204, 268–270.

REARDON, T. F. & ROBINSON, T. J., 1960. Seasonal variation in the reactivity to oestrogen of the ovariectomized ewe. *Aust. J. agric. Res.* 12, 320–326.

STEVENS, K. R., JACKSON, G. L. & NALBANDOV, A. V., 1968. Gonadotrophic activity of stalk median eminence extracts in hypophysectomized rats. *Endocrinology* 83, 225–233.

SUGAWARA, S., UMEZU, M. & TAKEUCHI, S., 1969. Effect of a single dose of human chorionic gonadotrophin on the ovulatory response of the immature rat. *J. Reprod. Fert.* 20, 333–335.

UILENBROEK, J. Th. J. & VAN DER WERFF TEN BOSCH, J. J., 1972. Ovulation induced by pregnant mare serum gonadotrophin in the immature rat treated neonatally with a low or a high dose of androgen. *J. Endocr.* 55, 533–541.

PART C

SEASONAL AND ENVIRONMENTAL EFFECTS ON REPRODUCTION

INTRODUCTION

In the late 1950's F.N. Bonsma and D.M. Joubert proposed that a regionalized system should be adopted for beef production in South Africa. Such a system would be based on breeding herds in the extensive, dry areas providing animals (stores) to be finished in the more intensive cropping areas. The objective in Paper 23 was to evaluate such a system. The significance to the theme in this dissertation is that reliable results were obtained regarding reproductive rates for *Bos taurus* and *Bos indicus* cattle under the same management system and in the same environment. It also became possible to compare environments.

Paper 23

Essentially, the experiment incorporated Afrikaner and Simmentaler breeding herds, located in the Highland Sourveld (Intensive area) and in the Lowland Thornveld (Extensive area)

Findings :-

1. The weight loss for the Simmentaler cows during lactation in the Lowland Thornveld was twice that seen for Afrikaner cows. However, the calving rate (over three years) was 5% lower for Afrikaner cows.
2. In the Highland Sourveld the weight loss during lactation for the Simmentaler cows was similar to that shown by the Afrikaners. In spite of this, the calving rates were 10% higher for Simmentaler cows.

Implications/Significance :-

1. Afrikaner cows were clearly more sensitive (in terms of calving rate) to nutritional deficiencies than Simmentaler cows. In a commercial situation the differences

would have been greater. This was because in the experimental situation cows that failed to conceive were not culled from the breeding herd, unless they had not produced a calf in two consecutive years. Such a practice was necessitated by the need to provide sufficient numbers of weaners for the second part of the investigation (not discussed here).

Paper 24

It is generally believed that during domestication cattle have lost the seasonal breeding habits. Although this may be true for dairy cattle, there may still be seasonal effects in beef breeds, particularly in sub-tropical areas. Farmers continue to report that during hot weather beef cows fail to exhibit oestrus and this has been confirmed by personal observation. Evidence has also been accumulated to show that in dairy cows fewer inseminations (per cow) are required during winter breeding than where cows are bred in summer.

The experiment outlined in Paper 24 examined seasonal effects on pituitary and ovarian hormones in dairy cows.

Findings :-

1. Tonic LH levels during days 6 to 15 of the oestrous cycle were significantly higher during autumn than during summer or spring.
2. Progesterone concentrations were not affected by season.

Implications/Significance :-

1. Even in dairy cows which are reputed to have lost the seasonality of breeding there was a clear seasonal effect on LH levels during the luteal phase of the oestrous cycle.
2. Environmental temperatures and relative humidities did not appear to influence LH levels.

If similar seasonal patterns apply to beef cows and the changes can be related to the fertility of the animals, then beef cows are at a decided disadvantage in being bred during late spring - early summer. Breed differences may also be important.

The reproductive responses of two breeds of beef cows and the performance of their progeny in two contrasting environments

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The experiment was carried out over three seasons during which a total of 889 Afrikaner and 805 Simmentaler 'cow years' were studied. Nucleus breeding herds of each of these breeds were maintained at stations in the Highland Sourveld and the Valley Thornveld. At weaning, half of the progeny of each breed were transferred to the alternate location; the other half were retained at their original location. Afrikaner and Simmentaler cows had calving rates of 53,59% and 58,85% respectively in the Thornveld and 65,82% and 76,24% in the Sourveld. Significantly more Afrikaner (44%) and Simmentaler (21%) calves were weaned in the Thornveld than the Sourveld. Breed and feed situation (stalls vs. veld) had a highly significant effect ($P < 0{,}01$) on ADG from weaning to final mass. Both the Simmentaler and the Afrikaner calves consumed significantly more DM/kg gain in the Sourveld, than their counterparts in the Thornveld.

S. Afr. J. Anim. Sci. 1986, 16: 209 – 214

'n Totaal van 889 Afrikaner en 805 Simmentaler 'koei-jare' is oor drie seisoene bestudeer. Kernkuddes van Simmentaler en Afrikaner koeie is op stasies in die Hoogland-suurveld en die Vallei-doringveld onderhou. Die helfte van die kalwers van elke ras is met speentyd na die alternatiewe lokaal verplaas. Die ander helfte is op hul geboorteplaas gehou. Afrikaner en Simmentaler koeie het onderskeidelik kalfpersentasies van 53,59% en 58,85% in die doringveld, en 65,82% en 76,24% in die suurveld gehad. Meer Afrikaner (44%) en Simmentaler (21%) kalwers is in die doringveld as in die suurveld gespeen. Ras en voerplek (stalle vs. veld) het 'n hoogs betekenisvolle ($P < 0{,}01$) effek uitgeoefen op die GDT van speen tot finale massa. Beide die Simmentaler en die Afrikaner diere het betekenisvol meer DM/kg massatoename in die suurveld ingeneem.

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Keywords: Environment, beef cows, calving rates, feed conversion

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Introduction

Most of the environmentally orientated research with beef cattle, in South Africa, has concentrated on the effects of heat stress and related problems (Bonsma, 1949; Bonsma, 1980). This is understandable because most of the important beef-producing areas experience either tropical or sub-tropical climates. Consequently, the effects of cold stress on beef production in the cooler, moister areas have largely been overlooked. The question may legitimately be asked whether cold stress is a problem in this country, and if so, to what extent? This particular question is pertinent in situations where cattle are moved from the Sourveld to the Thornveld areas during the winter months. Unfortunately, little is known or understood about the responses of beef cattle to translocation from one bioclimatic area to another.

In 1957 Bonsma & Joubert advocated a regionalized beef production system. They suggested that certain bioclimatic areas are better suited to breeding animals, whilst other areas closer to supplementary feed sources and markets, could probably be used better for the growing-out and finishing of beef animals.

The purpose of this paper is therefore two-fold. Firstly, an attempt is made to provide some biological evidence upon which decisions can be made regarding regionalized beef production. Secondly, this paper attempts to quantify the effects of stress, particularly cold stress, on traits of economic importance.

Procedure

Experimental sites

The two sites chosen for the experiment were the Thabamhlope Research Station — representative of Highland Sourveld and falling within Bioclimate area 4e, and the Onverwacht Research Station — representative of Lowland Thornveld and contained in Bioclimate area 10 (Philips, 1973).

Topography

The veld area classified as Highland Sourveld is approximately 1,83 million hectares in extent or 20,59% of the total area of Natal. Eighty-eight per cent (1,61 m ha) of the Highland Sourveld is natural grassland. The veld area assigned to the experiment consisted of gently rolling hills with a general absence of trees and bushes. The animals were therefore exposed to the prevailing Berg wind conditions. The altitude of the experimental area varies between 1457 and 1524 m above sea level.

The Thornveld area of Natal covers an area of 1,84 million hectares. Approximately 76% of the total area is covered by grasslands and about 23,5% is under cultivation. The area used for the experiment varied between flat open areas with occasional bushes to areas where grass cover was good and thornbushes averaging 3,5 m in height were abundant. Adequate shade and protection were therefore available at all times.

Daily rainfall measurements were used to calculate mean monthly figures. Temperature and humidity figures were collected at 08h00 and 14h00 each day.

Experimental animals

Breeding herds

Nucleus breeding herds of Simmentaler and Afrikaner cows were maintained at each location. Eight hundred and eighty-nine Afrikaner and 805 Simmentaler 'cow years' were studied over three seasons. The mating season extended from the 1st of December to the end of February. The bulls were rotated between the two stations on a fortnightly basis in an attempt to reduce the genetic affect of the sire. The bull to cow ratio was 1:25.

Calves

A total of 457 Simmentaler and 398 Afrikaner calves were involved during the 3-year experimental period. Weaning took place during May and June. At weaning half of the calves of each sex and breed were translocated to the alternate location. The other half were retained at their original location. At each location, half of the calves were allocated to the veld treatments, whilst the other half were fed in stalls. The stalls were constructed to allow individual penning of the animals so that accurate feed intake recordings were facilitated.

Feed regimes

Cows

Cows cannot maintain body mass during the winter months (May – September) on Highland Sourveld. Consequently, at weaning the cows were transferred to open kraals where they were fed conserved feed. During the first season the cows in the Sourveld were fed veld hay *ad lib.* plus 2,3 kg lucerne hay per head. These animals also had access to a NPN lick. The basic diet was changed for the second and third seasons for two reasons. Firstly, lucerne hay was becoming increasingly difficult to obtain and secondly, it was felt that the diet should be more in keeping with the potential of the area. The diet became one consisting of *Eragrostis curvula* hay, fed *ad lib*; 10 kg/head grass silage and an NPN lick which was freely available. In the Thornveld, the cows remained on veld during the winter months, but had NPN blocks freely available.

In both the Sourveld and the Thornveld, a molasses-based lick was available between September and January. The intake was restricted to between 0,68 and 0,77 kg per head per day.

Calves

During the first season the calves fed in the stalls received a basic diet consisting of *E. curvula* hay fed *ad lib.*, lucerne hay fed at the rate of 2,3 kg per head per day and 0,5 kg maize meal. The calves' diets were also changed after the first season. The diet for the following two seasons consisted of *E. curvula* hay (*ad lib.*), 3,2 kg/head/day maize meal and 0,5 kg per head high protein concentrate (HPC). The composition of the HPC was adjusted within three live mass categories concomitant with the animal's changing nutritional requirements. The three categories were: weaning – 273 kg; 274 kg – 364 kg; 365 kg – slaughter.

In the Sourveld the calves in the veld-grazing treatments were moved to open kraals during the winter months and fed *E. curvula* hay, grass silage plus an NPN lick. The calves in the Thornveld remained on veld, but were supplied with NPN blocks.

Afrikaner steers were raised to a mass of approximately 410 kg, and Simmentaler steers to a mass of 455 kg before being slaughtered. The Simmentalers required additional feeding in order to achieve the necessary 'finish' for the top grades. Heifers were raised to a mass of about 318 kg, the mass at which they were assumed to attain puberty. Heifers not absorbed into the breeding herd were culled.

Diet components

Roughages

The *E. curvula* hay used in the experiment was grown at the Thabamhlope Research Station. Each cut of hay was equally divided between the two stations, to ensure, as far as possible, uniform quality at each location. Lucerne hay was purchased from a single source and equally divided between the two stations. Samples of both *E. curvula* and lucerne hay were analysed for crude protein (CP) on a regular basis. Veld samples were cut and analysed on a weekly basis for CP.

Statistical analyses

Factors affecting mass 12 months after weaning and average daily gain (ADG) from weaning to final mass were analysed by multiple regression analysis. Analysis of covariance were used to correct for differences in weaning mass. Each season was analysed separately in three single analyses. In the fourth analysis all the data were pooled and season was then included as an independent variate.

Results and Discussion

Climatological data

Rainfall

In the Sourveld, 70% of the annual rainfall is recorded between December and March (Natal Ag. Met. 1979/80 – 1983/84). Recordings at the Thabamhlope Research Station indicated that the mean annual rainfall over the experimental period was 854 mm. The rainfall distribution in the Thornveld was similar to that in the Sourveld. However, the mean annual rainfall was only 605 mm, 250 mm per annum less than that recorded in the Sourveld.

Temperature

Mean maximum temperatures during the summer in the Thornveld were in the order of 33,5°C and winter mean minimum temperatures between 4 and 6°C. In the Sourveld, mean maximum temperatures ranged between 26 and 30°C and winter minimum temperatures between −3 and 0°C. Extreme temperatures of 34,5°C and −4°C were recorded on individual days but never for long periods at a time. It is important to note that the Sourveld experiences mean minimum temperatures below 10°C for 7 months of the year (April – October), whilst similar temperatures are experienced in the Thornveld from May to August; a period of only 4 months.

Effective ambient temperature (EAT) is an index used to describe the collective thermal impact of the animal's total environment (NRC, 1981). Such an index has become necessary because animals are exposed to and affected by several components of the climatic environment. The most important factors, in addition to air temperature, include thermal radiation, humidity, air movement, contact surfaces and precipitation.

The importance of air movement is particularly significant in the present study. Extrapolating from results presented by Bowden, Hironaka, Martin & Young (1979), it appears that a wind speed of 25 km/h would reduce a still-air temperature of about 15°C to 4°C, which is the lower limit of the acceptable temperature range (4 – 26°C) suggested for beef cattle for minimum production (Hahn, 1974). The EAT would, however, be considerably lower than 4°C and consequently, the effect on production could be even more severe. It must be emphasized that this paper is more concerned with EAT's that affect production, which are considerably higher than the lower critical temperatures (LCT's) given for various classes of beef cattle (Young, 1981). Winter mean monthly temperatures in the Sourveld fall well within this 'stress' zone.

Quality of dietary components

The mean CP content of the *E. curvula* hay fed at Thabamhlope was 9,33% (± 1,01) and 9,37% (± 0,63) at Onverwacht. Lucerne hay averaged 15,32% (± 1,63) CP in the Sourveld and 14,04% (± 1,25) in the Thornveld. The veld analyses indicated a typical seasonal pattern, peaking in spring and reaching a low point during the winter. The mean CP of the veld samples in the Sourveld between October and March was 7,94% (± 1,20) and 3,38% (± 0,79) between April and September. In the Thornveld the mean CP content of the grass sampled between October and March was 6,61% (± 1,25) and between April and September 3,50% (± 0,75).

Breeding cow herds

Calving rates

Data reflecting the number of cows that calved and the number of calves weaned are presented in Table 1. Calving rates were disappointingly low, particularly in the Thornveld. A number of factors are possibly responsible for these low calving percentages. Lamond (1970) has suggested that each cow has an optimum body mass for conception. The ability of a cow to reproduce decreases as body mass declines below this target mass. Steenkamp, van der Horst & Andrew (1975) found that post-partum mass in Afrikaner cows was the most significant factor influencing reconception. Figures 1a and 1b show the mean (3 years) live mass changes (expressed over a period of 12 months) of the lactating Afrikaner and Simmentaler cows involved in the experiment. The mass change patterns for the two breeds in the Sourveld are remarkably similar (Figure 1a). The Simmentaler cows lost 6,05% of their body mass during the calving season and the Afrikaner cows 8,18%. Average masses during the mating season were 415 kg for the Simmentalers and 410 kg for the Afrikaners. The difference in conception rate between the Simmentalers and the Afrikaners (10,42%, Table 1) cannot therefore be easily explained by large differences in mass gain, mass loss or even actual body mass at the time of mating.

The mean mass changes of the cows in the Thornveld show a far more dramatic trend (Figure 1b). Afrikaner cows lost 7,69% of their body mass during the calving season, compared to a loss of 14,9% in the Simmentalers. Consequently, the average mass during the breeding season was 375 kg for the Afrikaners and 363 kg for the Simmentalers. This is 9,0 and 13,0% less respectively, than their counterparts in the Sourveld. The difference in mass between the cows in the Sourveld and the Thornveld at mating could account for the differences in conception rates between the two areas (Table 1). Although both breeds gained in mass during the breeding season, it is likely that many of the cows never achieved the target mating mass necessary for reconception (Lamond, 1970; Meaker, 1975).

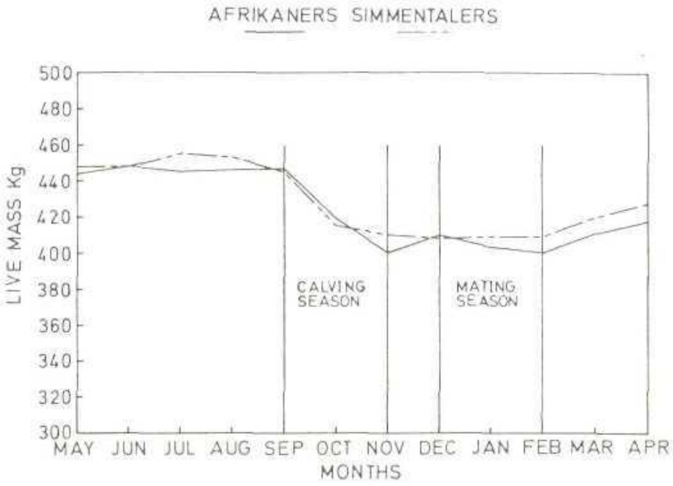

Figure 1a Mean mass changes of lactating cows which reconceived on the Sourveld

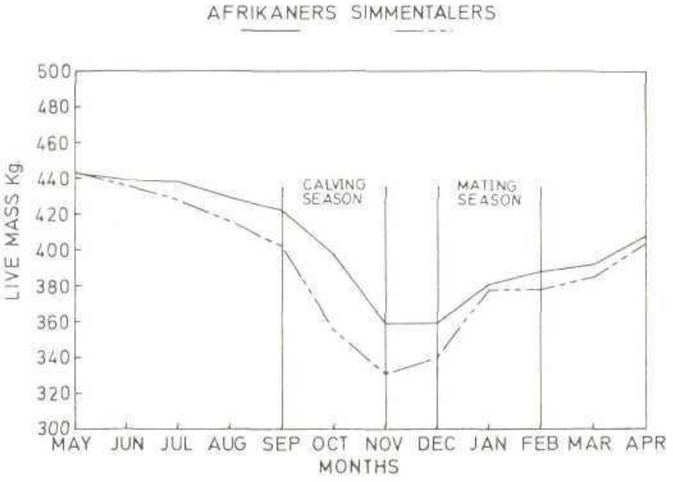

Figure 1b Mean mass changes of lactating cows which reconceived on the Thornveld

Table 1 Calving and weaning rates of Afrikander and Simmentaler cows in the Sourveld and Thornveld areas during the experimental period (3 years)

Area	Breed	Cows bred	Cows calved	%	Calves weaned	%
Sourveld	Afrikander	471	310	65,82	194	62,58
	Simmentaler	421	321	76,24	246	76,64
Thornveld	Afrikander	418	224	53,59	204	91,07
	Simmentaler	384	226	58,85	211	93,36

The data in Table 1 also show that the number of calves weaned in the Thornveld was significantly higher than in the Sourveld, despite the fact that the calving rates of both breeds were considerably higher in the Sourveld than the Thornveld. The high mortality rate of calves in the Sourveld can perhaps be ascribed to the climatic conditions prevailing during the early part of the calving season.

The mean temperature and rainfall recorded for September in the Sourveld was 15°C and 60 mm respectively. These factors combined with the strong cold winds normally encountered during this time of the year, would have reduced

Table 2 Post-weaning performance of Simmentaler (S) and Afrikaner (A) heifers fed in stalls and on veld at two locations

Origin	Location	Breed	Feed location	Corrected 12 months post-weaning mass	ADG weaning to final mass
Sourveld	Sourveld	Simm	Stalls	293,83 ± 7,31	0,38 ± 0,03
Sourveld	Thornveld	Simm	Stalls	253,20 ± 11,57	0,42 ± 0,03
Thornveld	Thornveld	Simm	Stalls	309,45 ± 10,11	0,42 ± 0,04
Thornveld	Sourveld	Simm	Stalls	285,97 ± 30,99	0,36 ± 0,01
Sourveld	Sourveld	Afrik	Stalls	274,91 ± 30,50	0,31 ± 0,03
Sourveld	Thornveld	Afrik	Stalls	299,79 ± 19,04	0,37 ± 0,04
Thornveld	Thornveld	Afrik	Stalls	293,95 ± 22,06	0,34 ± 0,04
Thornveld	Sourveld	Afrik	Stalls	253,33 ± 18,61	0,25 ± 0,04
Sourveld	Sourveld	Simm	Veld	271,92 ± 43,82	0,31 ± 0,04
Sourveld	Thornveld	Simm	Veld	273,20 ± 0,55	0,27 ± 0,06
Thornveld	Thornveld	Simm	Veld	276,87 ± 9,35	0,29 ± 0,05
Thornveld	Sourveld	Simm	Veld	262,63 ± 49,36	0,30 ± 0,03
Sourveld	Sourveld	Afrik	Veld	232,44 ± 49,69	0,23 ± 0,03
Sourveld	Thornveld	Afrik	Veld	253,24 ± 17,12	0,26 ± 0,05
Thornveld	Thornveld	Afrik	Veld	253,55 ± 15,36	0,26 ± 0,05
Thornveld	Sourveld	Afrik	Veld	229,03 ± 35,61	0,21 ± 0,02

the still-air temperature to an effective 0 – 5°C. This figure is close to the lower critical temperature (LCT) given for new-born calves (Young, 1981). The highest incidence of calf mortality was noted during the peri-natal phase. Subsequent experience has verified the fact that high calf mortality coincides with low temperatures and high winds. It is however unlikely that climatic effects *per se* would result in the death of new-born calves. A more reasonable explanation is that particularly cold spells lower the resistance of new-born calves, making them more susceptible to *E. coli* infection and Coccidiosis. This point however, requires further investigation.

These data show unambiguously that both breeds had higher calving rates in the Sourveld than the Thornveld, but that a greater number of calves were weaned in the Thornveld (Table 1). These results appear to support the hypothesis proposed by Bonsma & Joubert (1957). It is equally clear that management is a significant factor under these conditions. Manipulation of the physical or managerial environment to enhance animal productivity is a potentially important tool for use by animal scientists.

Calf performance

Mass 12 months after weaning

The mass of the animals 12 months after weaning was considered to be important. It was assumed that by this stage the translocated animals would have had sufficient time to adjust to their respective environments. This parameter therefore served as a measure of adaptability to the specific environments.

Breed and weaning mass were found to be highly correlated ($P < 0,01$; r value $-0,744$). It was important in these analyses to establish the precise role that breed played in affecting post-weaning performance. Breed was therefore included as an independent variate in the analyses. Weaning mass was excluded. The variates included in the regression analyses were breed (X_1), stalls/veld (feed situation, X_2), location (X_3), origin (X_4), and season (X_5).

Breed accounted for between 36,1% (season 1) and 60% (season 3) of the variation in Y (mass 12 months after weaning; Table 3). The effect of breed in all four analyses was highly significant ($P < 0,01$). These results substantiate the concept of selecting the right type of animal for certain environmental conditions.

The animals fed in the stalls would be expected to have higher mass gains than the animals on natural veld for two basic reasons. Firstly, the CP's of the conserved feed were higher than those of the natural veld; there was also no seasonal variation. Secondly, the animals on veld were far more susceptible to environmental elements than the animals in the stalls. It is not surprising therefore that the feeding situation (S/V, X_2) accounted for between 30,7% (season 1) and 12,5% (season 2) of the variation in Y.

Table 3 Regression equations of factors affecting mass 12 months after weaning

Season 1	$Y = 260{,}9 - 28{,}10\ X_1 - 25{,}92\ X_2 - 18{,}25\ X_3 + 7{,}59\ X_4$
Season 2	$Y = 284{,}24 - 28{,}05\ X_1 - 12{,}78\ X_2$
Season 3	$Y = 300{,}25 - 24{,}21\ X_1 - 21{,}51\ X_2$
Pooled data Seasons 1 – 3	$Y = 281{,}53 + 26{,}79\ X_1 + 20{,}07\ X_2 + 30{,}08\ X_3 + 8{,}95\ X_4 + 6{,}15 X_5$

X_1 = breed, X_2 = stalls/veld (S/V), X_3 = location, X_4 = origin, X_5 = season

When the data were pooled, location (X_3) and origin (X_4) had small, but significant ($P < 0,01$) effects on the mass 12 months after weaning. These trends are clearly evident in Table 2. Irrespective of breed and feed situation, animals born and raised in the Thornveld had higher 12 month post-weaning masses than animals born in the Thornveld and raised in the Sourveld. However, it is also clear from these data (Table 2), that animals raised in the Thornveld consistently had higher masses 12 months post-weaning than animals located in the Sourveld, with origin apparently playing a relatively minor role. Origin (X_4) contributed only 1,9% to the variation in Y. A total of 77,3% of the variation in Y was accounted for by the five parameters measured.

Average daily gain (ADG) from weaning to final mass

Final mass for the heifers is defined as the mass at which the animals were withdrawn from the trial and introduced into the breeding herd. The mass was in the region of 318 kg. The feeding situation, stalls vs. veld (X_1) had a highly significant ($P < 0,01$) effect on ADG from weaning to final mass (Y),

Table 4 Regression equations of factors affecting ADG (*Y*) from weaning to final mass

	Season			
	1 $\%R^2$	2 $\%R^2$	3 $\%R^2$	1 – 3 $\%R^2$
Stalls/veld (X_1)	20,6	47,3	32,0	32,1
X_1 + breed (X_2)	54,2	55,8	49,3	49,8
$X_1 + X_2$ + location (X_3)	55,5	61,2	50,4	52,0
$X_1 + X_2 + X_3$ + orogin (X_4)	57,6	61,3	51,5	52,7
$^*X_1 + X_2 + X_3 + X_4 +$ + season $^*(X_5)$				53,5
Season 1	$Y = 0{,}357 - 0{,}033X_1 - 0{,}042X_2$			
Season 2	$Y = 0{,}363 - 0{,}053X_1 - 0{,}022X_2$			
Season 3	$Y = 0{,}340 - 0{,}047X_1 - 0{,}035X_2$			
Season 1 + 2 + 3	$Y = 0{,}353 - 0{,}044X_1 - 0{,}033X_2 - 0{,}011X_3$			

*The data for seasons 1, 2 & 3 were pooled and season added as an independent variate (X_5)

accounting for between 20,6% (first season) and 47,3% (second season) of the variation in *Y* (Table 4).

A relatively small contribution (20,6%) to the variation in ADG was made by stalls/veld, whilst breed accounted for 33,6% of this variation, during the first season. This suggests that, when the effect of feeding site was reduced, the breed effect could be fully expressed. However, during the second season, although breed may still have been an important factor, the breed effect was supressed by the strong influence of the feeding site (S/V), which accounted for 47,3% of the variation in *Y* (Table 4). These results indicated the possibility of an interaction between breed and S/V. However, these interactions were found to be non-significant, as were all other interactions tested for. In the first three regression analyses (Table 4) neither location nor origin made a significant contribution to the variation in *Y*. However, when the data were pooled, location made a small, but significant ($P < 0{,}05$), contribution to the variation in ADG. The effect of origin was non-significant. When the two breeds and the two feeding situations were compared on a location basis (i.e. excluding origin), the pooled data in Table 2 showed clearly that the Simmentaler and Afrikaner heifers fed in the stalls in the Thornveld, had 13,5% and 26,78% higher ADG's respectively than those in the Sourveld. Contrasting results were obtained in the veld situation. Simmentaler heifers raised in the Sourveld had an 8,92% ADG advantage over the Simmentalers running on Thornveld (Table 2). The Afrikaner heifers however, had an 18,18% higher ADG in the Thornveld than in the Sourveld (Table 2). The reasons for the difference in breed response to the veld situation are not clear.

Efficiency of feed utilization

Heifers

Simmentalers generally required less dry matter per kilogram live mass gain than the Afrikaners. The Simmentalers in the Thornveld proved to be the most efficient and the Afrikaners in the Sourveld the least efficient in terms of feed utilization (Table 5). Afrikaner heifers in the Sourveld consumed 35,63% more DM/kg live mass gain than their counterparts in the Thornveld, whilst the intake of the Simmentalers was 24,83% more DM/kg gain in the Sourveld than the Thornveld (Table 5). In the Thornveld, Afrikaner heifers took in 25,66% more DM/kg gain than the Simmentalers, whilst in the Sourveld, Afrikaner heifers consumed 36,53% more DM/kg gain than the Simmentalers. If location is ignored, Afrikaner heifers consumed 26,02% more DM/kg gain than the Simmentalers.

Steers

Afrikaner steers consumed 44,43% ($P < 0{,}01$) more DM/kg gain in the Sourveld than in the Thornveld (Table 5). The Simmentaler steers on the other hand consumed only 10,38% more DM/kg gain in the Sourveld. Afrikaner steers consumed 36,17% ($P < 0{,}01$) more DM/kg gain than the Simmentalers in the Sourveld. The difference between the two breeds in the Thornveld was, however, relatively small. When location is ignored, the feed consumption of the Simmentalers was on average 12,67 (± 1,34) kg DM/kg gain and for the Afrikaners 15,98 (± 2,98) kg DM/kg gain, a difference of 26,12% (NS).

Table 5 Dry matter intakes per kilogram live mass gain (DM/kg gain) for heifers and steers fed in stalls in two different climatological areas

Origin	Location	Breed	Sex	Dry matter intake per kg live mass gain kg
Sour	Sour	Simm	♂	13,22 ± 1,28
Sour	Thorn	Simm	♂	12,20 ± 0,84
Thorn	Thorn	Simm	♂	12,84 ± 1,14
Thorn	Sour	Simm	♂	14,42 ± 1,28
Sour	Sour	Afrik	♂	17,99 ± 1,54
Sour	Thorn	Afrik	♂	13,32 ± 0,93
Thorn	Thorn	Afrik	♂	12,75 ± 0,36
Thorn	Sour	Afrik	♂	19,66 ± 2,16
Sour	Sour	Simm	♀	13,56 ± 1,17
Sour	Thorn	Simm	♀	10,22 ± 0,68
Thorn	Thorn	Simm	♀	11,44 ± 0,24
Thorn	Sour	Simm	♀	13,48 ± 1,00
Sour	Sour	Afrik	♀	17,12 ± 1,62
Sour	Thorn	Afrik	♀	13,84 ± 0,76
Thorn	Thorn	Afrik	♀	13,39 ± 0,80
Thorn	Sour	Afrik	♀	19,80 ± 2,32

It is clear that the Afrikaners, irrespective of origin, had difficulty in adapting to conditions in the Sourveld (Table 2). This is not altogether surprising, because it is generally accepted that Afrikaner animals, whilst having excellent heat tolerance, are not cold tolerant. Simmentalers on the other hand, are only reasonably heat tolerant, but have good cold tolerance (Bonsma, 1977). Of the five parameters measured that were regarded as having a significant effect on mass 12 months after weaning, breed was the most significant. Although the effect of breed varied from season to season, it was always highly significant ($P < 0{,}01$; Table 3).

The heifers fed in the stalls had significantly higher 12 month post-weaning masses than the animals running on the veld. Once again these results are not unexpected because the animals fed in the stalls had better, more uniform quality feed, and more protection from the environmental elements, than the animals on the veld. The data in Tables 3 and 4 show only the mean (3 year) 12 month post-weaning masses and the mean (3 year) ADG's from weaning to final mass, but when attention is focused on production within a season, distinct seasonal variations in performance are evident. The highest mass gains were recorded during the summer periods and the lowest gains during the winter phases. These trends were observed in both the stalls and the veld situations. The seasonal response of the animals fed in the stalls is of particular interest since the quality of feed was not subject to seasonal variations. Research findings have indicated that 40 to 60% of the seasonal variation in feedlot performance can be attributed to climatic variables (Milligan & Christison, 1974; Ames, Brink & Schalles, 1975).

The results from this experiment show unequivocally that both the Simmentalers and the Afrikaners consumed significantly more DM/kg gain in the Sourveld than their counterparts in the Thornveld (Table 5). The effect of location was greater on the Afrikaners than the Simmentalers. It is axiomatic that an increase in total feed intake would mean an increase in energy intakes as well. An increased energy uptake would be necessary because maintenance energy requirements increase linearly during cold, but nonlinearly during heat stress (Ray, Hale & Marchello, 1969; Ames & Ray, 1983). However, maintenance energy requirements increase more rapidly than their rate of voluntary energy intake during cold (Ames & Ray, 1983). Consequently, if the ration is deficient in energy, beef animals could be under considerable stress to maintain normal body functions.

This research has revealed nothing new in terms of the ability of certain breeds to adapt to certain environmental conditions. It has however, drawn attention to the fact that in some instances, the synchronization of genetic resources with the environment may be a primary factor affecting productivity. This study has shown that mean monthly winter temperatures of between 5 and 10°C were sufficiently low to have had a significant effect on beef cattle performance. However, the effective ambient temperatures (EAT's; NRC, 1981) were probably substantially lower than the recorded still-air temperatures. The question then remains: to what extent should the environment be modified to improve animal performance? Hahn (1974) differentiated between two distinct areas of environmental manipulation. 'Protective' modification such as solar radiation shades or winter wind breaks. This type of modification would suffice in the case of animals maintaining body functions. However, as production levels increase, so environmental factors gain in importance relative to nutrition and genetics (Hahn, 1974). In these situations, modifications are necessary to improve the 'productive' function of the animal. Such modifications often involve costly buildings and shelters. The degree of environmental modification needed will depend on the availability of strategic facts for decision making.

Environmental physiology and animal performance must be viewed in proper perspective. 'The efficiency of animal production and the fundamental questions of physiology and health that underlie the efficiency of animal production are determined by interactions between measurable elements of genetics, nutrition and the environment. In simple terms we seek to achieve the right feed for the right breed in the right place' (Webster, 1983).

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References

AMES, D.R., BRINK, D.R. & SCHALLES, R.R., 1975. Effects of severe winter conditions on performance of feedlot steers. *Can. J. Anim. Sci.* 54, 605.

AMES, D.R. & RAY, D.E., 1983. Environmental manipulation to improve animal productivity. *J. Anim. Sci.* 57, 209.

BONSMA, J.C., 1949. Breeding cattle for increased adaptability to tropical and subtropical environments. *J. Agric. Sci.* (Camb.) 39, 204.

BONSMA, J.C., 1977. Hoe kan die veeboer realisties wees? *Vleis/Meat*, Aug. p. 10.

BONSMA, J.C., 1980. Livestock Production, A Global approach. Tafelberg Publishers, Cape Town.

BONSMA, F.N. & JOUBERT, D.M., 1957. Factors influencing the regionalization of livestock production in South Africa. Science Bulletin No. 380, Dept. Agric.

BOWDEN, D.M., HIRONAKA, R., MARTIN, P.J. & YOUNG, D.M., 1979. Feeding beef cows and heifers. Publication 1670. Agriculture Canada, Ottawa.

HAHN, G.L., 1974. Discussion of environmental effects on ruminant production — Rational decisions based on current knowledge. In Livestock Environment. Proc. Int. Livestock Environ. Symp. ASAE, St. Joseph, Michigan.

LAMOND, D.R., 1970. The influence of undernutrition on reproduction in the cow. *Anim. Breed Abstr.* 38, 359.

MEAKER, H.J., 1975. Relationship between body mass and conception in beef cows. *S. Afr. J. Anim. Sci.* 10, 105.

MILLIGAN, J.D. & CHRISTISON, G.I., 1974. Effects of severe winter conditions on performance of feedlot steers. *Can. J. Anim. Sci.* 54, 605.

NATAL AGROMETEOROLOGICAL SECTION (SIRI). Yearly meteorological reports, 1979/80 – 1983/84. Dept. Agric. Natal Region, Cedara.

NATIONAL RESEARCH COUNCIL, 1981. Effects of environment on nutrient requirements of domestic animals. National Academy Press, Washington, DC.

PHILIPS, J., 1973. The agricultural and related development of the Tugela Basin and its influent surrounds. Natal Town and Regional Planning Rep. Vol. 19.

RAY, D.E., HALE, W.H. & MARCHELLO, J.A., 1969. Influence of season, sex and hormonal growth stimulants on feedlot performance of beef cattle. *J. Anim. Sci.* 29, 490.

STEENKAMP, J.D.G., VAN DER HORST, C. & ANDREW, M.J.A., 1975. Reconception in grade and pedigree Afrikaner cows of different sizes. 1. Postpartum factors affecting reconception. *S. Afr. J. Anim. Sci.* 5, 103.

YOUNG, B.A., 1981. Cold stress as it affects animal production. *J. Anim. Sci.* 15, 722.

WEBSTER, A.J.F., 1983. Environmental stress and the physiology, performance and health of ruminants. *J. Anim. Sci.* 57, 1584.

PAPER 24

The influence of season on tonic luteinizing hormone (LH) and progesterone levels in cattle in a subtropical environment

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Plasma LH and progesterone levels were measured daily from the sixth to the fifteenth day of the oestrous cycle in Friesland cows during December (summer) of one year, and April/May (autumn), June/July (winter) and September (spring) of the following year. Five to seven cows were sampled per season, and they were subjected to a plane of nutrition designed to maintain body mass throughout the experimental period. Mean tonic LH levels during autumn (2,26 ± 0,07 ng/mℓ) were significantly ($P \leqslant 0{,}01$) higher than those obtained during summer (1,95 ± 0,06 ng/mℓ) and spring (1,94 ± 0,07 ng/mℓ), but not significantly higher than those obtained during winter (2,15 ± 0,07 ng/mℓ). The mean area under the LH curve for cows sampled during autumn was significantly ($P \leqslant 0{,}05$) greater than that for cows sampled during summer and spring. The season did not influence progesterone concentrations. The possible reasons for the seasonal differences in tonic LH secretion are discussed.

S. Afr. J. Anim. Sci. 1983, 13: 253 – 256

Plasma LH- en progesteroonvlakke is daagliks vanaf die sesde tot die vyftiende dag van die estrussiklus by Frieskoeie gedurende Desember (somer) van een jaar, en April/Mei (herfs), Junie/Julie (winter) en September (lente) van die daaropvolgende jaar gemeet. Vyf tot sewe koeie per seisoen is bestudeer. Die voeding van die diere het min gevarieer oor die eksperimentele periode. Gemiddelde basale LH-vlakke gedurende die herfs (2,26 ± 0,07 ng/mℓ) was betekenisvol ($P \leqslant 0{,}01$) hoër as dié gedurende die somer (1,95 ± 0,06 ng/mℓ) en die lente (1,94 ± 0,07 ng/mℓ), maar nie betekenisvol hoër as dié gedurende die winter (2,15 ± 0,07 ng/mℓ) nie. Die gemiddelde area onder die LH-kurwe vir koeie waarvan monsters gedurende herfs verkry is, was ook betekenisvol ($P \leqslant 0{,}05$) groter as dié vir koeie gedurende die somer en die lente. Seisoen het nie progesteroonvlakke beïnvloed nie. Die moontlike redes vir die seisoensgebonde verskille in basale LH-afskeiding word bespreek.

S.-Afr. Tydskr. Veek. 1983, 13: 253 – 256

Keywords: Season, LH, progesterone, cattle

Introduction

Evidence has been obtained to support the viewpoint amongst farmers that the season of the year exerts an influence on the reproductive efficiency of cattle. Various authors, including Hillin & Rupel (1960), Labhsetwar, Tyler & Casida (1963) and Gwazdauskas, Wilcox & Thatcher (1975) have reported reduced reproductive performance during the summer months and attributed this effect mainly to temperature. Mercier & Salisbury (1947) and Sweetman (1950) reported lowest reproductive efficiency during the winter period and Bonsma (1951) concluded that conception rates were higher during autumn and spring than during the other seasons. The conflicting and often confusing results relating season to reproductive efficiency in cattle may be due to differences in climatic conditions during studies, and the failure to recognize the level of nutrition as a factor affecting reproductive performance (Terblanche, 1974). Season exerts an influence on the sexual activity of sheep (Hunter, 1962; Watson & Radford, 1966). A knowledge of the influence of season on the reproductive ability of cattle is important to obtain maximum breeding efficiency in the dairy herd and in intensive beef systems, especially in tropical and subtropical regions. It is known for example that excessively high temperatures change the duration of the oestrous cycle, shorten the oestrous period, diminish the intensity of oestrous, and if the heat stress is of a sufficient magnitude, it can induce anoestrus (Fuquay, 1981). The object of this investigation was therefore to study the influence of season on tonic LH and progesterone secretion since the secretory patterns of these two hormones have been related to conception rates in cattle (Carstairs, Morrow & Emery, 1980).

Procedure

The experiment was conducted from December of one year to September of the following year at the Cedara Agricultural Research Station which is situated approximately 1 060 m above sea level, and at latitude 29°32′ and longitude 30°17′. The area receives a mean annual rainfall of 900 mm, mainly during the summer (October – March) months. Summers are hot and winters cold with frequent frost (Figure 1). The area falls within Bioclimatic Group 3, as defined by Phillips (1973).

Minimum and maximum temperatures, relative humidity and daylight length during the course of this experiment were obtained from the meteorological data section at Cedara Agricultural Research Station.

To avoid the confounding effects of lactation and pregnancy on LH and progesterone secretion, a herd of twenty non-lactating and non-pregnant Friesland cows (four to nine years of

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age) was used in this study. All the cows were cycling normally at the commencement of the experiment. The feeding regime imposed on the herd consisted of natural veld grazing, supplemented with mineral or urea-containing licks from the fifteenth of October to the fifteenth of May and *Eragrostis curvula* hay (fed *ad lib.*) supplemented with a mineral lick from the sixteenth of May to the fourteenth of October. This programme aimed at mass maintenance in the cows, thereby eliminating the influence, if any, of gain or loss in body mass on LH and progesterone secretion. The body mass of the cows was measured at fortnightly intervals throughout the experiment. Blood samples were obtained from a proportion of the cows during each of four sampling periods, i.e. during summer (11 – 24 December), autumn (26 April – 7 May), winter (29 June – 11 July) and spring (8 – 22 September).

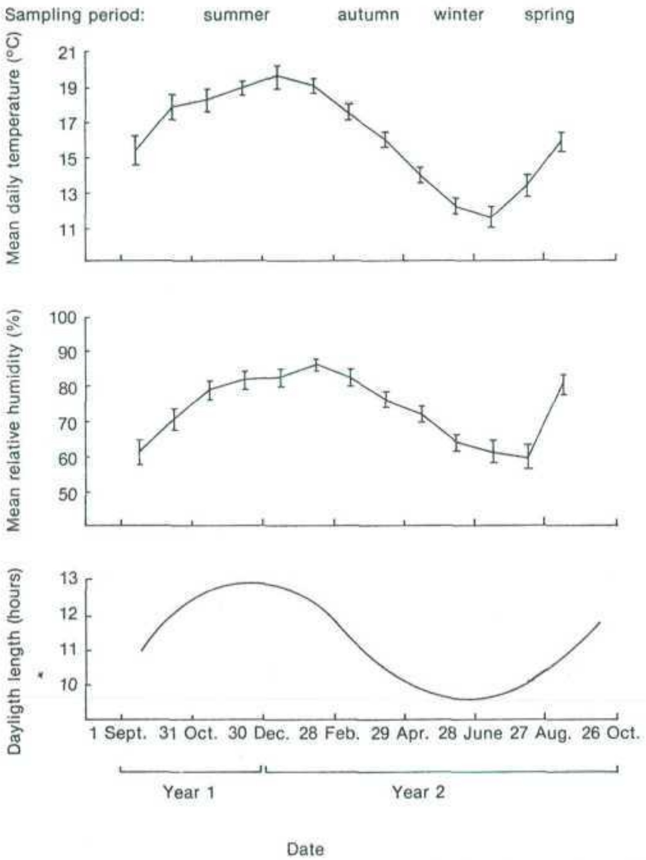

Figure 1 Changes in climatological parameters during the experimental period.

To establish the occurrence of oestrus prior to each sampling period the cows were joined with an active, masculinized teaser cow in the early morning (05h30 – 07h00) and later afternoon (17h00 – 18h30). From days six to fifteen of the oestrous cycle (day of oestrous = day one) blood samples were obtained daily at 07h00 from seven cows during the summer, five cows during the autumn, six cows during the winter and six cows during the spring sampling periods. During each sampling period a different set of cows was drawn from the herd for blood collection, on the basis of them having exhibited heat within a four to five day period. This procedure was adopted to prevent the use of excessively long sampling periods. Certain cows were sampled in more than one sampling period. The blood samples were heparanized and centrifuged within 30 minutes of collection. The plasma obtained was stored at – 15 C° pending analysis.

All plasma samples obtained were analysed for progesterone and LH. The method described by Butcher (1977) was used for progesterone, and the levels were corrected for recovery of tritiated progesterone added to plasma, which varied from 72 to 86 %. Progesterone concentrations were not corrected for water blank values, which varied from 15 to 25 pg/mℓ water. The intra- and inter-assay coefficients of variation for the progesterone level of a poolled plasma sample measured in each of the individual assays were 8,6 and 10,7 % respectively. LH was measured according to the method described by Niswender, Reichert, Midgley & Nalbandov (1969) and validated by Lishman (1972) in this laboratory, except for a few modifications to procedure to attain greater sensitivity. The initial dilution of anti-serum to LH was changed from 1:100 000 to 1:160 000, and the incubation of the anti-LH serum with standards and unknown plasma samples prior to the addition of labelled LH was increased from 24 to 48 hours. The sensitivity was 0,24 ng LH/mℓ plasma. The LH levels in all plasma samples collected in this study were measured in a single assay, thereby eliminating inter-assay variation. The intra assay coefficient of variation was 11,8 %.

Hormone levels, cow masses and meteorological parameters obtained during the four seasons were subjected to analyses of variance. Regression analysis was used to study the relationship between hormone levels and meteorological parameters.

Results

Figure 1 illustrates various climatological parameters measured over the experimental period. Mean daily temperatures during the spring (16,7 ± 0,6°C), summer (17,9 ± 0,3°C) and autumn (16,1 ± 0,7°C) sampling periods did not differ significantly from each other, but were significantly ($P \leqslant 0,05$) higher than the mean temperature during the winter sampling period. The mean daily relative humidity during the spring (78,9 ± 3,4 %), summer (83,0 ± 3,3 %) and autumn (75,6 ± 2,2 %) was also significantly higher than that measured during the winter sampling period (63,9 ± 3,3 %). As expected season exerted a significant ($P \leqslant 0,01$) influence on daylight length (Figure 1). It is evident from Figure 2, in which mass changes in the herd of cows prior to and during the experimental period are presented, that relatively small mass changes occurred, and that these were unrelated to season. The mean masses of the cows sampled during each of the four sampling periods did not differ significantly, and the body mass of cows was not significantly correlated with LH or progesterone levels.

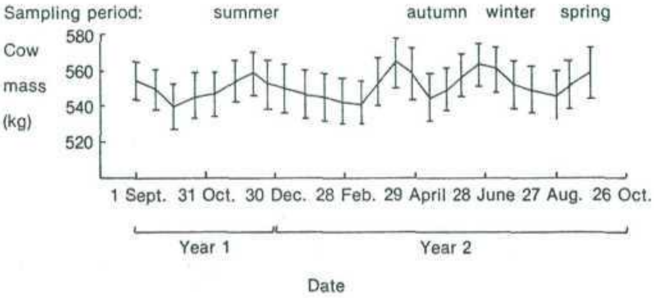

Figure 2 Changes in body mass of cows during the experimental period.

Tonic LH levels measured from day six tot fifteen of the oestrous cycle fluctuated from day to day at relatively low levels (Figure 3). The day of the oestrous cycle did not significantly influence LH levels, although there was a trend for these to decline between days six and fifteen of the cycle, notably during the summer and autumn sampling periods. Mean LH levels obtained over the ten day sampling period during autumn (2,26 ± 0,07 ng/mℓ) were significantly ($P \leqslant 0,01$) higher than those obtained during summer (1,95 ± 0,06 ng/mℓ) and spring (1,94 ± 0,07 ng/mℓ) but not those obtained during winter (2,15 ± 0,07 ng/mℓ). The mean area under the LH curve (an indication of the total quantity of LH released) for autumn (20,1 ± 0,7 mm^2) was also significantly ($P \leqslant 0,05$) greater than the mean for summer (17,7 ± 0,59 mm^2) and spring (17,4 ± 0,64 mm^2). The day of the oestrous cycle significantly ($P \leqslant 0,05$) influenced progesterone secretion, and mean levels on each of days thirteen to sixteen were significantly ($P \leqslant 0,05$) higher than those measured on each of days five to eight of the cycle. It is however evident from Figure 3 that season did not significantly influence the pattern or the total quantity of progesterone secreted.

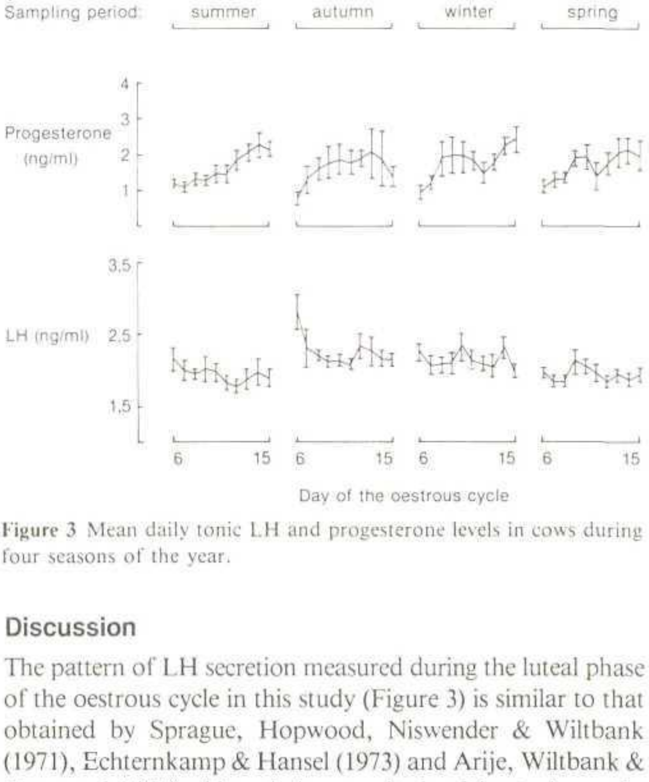

Figure 3 Mean daily tonic LH and progesterone levels in cows during four seasons of the year.

Discussion

The pattern of LH secretion measured during the luteal phase of the oestrous cycle in this study (Figure 3) is similar to that obtained by Sprague, Hopwood, Niswender & Wiltbank (1971), Echternkamp & Hansel (1973) and Arije, Wiltbank & Hopwood (1974), although the magnitude of day to day variations in tonic LH levels in the present study was smaller than that obtained by the aforementioned workers. Schams & Karg (1969) and Hansel & Snook (1970) noted that secondary LH surges, the peak levels of which were significantly higher than normal tonic levels, occurred during the luteal phase of the cycle in certain cows. More frequent sampling than was performed in the present study may be necessary to detect such secondary surges. In the present study the day of the oestrous cycle did not significantly influence tonic LH levels, a finding in agreement with that of Rahe, Owens, Fleeger, Newton & Harms (1980), although these workers found that the frequency and amplitude of individual surges varied significantly during different stages of the luteal phase of the cycle.

Season exerted a significant influence on tonic LH secretion in the present study (Figure 3). To our knowledge no such influence of season on LH secretion has previously been reported, although Randel & Harrison (1981) found that the incidence of pre-ovulatory LH peaks varied amongst five consecutive months of the year, but concluded that this phenomenon was controlled by nutritional factors. Madan & Johnson (1973) have noted that a heat load sufficient to raise the core temperature of heifers by 1 to 1,5°C depressed both tonic and pre-ovulatory peak LH levels. Results obtained in the present study suggest that factors other than temperature and relative humidity might exert an influence on tonic LH secretion. Thus, the correlation coefficient describing the relationship between these climatological parameters and tonic LH levels were not significant, and although autumn tonic levels were significantly higher than those measured during spring and summer, daily temperatures and relative humidities did not differ significantly amongst these seasons. On examining the climatological data presented in Figure 1 together with LH levels illustrated in Figure 3 it appears more likely that the gradual decrease in daylight length which preceded the autumn sampling period resulted in the significantly higher tonic LH levels during this season, as opposed to the lower LH levels measured during spring and summer, the two seasons preceded by an increase in daylight length. Tonic LH levels and daylight length measured over the four seasons were significantly ($P \leqslant 0,01$) correlated ($r = -0,67$). It is a well established fact that a decrease in daylight length results in increased sexual activity in sheep at high latitudes (Hafez, 1952).

Season exerted no influence on progesterone levels measured during the present study (Figure 3), but in view of the considerable animal to animal variation in progesterone levels obtained, a larger sample may be necessary to accurately measure a seasonal effect in cows. Rhodes, Randel & Long (1982) have recently found that corpora lutea removed from cattle during winter have a lessened capacity to release progesterone *in vitro* than those removed during summer. Previous research results relating climate, and notably temperature, to progesterone secretion were somewhat inconsistent. It was observed by Rosenberg, Herz, Davidson & Folman (1977) that progesterone levels were lower during summer than during winter and Stott & Wiersma (1973) found that high environmental temperatures depress progesterone secretion. Conversely Mills, Thatcher, Dunlap & Vincent (1972), Gwazdauskas, Thatcher & Wilox (1973) and Abilay, Johnson & Madan (1975) showed that the stress of a relatively high temperature is associated with increased progesterone secretion. There is clearly a need to accurately establish the influence of temperature on progesterone secretion in different localities, since a positive causal relationship has been established between conception rate and the quantity of progesterone secreted during the oestrous cycle preceeding conception (Folman, Rosenberg, Herz & Davidson, 1973; Rosenberg *et al.,* 1977; Carstaris *et al.,* 1980).

Convey, Beck, Neitzel, Bostwick & Hafs (1977), Roche & Ireland (1981) and Ireland & Roche (1982) obtained evidence indicating that progesterone plays a leading role in the regulation of tonic LH secretion in the cow. In this context it is interesting to note that the inverse relationship between progesterone and LH levels measured in the present study was flexible (Figure 3), and it therefore appears that factors other than progesterone may be involved in the control of tonic LH secretion in cattle.

The finding that season influenced tonic LH secretion under subtropical conditions (present study), and the ability of the corpus luteum to secrete progesterone *in vitro* (Rhodes *et al.,* 1982) points to the need for further research into the influence of season on the secretion of these and other reproductive hormones. Furthermore, it should be established whether seasonal differences in the secretory patterns of these hormones are related to the inherent fertility of cows during different seasons of the year.

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References

ABILAY, T.A., JOHNSON, H.D. & MADAN, M., 1975. Influence of environmental heat and peripheral plasma progesterone and cortisol during the bovine oestrous cycle. *J. Dairy Sci.* 58, 1836.

ARIJE, G.R., WILTBANK, J.N. & HOPWOOD, M.L., 1974. Hormone levels in pre-and post-parturient beef cows. *J. Anim. Sci.* 39, 338.

BONSMA, J.C., 1951. The way to a higher calving percentage. *Farmers Weekly,* Aug 1, 1951, 10.

BUTCHER, R.L., 1977. Changes in gonadotropins and steriods associated with unilateral ovariectomy of the rat. *Endocrinology* 101, 830.

CARSTAIRS, J.A., MORROW, D.A. & EMERY, R.S., 1980. Postpartum reproductive function of dairy cows as influenced by energy and phosphorous status. *J. Anim. Sci.* 51, 1122.

CONVEY, E.M., BECK, T.W., NEITZEL, R.R., BOSTWICK, E.F. & HAFS, H.D., 1977. Negative feedback control of bovine serum luteinizing hormone (LH) concentration from completion of the preovulatory LH surge until resumption of luteal function. *J. Anim. Sci.* 45, 792.

ECHTERNKAMP, S.E. & HANSEL, W., 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum oestrous cycle. *J. Anim. Sci.* 37, 1362.

FOLMAN, Y., ROSENBERG, M., HERZ, L. & DAVIDSON, M., 1973. The relationship between plasma progesterone concentration and conception in post partum dairy cows maintained on two levels of nutrition. *J. Reprod. Fert.* 34, 267.

FUQUAY, J.W., 1981. Heat stress as it affects animal production. *J. Anim. Sci.* 52, 164.

GWAZDAUSKAS, F.C., THATCHER, W.W. & WILCOX, C.J., 1973. Physiological, environmental, and hormonal factors at insemination which may affect conception. *J. Dairy Sci.* 56, 873.

GWAZDAUSKAS, F.C., WILCOX, C.J. & THATCHER, W.W., 1975. Environmental and managemental factors affecting conception rate in a subtropical climate. *J. Dairy Sci.* 58, 88.

HAFEZ, E.S.E., 1952. Studies on the breeding season and reproduction of the ewe. *J. Agric. Sci.* 42, 189.

HANSEL, W. & SNOOK, R.B., 1970. Pituitary ovarian relationships in the cow. *J. Dairy Sci.* 53, 945.

HILLIN, J.M. & RUPEL, J.W., 1960. Differential effect of season on conception rate in Holstein and Jersey cattle. *J. Dairy Sci.* 43, 442.

HUNTER, G.L., 1962. Observations on oestrus in Merinos. *Proc. S. Afr. Soc. Anim. Prod.* 1, 67.

IRELAND, J.J. & ROCHE, J.F., 1982. Effect of progesterone on basal LH and episodic LH and FSH secretion in heifers. *J. Reprod. Fert.* 64, 295.

LABHSETWAR, A.P., TYLER, W.J. & CASIDA, L.E., 1963. Genetic and environmental factors affecting quiet ovulations in Holstein cattle. *J. Dairy Sci.* 46, 843.

LISHMAN, A.W., 1972. The influence of variations in the level of nutrition on reproduction in the ewe. Ph.D. Thesis, University of Natal.

MADAN, M.L. & JOHNSON, H.D., 1973. Environmental heat effects on bovine luteinizing hormone. *J. Dairy Sci.* 56, 1420.

MERCIER, E. & SALISBURY, G.W., 1947. Fertility level in artificial breeding associated with season, hours of daylight, and the age of cattle. *J. Dairy Sci.* 30, 817.

MILLS, A.G., THATCHER, W.W., DUNLAP, S.E. & VINCENT, C.K., 1972. Influence of postbreeding thermal stress on peripheral plasma progestin concentrations in heifers. *J. Dairy Sci.* 55, 400.

NISWENDER, G.D., REICHERT, L.E., MIDGLEY, A.R. & NALBANDOV, A.V., 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology* 84, 1166.

PHILLIPS, J.F.V., 1973. The agricultural and related development of the Tugela Basin and its influent surrounds. Natal Town & Regional Planning Rept. No. 19.

RAHE, C.H., OWENS, R.E., FLEEGER, H.L., NEWTON, H.J. & HARMS, P.G., 1980. Pattern of plasma luteinizing hormone in the cyclic cow: Dependance upon the period of the cycle. *Endorcrinology,* 107, 498.

RANDEL, R.D. & HARRISON, L.M., 1981. Effect of season and monensin on the pre-ovulatory luteinizing hormone surge in Brahaman cows. Beef cattle research in Texas. College Station, U.S.A., Texas Agr. Exp. Sta. 1981, 80.

RHODES, R.C., RANDEL, R.D. & LONG, C.R., 1982. Corpus luteam function in the bovine: *in vivo* and *in vitro* evidence for both a seasonal and breedtype effect. *J. Anim. Sci.* 55, 159.

ROCHE, J.F. & IRELAND, J.J., 1981. The differential effect of progesterone on concentrations of luteinizing hormone and follicle stimulating hormone in heifers. *Endocrinology* 108, 568.

ROSENBERG, M., HERZ, L., DAVIDSON, M. & FOLMAN, Y., 1977. Seasonal variations in post-partum plasma progesterone levels and conception in primiparous and multiparous dairy cows. *J. Reprod. Fert.* 51, 363.

SCHAMS, D. & KARG, H., 1969. Radioimmunologische LH-bestemmung in blutserum vom rind unter besondere beruchsichtigung des bunstzyklus. *Acta endocr., Copenh.* 61, 96.

SPRAGUE, E.A., HOPWOOD, M.L., NISWENDER, G.D. & WILTBANK, J.N., 1971. Progesterone and luteinizing hormone levels in peripheral blood of cycling beef cows. *J. Anim. Sci.* 33, 99.

STOTT, G.H. & WIERSMA, F., 1973. Climatic thermal stress, a cause of hormonal depression and low fertility in the bovine. *Int. J. Biometeor.* 17, 115.

SWEETMAN, W.J., 1950. Artificial breeding in Alaska and effect of extra light during short winter days. *J. Dairy Sci.* 33, 391 (Abstract).

TERBLANCHE, H.M., 1974. Die invloed van voeding op die vrugbaarheid en voortplantingsvermoë van die koei. *J. S. Afr. Vet Med. Ass.* 45, 65.

WATSON, R.H. & RADFORD, H.M., 1966. Seasonal variation in fertility in Merino ewes: The reproductive wastage associated with mating in winter, spring, summer or autumn. *Aust. J. Agric. Res.* 17, 335.

PART D

USE OF EXOGENOUS HORMONES TO INDUCE OVULATION POST PARTUM

INTRODUCTION

Research, particularly in the USA, had for many years been focused on attempts to induce early post partum breeding in lactating beef cows through the use of exogenous hormones. In most of this work the "shot-gun" approach, where treatment was based largely on guesswork, had been used with variable success. Researchers were thus usually at a loss to explain why a given regime of treatment was successful in one case, but not in others. In our hands much the same situation applied.

Paper 25

In 1966, G.L. Hunter prepared a review of work done in sheep and following the writer's participation in gathering published information for this review, it was decided to test some of the recommended procedures. Again, lactating ewes were utilized, not only because of their availability, but also because much interest was being shown in accelerated lambing programmes. Such schemes required that lactating ewes conceive soon after lambing. Certain of the newer products such as progestogen implants and GnRH were also incorporated in this trial, together with PMSG and oestradial benzoate (ODB).

Findings :-

1. Compared to untreated controls all the hormone regimes, except two GnRH injections at a 16-day interval, reduced the interval to first oestrus.
2. Overt oestrus could be more successfully induced when treatments incorporated PMSG and a progestogen implant, but lambing rates did not follow this trend.
3. Exogenous hormones failed to shorten the inter-lambing interval.

Implications/Significance :-

1. It was surprising to note that in ewes in which oestrus was not successfully induced the anoestrous period was prolonged. This observation has been repeated in several subsequent studies, including those utilizing cows. It thus appears that if attempts are made to induce reproductive function, before a state of spontaneous readiness has been attained, then the animal goes into a deepened state of anoestrus. Perhaps the meagre pituitary stores of LH are depleted and it may take longer than expected to replenish same. The rapidity with which pituitary reserves return to normal, soon after calving/lambing does not favour the aforementioned conclusion.
2. The superiority of treatments incorporating progestogen pre-treatment was obviously due to the elimination of sub-normal luteal function. At the time the concept had not been identified.

Paper 26

The objective here was to study, more closely, the release of LH when treatments such as those utilized in Paper 24 are utilized to induce oestrus.

Findings :-

1. More than 50% of the ewes that received daily injections of progesterone did not show a pre-ovulatory release of LH. Oestrus in such animals occurred only provided oestrogen had also been administered, but even then not all ewes exhibited oestrus.

Implications/Significance :-

1. Progesterone in oil, as opposed to SC9880 via pessary, was not effective in triggering an LH release in anoestrous ewes. Unfortunately, this did not shed much light on what had happened in the lactating ewes that remained anoestrus.

Paper 27

The experiment described in Paper 27 was a follow-up to that detailed in Paper 25. Here the object was to examine the effect, on the pre-ovulatory LH release, of oestrogen priming (Oestradiol benzoate, ODB) and of administering GnRH as a divided dose rather than a single injection. Attention was also given to the resulting luteal function.

Findings :-

1. Administration of 30µg ODB as three 10µg injections (4 hours apart), prior to administering 50µg GnRH, in two equal doses, four hours apart, yielded the best LH response (number of ewes responding, peak LH level, duration of release and total quantity released).
2. No treatment resulted in more than 45% of the ewes exhibiting luteal activity and in general the responses were poor.

Implications/Significance :-

1. Lack of pituitary LH stores did not appear to be a factor limiting early post partum breeding. This is based on the finding that oestrogen priming (for 24 hours) followed by GnRH (in two doses, four hours apart) resulted in LH releases equivalent to those seen in ewes cycling spontaneously. This has of course been confirmed by the work of others.
2. Unfortunately, the sampling procedure did not allow much light to be shed on luteal function, except for the general finding that the treatments applied did not initiate normal luteal function in this type of animal and at this time of the breeding season (onset of annual season).

PART E

LUTEAL FUNCTION AFTER INDUCED OVULATION

INTRODUCTION

The early work on the use of GnRH after lactation anoestrus in beef cows had suggested that ovulation could be induced in a proportion of the cows treated. In focusing on why some animals responded while others did not E. K. Inskeep proposed in 1976 that provided the ovaries contained a follicle 10mm in diameter then such cows would ovulate in response to GnRH. Obviously, the presence of a follicle that large would indicate that the animal was close to initiating ovarian cycles spontaneously and had been exposed to the appropriate endogenous gonadotropic stimulation.

Paper 28

Experiment I :-

The objective in the first experiment was to incorporate the suggestion by E. K. Inskeep and to promote the development of follicles prior to their ovulation via GnRH. Additional objectives were to examine more closely the poor luteal function during early cycles and to test whether undernutrition reduced the response to gonadotropic stimulation.

Findings :-

1. Limiting the level of feeding during lactation to 60% of NRC recommendation reduced the LH response to GnRH.
2. FSH treatment prior to ovulation induction did not increase the proportion of cows responding. However, follicle size prior to GnRH administration, influenced both the pattern of LH release and the total LH release. The proposal that a medium to large sized follicle needed to be present, prior to ovulation-induction, in order to favour successful ovulation, was confirmed.

3. None of the factors examined influenced the occurrence of corpora lutea (CL) after GnRH.
4. In 95% of the cows the CL present seven days after GnRH had regressed by day 14 and plasma progesterone concentrations confirmed the shortened CL life span.

Experiment II :-

This investigation utilized the animals from experiment I which had not ovulated. The stage post partum was later and the dose of FSH was increased from a daily maximum of 0.5 mg to 4mg.

Findings :-

1. FSH pre-treatment increased the size of the largest follicle and the number of cows ovulating by 32%.
2. Induced CL were again short-lived.

Experiment III :-

This experiment was conducted a year later and included ODB treatment in addition to FSH pre-treatment.

Findings :-

1. Treatment affected neither follicular development, oestrogen secretion prior to ovulation or LH release after GnRH.
2. Forty five percent of the induced CL were short-lived, without treatment effects on lifespan.

Implications :-

1. Neither bodyweight postpartum nor bodyweight change during lactation appeared to determine whether cows would ovulate in response to GnRH or not. This was surprising in view of the results outline in Papers 18 where inadequate feeding had major effects on calving rates. Prior to the initiation of these experiments it was

believed that provided a lactating cow could be induced to ovulate, this would result in recurrent cycles. Early post-partum conception would thereby be promoted. However, the occurrence of short-lived CL showed the assumption to be incorrect. Possible reasons for the inadequate CL function were proposed and included deficient pre-ovulatory LH release or deficiency of the follicle ovulated, or insufficient oestrogen priming of the follicle.

Paper 29

This investigation (two experiments) considered the possibility of incorrect pre-ovulatory LH release. Short-term calf removal (CR) and/or progestogen pre-treatment were used in an attempt to increase the pre-ovulatory release of LH and to improve luteal function in response to GnRH. The subject of how suckling influences postpartum breeding will be reviewed in more detail in Part F.

Findings :-

1. In the first experiment, when combined with or without 24 hour calf removal, progestogen pre-treatment increased the release of LH, compared to calf removal alone or GnRH alone.
2. Removal of calves for 32 hours, in the second experiment, resulted in a greater release of LH than 24 hour CR or no CR.
3. Only 50% of the cows having a largest follicle less than 10mm in diameter formed CL. Above this diameter the % response was correlated with the diameter of the largest follicle.
4. Only 22% of the CL survived to day 14.

Implications/Significance :-

1. From the results obtained it was clear that the magnitude of the pre-ovulatory LH release was not related to subsequent luteal function.
2. The importance of adequate follicle development prior to ovulation induction was confirmed.

3. Calf removal is beneficial to GnRH - induced LH release, provided suckling is not resumed during the time of LH release.
4. The ability of progestogen pre-treatment to increase pre-ovulatory LH release might reside in improved oestrogen sensitization of the pituitary. The importance of this sensitisation has already been outlined in Papers 17 and 27. This aspect received further attention in Papers 31 and 33.

Paper 30

This experiment is probably one of the first, if not **the** first, to consider the possibility that the lifespan of CL induced by GnRH is shortened by an unexpected release of prostaglandin $F_2\alpha$ ($PGF_2\alpha$).

Two experiments were conducted with lactating ewes and the treatments involved pre-treatment with progestogen and weaning of lambs.

Findings :-

1. The pattern of LH release and the mean concentration were affected by progestogen treatment, but not by weaning of lambs.
2. The number of CL induced by GnRH was not affected by treatment.
3. Pre-treatment with progestogen reduced progesterone concentrations, both in ewes showing short lived luteal and in those experiencing normal luteal phases.
4. Administration of progestogen, before GnRH, reduced the concentration of $PGF_2\alpha$ in uterine venous plasma. However, this could not be related to concentrations or patterns of progesterone secretion.

Implications/Significance :-

1. Observation of a reduction in luteal function (qualitative) following progestogen treatment does not fit subsequent work with lactating cows.
2. The finding that progestogen pre-treatment reduces $PGF_2\alpha$ on day 7 after GnRH injection was an important step forward. Unfortunately, it was largely ignored until

1990 when D.A. Cooper and E.K. Inskeep demonstrated its significance in lactating beef cows.

3. The reason why progesterone production by induced CL was not related to $PGF_2\alpha$ on day 7 is more than likely due to the late stage at which sampling took place.

Paper 31

The investigation described here focused again on the possible need to increase oestrogen levels prior to ovulation induction. An attempt was made to mimic the effect of progestogen on oestrogen by implants containing oestradial-17ß.

Findings :-

1. The oestrogen implants achieved the same level of oestrogen as that seen following insertion of progestogen pessaries.
2. Progestogen pre-treatment improved luteal quality, while oestrogen had a reduced effect.

Implications/Significance :-

1. The beneficial effects of progestogen pre-treatment were probably not mediated via the increased pre-ovulatory secretion of oestradial-17ß and agreed with the findings B.J. McLeod in anoestrus sheep and B.R. Pratt in lactating cows. The mechanism through which progestogen acted was considered to be via the pre-ovulatory follicle.

Paper 32

The research described here continued to focus on luteal function in lactating ewes. The hypothesis under test was that the variable responses (in terms of induced oestrus) often seen when PMSG was used, could be due to inadequate luteal functions as observed following GnRH treatment.

Findings :-

1. Almost as many ewes treated with PMSG alone exhibited inadequate luteal activity as obtained with GnRH alone (60% vs 70%).
2. When combined with progestogen pre-treatment, PMSG induced luteal activity equivalent to that seen in spontaneously cycling, non-lactating ewes.
3. Prostagladin administration, eight days after an ovulating dose of PMSG apparently failed to regress many of the induced CL.
4. Attempts to improve luteal function by providing additional luteotropic support (60 IU PMSG/day for 16 days) were unsuccessful.

Implications :-

1. Conception rates to induced ovulations do not appear to be reduced because of poor luteal support only.
2. The dose of PMSG may have been too low to have had a luteotropic effect. In view of the long half-life of PMSG in sheep, frequency of administration (twice daily) is unlikely to have been a problem. The possibility that inadequate luteal function is determined, at or soon after ovulation, was also considered as a possibility.
3. A question that requires answering is why did the $PGF_2\alpha$ not regress the induced CL. It may have done so in three of the 10 ewes, but in six it did not. The only explanation that appears to fit the situation is that the dose of 125 μg was too low.

Paper 33

This study represents a serious attempt to improve the function of GnRH-induced CL, by providing post-ovulatory luteotropic support. This approach is based on the assumption that lactation anoestrus is due to deficiencies in the release of LH. When ovulation is induced, artificially, under such circumstances, LH is likely to remain deficient after ovulation. Such a situation would be exacerbated by suckling.

At the same time, an attempt was made to improve the competence of the follicle (s) destined to ovulate (in response to GnRH) by gonadotropic stimulation and/or oestrogen priming. Because infrequent injections do not approximate the physiological situation, particularly as regards gonadotropins, the hormones were administered by intravenous infusion over a protracted period. In order to achieve this a system had to be developed whereby relatively large numbers of animals (ewes) could be infused, simultaneously and at low cost.

Findings :-

1. In terms of luteal function, provision of luteotropic support was superior to PMSG priming of the preovulatory follicle.
2. In the second experiment PMSG priming resulted in luteal function equivalent to that of normal cycling, non-lactating ewes. A further improvement was seen when oestrogen was combined with PMSG.
3. Pre-ovulatory LH release was reduced by PMSG treatment.

Implications/Significance :-

1. The results suggest that stimulation of the pre-ovulatory follicle improves early (first week) luteal function, whereas luteotropic support improves function after this phase.
2. Compared to spontaneously cycling ewes, stimulation of the follicle produces the more normal response.

Paper 34

The investigation reported in Paper 34 was a follow-up to Papers 32 and 33. An attempt was made to duplicate the responses seen in Paper 33 by using a less demanding technique than continuous infusion. Since twice-daily injection of 30 IU PMSG was ineffective, the dose was increased to 100 IU. Attention was also given to the stage of the cycle at which luteotropin became important. An attempt was made to raise endogenous LH levels by counteracting opioid suppression. The opioid antagonist, Naloxone (NAL) was used.

Findings :-

1. Naloxone (either before or after) and PMSG before GnRH did not improve luteal function.
2. PMSG on days 3 - 5 after GnRH was more effective in improving luteal function than on days 6 - 8 or 9 - 11.

Implications/Significance :-

1. In order to promote luteal function, exogenous luteotropin support needs to be provided during the early growing phase of the CL.
2. Except for differences in mode of PMSG administration, no reason can be offered for why pre-ovulatory PMSG stimulated luteal function in Paper 34, but not here.

Paper 35

This paper reviews the subject of deficient luteal function after re-initiation of breeding activity, particularly in beef cows and suggests mechanisms which could result in this phenomenon.

Since writing of the review no serious advances have been made. Additional evidence demonstrating deficiencies of the pre-ovulatory follicle has been obtained by others and Inskeep's group has shown the important effects of the CL on its own lifespan.

Paper 36

In experiments which were conducted at Henderson Research Station (Zimbabwe) and which will be described later, it was established that lactation anoestrus could be shortened by manipulation of suckling. However, the likelihood of conception to ovulations that occurred prior to 50 days post partum was low. Similarly, experiments conducted in the USA had shown that complete weaning of calves in early lactation could induce oestrous behaviour and ovulation within a few days of calf removal. However, unless weaning was preceded by a period of progestogen priming the resultant CL were short lived. Pregnancy seldom resulted from service at the first induced oestrus.

A series of four experiments was conducted at West Virginia University to examine the role of ovulation, fertilization, transport to the uterus, quality of the embryo and of progestogen replacement therapy in the poor conception rates at first oestrus induced by weaning of the calf. Cows pre-treated with Norgestomet had shown good fertilization rates following weaning and were used as controls in these studies.

Findings :-

1. Recovery rates, fertilization rates and development of embryos to 4-8 cell stage were not reduced in cows receiving no progestogen priming and embryo transport to the uterus was apparently unchanged.
2. Neither feeding of progestogen nor daily injection of progesterone could maintain pregnancy in cows having short-lived CL after mating.

Implications/Significance :-

1. Follicles, which after ovulation are converted to CL that survive for only about a week, produce oocytes that are capable of being fertilized and of undergoing early development within the oviduct. Transport within the oviduct also appears to be normal.
2. Replacing the regressing CL with exogenous progestogen did not allow pregnancy to be maintained. The possibility remained that early regression of induced CL may not be the only explanation for the low fertility seen at induced ovulations. It needed to be shown whether the embryo produced under such circumstances was defective or whether the reproductive tract, particularly the uterus, was hostile to embryo survival and implantation.

Paper 37

Paper 37 considered the preparation of the uterus to support pregnancy, with short-lived CL being eliminated by providing exogenous progestogen (Norgestomet implants) prior to weaning of calves. In experiment 1, good quality embryos (two per cow) were transferred to the uteri of cows (calf weaned only) expected to have short-lived or normal CL after calf weaning (Norgestomet implants for 9 days).

In experiment 2 the level of exogenous progestogen was increased through the medium of daily injections at a dosage known to maintain pregnancy in ovariectomized cows (100mg; twice daily)

Experiment 3 examined the persistence of large follicles, in the absence of CL, when cows received Norgestomet implants.

Findings :-

1. Only 18% of the cows were pregnant 35 days after oestrus.
2. During the first 24 days after first oestrus, oestrous activity, without standing oestrus, was observed in more than 60% of the cows. This was in the presence of Norgestomet implants.
3. In the second experiment the pregnancy rate was twice as high in Norgestomet pre-treated cows compared to cows not primed. Additional progestogen could maintain pregnancy, but not in all cows.
4. Persistent follicles and oestrous activity were seen (Experiment 3) in non-lactating cows implanted as for Experiment 1.

Implications/Significance :-

1. Short-lived CL are not the only cause of low pregnancy rates in cows induced to ovulate by weaning of the calf. A poor quality oocyte or a hostile environment (oviduct and/or uterus) before day 7 may play a role,
2. If good quality embryos are introduced after the first post-partum ovulation and exogenous progestogen is provided, then cows likely to have short luteal phases can maintain a pregnancy.

Paper 38

E.K. Inskeep and the author of this dissertation reviewed the factors affecting post-partum anoestrus in beef cattle (Paper 40). One of the aspects evaluated was the physiological mechanisms which appeared to control the duration of post partum anoestrus. Paper 38 was designed to provide more information concerning the mechanisms that appeared to be important. In addition, special attention was given to *Bos indicus* cattle, since it appeared that lactation anoestrus was more serious in these types than in *Bos taurus* breeds.

Findings :-

1. There was a tendency for more cows suckled once daily to ovulate in response to GnRH stimulation than cows suckled normally.
2. The conception rate was also higher and incidence of short luteal cycles was lower when Drakensberger (*Bos indicus*) cows were suckled once daily.
3. No treatment effects (Breed, suckling type, stage postpartum) were evident as regards tonic LH, pre-ovulatory LH and oestrogen secretion prior to ovulation.

Implications/Significance :-

1. The greater tendency of cows suckled once daily to ovulate in response to GnRH suggests that this technique has potential as a means of improving the low reproductive rate of *Bos indicus* breeds used in this country. This topic will be considered in detail in the next section.

Paper 39

The development of a radioimmunoassay for milk progesterone allowed research to be initiated on the reasons why dairy cows were completing, on average, only three lactations before being culled. It was hoped that research in this direction would cast light on poor conception rates to early postpartum matings in beef cows. Early post-oestrus embryonic death before day 28 of the cycle was examined by progesterone assay of milk samples. In addition, the changes in distribution of oestrous cycle length amongst cows inseminated at oestrus versus those not inseminated was examined.

Findings :-

1. Progesterone assay indicated an embryo/foetal loss of 15.2% between days 28 and

75 post mating. Based on delayed returns to oestrus the figure obtained was 21.6%.

2. The number of cycles extended beyond 22 - 28 days indicated that early embryo mortality was of the order of 23.3%.

Implications/Significance :-

1. Taken together these estimates indicate that embryonic/foetal mortality could be as high as 40%. In the beef cow the opportunity for mating at successive oestrous periods is decidedly limited, because of the short duration of the mating period and due to the prolongation of lactation anoestrus. If the findings in dairy cows can be extrapolated to beef cows then it is perhaps not entirely surprising that low calving rates are common. Admittedly, beef cows are under less stress than high producing dairy cows, but there are many other factors that count against them.

PART F

MANIPULATION OF SUCKLING TO REDUCE THE DURATION OF LACTATION ANOESTRUS

Paper 40

A review published in 1979 (Paper 40) considered the effects of breed and age of cow, suckling and plane of nutrition. It was clear that even amongst *Bos taurus* breeds there were important differences in the duration of lactation anoestrus. Although suckling a calf was shown to prolong anoestrus the benefit of weaning the calf (at 55 days of age) was important only in two-year old cows that were not expected to have neared the end of lactation anoestrus by the time of weaning. The level of energy feeding (both pre-and post partum) was seen to have important consequences as regards the duration of post partum anoestrus and this formed the basis of Paper 41.

Paper 41

In a review by P.J. Chenoweth it was proposed that weaning strategies were likely to be more beneficial in shortening lactation anoestrus in first-calvers than in older cows. The adequacy of feeding of such cows was also likely to be an important modifying factor. Accordingly, a trial was conducted in which *Bos taurus* type heifers were fed to achieve different body conditions at calving. At two stages post partum (50 and 80 days) the calves were removed for seven days.

Findings :-

1. Although differences in body condition were achieved no cows ovulated in response to temporary calf removal while only two cows showed oestrus after calf weaning at 80 days post partum.

Implications :-

1. Reasons were sought for the lack of response the most probable explanation being the absence of stimulation by bulls. This is supported by the results reported in Paper 44.

Paper 42

The following series of papers represents the climax of the research programme described in this thesis.

When P.L. Wells was considering initiating a programme on which a PhD thesis could be based he was advised to look at the relationship between the Afrikaner cow and her calf. The question that needed answering was whether there was a special relationship in this breed which could explain the prolonged inhibition of suckling on resumption of oestrous cycles, post partum?

The Afrikaner breed was being used extensively in Zimbabwe and South Africa, in spite of the record of poor calving rates (55 - 65%). In contrast, the Mashona breed showed good calving rates (80% plus) under the same conditions in Zimbabwe. Consequently, Mashona cows were included for comparative purposes.

It had long been known that Afrikaner cows that lost their calves, at or soon after calving, were able to conceive in the immediately subsequent breeding period. In contrast, conception rates were poor in cows which nursed their calves to weaning age (about seven months) with cows often calving only in alternate years. It was thus obvious that the inhibitory effects of calf suckling on conception rates needed to be counteracted. Alternatives such as short-term calf removal or reduced suckling frequency were considered. However, it was deemed essential to establish, in the initial study, what the maximum effect of weaning would be. Accordingly, calves were weaned at three days of age, while cows which suckled their calves normally, served as controls.

The results for only the Afrikaner cows are presented in Paper 42, but for the sake of completeness those pertaining to the Mashona have been taken from the PhD thesis written by P.L. Wells.

Findings :-

1. Non-suckled Afrikaner cows ovulated sooner (18.1 ± 5.2 days) after parturition than normally suckled cows (53.2 ± 19.7 days). In Mashona cows the difference was not significant (19.2 vs 37.6 days).
2. Conception rates were higher in non-suckled (80%) than in suckled Afrikaner cows (50%), but no difference occurred in Mashona cows (100% vs 90%).
3. Interval to conception was significantly shorter in weaned Afrikaner cows (45.8 ± 15.8 days) than in cows suckled normally (70.9 ± 17.9 days). Again, the difference in Mashona cows was not significant (40.2 vs 60.0 days).
4. Conception rates, regardless of treatment, were highest between 50 and 70 days post partum with non-suckled cows ovulating more times (2.5 times) before they conceived than suckled cows (2.1 times).
5. Suckling treatment did not affect the incidence of short luteal cycles.

Implications/Significance :-

1. Suckling is clearly an important factor in prolonging lactation anoestrus in Afrikaner cows and thereby limiting conception rates to 50%.
2. Although more non-suckled cows were induced to ovulate than suckled cows, both treatments showed a peak in conception rates at 50 - 70 days post partum. Several explanations for this situation appear feasible. Either, the ovulations were not accompanied by satisfactory service and the ova were not fertilized or the embryos did not survive due to inadequate luteal support. The first possibility will be reviewed in Part G and the second alternative has been considered in Part E.
3. The unexpectedly high improvement in the CR of Afrikaner cows, resulting from weaning of the calf, suggests that the "maternal instinct" could be more suppressive than in other *Bos indicus* breeds and in *Bos taurus* types. Both endocrine and behavioural mechanisms may be contributing to the high suckling-induced suppression of breeding during lactation.

Paper 43

Most of the Afrikaner cows incorporated in the previous study and weaned at three days after calving, conceived 60 - 65 days post partum. Thus, any weaning strategy should be timed such that the first ovulation occurs 15 - 20 days earlier than the conception date. The study outlined in Paper 43 was based on this proviso and included a "weaning" strategy that could easily fit into a commercial production system.

The procedure adopted was to limit suckling (Partial weaning) to twice daily for the period 28 - 41 days post partum and thereafter, once daily until 100 days post partum. Only Afrikaner cows were used in this study.

Findings :-

1. Partial weaning (PW) reduced the proportion of Afrikaner cows anovulatory for 100 days after calving from 40.6% in cows suckled normally to only 6.1%.
2. Conception rates were also improved by PW (90.9 vs 50%).
3. The delay to first rise in progesterone was not shortened by PW (55 vs 52 days).
4. The mean interval to conception was not reduced by PW (69 vs 72 days)
5. The probability of conception again appeared to be highest at 55 - 65 days post partum.

Implications/Significance :-

1. The primary reason why normally suckled Afrikaner cows fail to reconceive during a limited breeding period appears to be because they do not ovulate while exposed to bulls. This is based on the finding that PW could increase the conception rate by 40% through a stimulation of ovarian activity in about 33% of the cows. These cows would have remained anoestrus for at least 100 days post partum if they had been suckled in the normal manner. This agrees with our unpublished observation where rectal palpation has revealed no ovarian activity in many Afrikaner cows at 120 days post partum.

Paper 44

The investigations outlined in Papers 42 and 43 involved reduction in the suckling stimulus at the time that breeding bulls were introduced into the herds. Since joining with bulls was a factor common to all treatment the responses seen were attributed solely to manipulation of suckling. However, in view of the findings reported in Part A of this thesis, and because of the findings of others showing that bulls can stimulate cows, it was deemed advisable to give attention to this aspect. Accordingly, cows were PW without bulls being joined with them and this was compared to the procedure applied in Paper 43. Again, Mashona cows were included for comparative purposes.

Findings :-

1. PW on its own did not reduce the incidence of anovulatory cows compared to cows suckled normally. When introduction of bulls was combined with PW, the proportion of anovulatory Afrikaner cows was reduced, the proportion ovulatory for the first time increased and the interval to conception for Mashona cows was reduced.
2. Conception rates were 47% for Afrikaner cows and 90% for Mashona cows and were not significantly affected by weaning treatment.
3. The lower conception rate in Afrikaner cows was due to an increase in cows anovulatory (39%) and to a reduction in the conception rate (79 vs 89%).
4. PW before joining with bulls significantly shortened the interval from joining to conception for both breeds. However, the low conception rates to first ovulations resulted in Afrikaner cows PW at bull introduction not exhibiting a reduced interval from joining to conception.
5. In Mashona cows PW during breeding shortened the intervals from joining to conception.
6. Luteal activity was improved by restricting suckling after fertile breeding.

Implications/Significance :-

1. PW has only limited potential for eliminated anovulatory Afrikaner cows unless it

is accompanied by the stimulus of joining with bulls.

2. Limitation in suckling improves luteal activity after conception and thereby probably favours continued pregnancy.

Paper 45

This paper considers the possibility that low conception rates to ovulations that occur at an early stage post partum are due to mating either not occurring at all or not frequently enough. There was the added possibility that the continuous presence of the suckling calf might modify cow behaviour at oestrus. The results reported here form part of the experiments outlined in Papers 42, 43 and 44. The emphasis here is placed on oestrous behaviour only in cows actually seen to be served by bulls.

Findings :-

1. Cows suckled normally exhibited low levels of homosexual activity.
2. In Afrikaner cows, PW while bulls were present, increased the intensity of homosexual behaviour.
3. Mounting of bulls by cows in oestrus also occurred at a low frequency (< 40% of oestrous periods) and no consistent effect of PW was seen.
4. Behaviour that was initiated by bulls also occurred infrequently and in many cases a behavioural component, directed at a particular cow, occurred only once at any one oestrus.
5. Forty percent of all the full ovulations recorded were not associated with observed service. Complete and partial weaning increased the incidence of observed service, without having a consistent effect on the number of services per oestrus.

Implications/Significance :-

1. Oestrus in Afrikaner cows is characterised by a low incidence of interactive behaviour between cows and between cows and bulls and by a short duration (3-4hrs) of heterosexual activity. Thus, in the Afrikaner cow, oestrus is a phenomenon which is associated with a minimum of interactive behaviour. With many periods

of oestrus being accompanied by a single service only, the definition needs to be modified from oestrous period to oestrous event.

2. With normal suckling, service was NOT seen at about 50% of the full ovulations. The fact that service probably occurred outside the surveillance period (05:00 to 17:00h) was deduced from the finding that numerous cows calved without service having been observed.
3. The results do not support the conclusion that the low conception rate, to ovulations before 50 days post partum might be due to cows not being mated.
4. Taken as a whole the results provide good evidence, though not always statistically significant, that limited suckling enhances the intensity of oestrous behaviour and attractiveness of cows to bulls.

Paper 46

At the outset of this section it was emphasised that the major aim was to establish whether cow-calf behaviour in Afrikaner cows could explain the severe limitation of suckling on sexual activity during lactation. A secondary objective was to determine whether suckling behaviour changed as the calf became less dependent on its dam and thereby allowed the cow to escape from the inhibition on sexual activity.

In order to examine these aspects, attention has been focused in Paper 46 on suckling behaviour as the calves approached the age at which cows could be expected to show first post-partum oestrus.

Findings :-

1. Two distinct phases during a suckling episode were identified viz., an extraction phase and a stripping phase. Thus, milk removal by the calf could be likened to hand milking. The duration of the stripping phase was about 50% longer than the extraction phase and nearly six times as many teat changes were made.
2. Wide variations in suckling behaviour between the three breeds (Afrikaner, Mashona, Hereford) were seen.
3. No consistent trend in behavioural changes was seen as calves grew older and thus there was no apparent behavioural change that might be associated with a return to

cyclicity in the dam.

4. Suckling stimulation experienced by the cow depended more on the number of sucklings per day rather than on variations within an individual pattern, from calf to calf.
5. There was little variation in the suckling pattern between Afrikaner and Mashona cows.

Implications/Significance :-

1. The severe prolongation of lactation anoestrus in Afrikaner cows could not be explained in terms of behavioural differences associated with suckling by the calf.
2. The udder stimulation that a cow received at suckling appeared to be independent of the cow's milk yield.
3. No obvious changes in suckling behaviour could be used to explain why there was a greater probability of cows resuming sexual activity with advance in the post partum period.

Paper 47

In the paper reviewed above, observations were confined to daylight hours. The uncertainty thus remained as to what could be happening during hour of darkness.

The opportunity became available to observe *Bos taurus* type females under intensive grazing conditions. It was decided to extend the observations that had been made under extensive grazing situations to a situation where cows and calves were likely to be in relatively close contact throughout the day and night. In addition, it was considered vitally important to extend observations into the dark period. The need to avoid disrupting any seasonal effects due to day length, by using bright lights, was appreciated.

Findings:-

1. Over a 24-hour period there was a wide variation in observed frequency of suckling, from twice to 11 times.

2. The mean suckling frequency decreased significantly after 45 days of age.
3. The mean duration of a suckling event was 9.6 ± 2.2 min. with a range of 5.6 to 14.8 min.
4. Duration of suckling was negatively correlated ($r = -0.21$; $P < 0.01$) with frequency.
5. Suckling events were not evenly distributed over the 24-hour period.
6. As the age of the calf increased so the longest interval between sucklings became extended. With few exceptions, this long inter-suckling period occurred in the early hours of the morning.

Implications/Significance :-

1. Over a 24-hour period the total time spent suckling varied widely. It is thus unlikely that a relationship exists between suckling frequency and onset of first oestrus post-partum.
2. The daily distribution of sucklings over the 24-hour period warranted attention. Of particular interest was the nadir in incidence of calves suckling between midnight and 04:00h and the apparent lengthening of this interval as calves became older. This observation became the focal point of subsequent research.

Paper 48

Onset of oestrus, post-partum, has been successfully induced by others through temporary removal of the calf or a limitation in the number of sucklings per day. Cows that suckle their calves normally do eventually ovulate and exhibit oestrus. It was hypothesised that some change in suckling inhibition of sexual activity occurred naturally and that the cue might be a lengthening in the non-suckling interval between midnight and dawn. Accordingly, in the experiment described in Paper 48 calves were separated from their dams either during the night or during the day.

Findings :-

1. Calves that were separated from their dams for 12-hour periods did not alter their behaviour patterns to compensate for the missed sucklings.

2. The proportion of cows observed in oestrus during the first 30 days of the breeding period was 2.5 times greater in the cows suckled during the day only than where calves were present at night.

It thus appeared that suckling-induced prolongation of post-partum anoestrus is somehow dependent on calves suckling at night.

Paper 49

This paper examined the possibility that suckling late at night was more important on prolonging lactation anoestrus than frequent suckling per 24 hours. Accordingly, cows suckled their calves five times per 24 hours with a long interval between sucklings either from 21:00h to 05:00h or from 09:00h to 17:00h. Cows that suckled their calves without interference were not used as controls since a wide variation in suckling frequency usually occurred under such circumstances. Instead, cows that suckled their calves only once daily served as controls.

Findings :-

1. Only two of the 15 cows that were suckled at 24:00h exhibited oestrus within 80 days after calving. In contrast, 100% of those cows not suckled at this time cycled within this period.
2. The mean post-partum interval to first oestrus for cows not suckled at 24:00h was not different to that seen in cows suckled only once daily.

Implications/Significance :-

1. Suckling late at night prolongs lactation anoestrus.
2. Prevention of suckling between 21:00 and 05:00h, even if accompanied by suckling five times in a 24-hour period, is as effective in stimulating onset of sexual activity, post-partum, as once daily suckling at 17:00h.
3. There may be a time of night when beef cows are particularly sensitive to the inhibitory influence of suckling on resumption of oestrous cycles.

FOLLICULAR DEVELOPMENT AND FUNCTION OF INDUCED CORPORA LUTEA IN UNDERFED POSTPARTUM ANESTROUS BEEF COWS[1]

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SUMMARY

Effects of energy restriction during late gestation and early lactation on pituitary and ovarian responses to FSH and GnRH, and the effects of exogenous FSH or estradiol on follicular growth and luteal function after GnRH were examined in three experiments. In experiment I, 43 2-year-old Hereford heifers were assigned randomly 30 days prior to expected calving to be fed either 115% or 60% of the NRC recommended energy requirements. Although cows on the low plane lost more weight ($P<.01$) through the first 30 days after parturition, level of feeding had no effect on size of the largest ovarian follicle or concentration of plasma estradiol-17β at 30 days postpartum.

At 30 days postpartum cows received injections twice daily of 0, .125 or .25 mg FSH for 3 days and were then treated with 300 μg GnRH. Dose of FSH did not affect estradiol-17β in daily plasma samples. Concentrations of LH in jugular blood samples taken every 30 min for 7 hr after GnRH were affected by plane of nutrition ($P<.01$). There was a positive association between follicle size and both the pattern of release of LH ($P<.01$) and the area under the LH response curve ($P<.05$).

Occurrence of CL after GnRH was not related to body weight postpartum, change in weight during lactation, amount of LH released, follicle size, nutrition or dose of FSH. In 22 of 23 cows the CL palpated on day 7 had regressed by day 14 and progesterone in plasma at 3- or 4-day intervals after GnRH reflected a shortened life span of the induced CL.

In experiment II, 17 cows that did not have CL in experiment I were injected twice daily for 3 days with either 0 or 2 mg FSH followed by injection 300 μg GnRH. Concentrations of estradiol-17β in plasma collected prior to FSH were correlated with body weights at 48 days postpartum ($r=.70$). Estradiol prior to GnRH, was correlated with size of the largest ovarian follicle ($r=.53$) at that time. Treatment with FSH (4 mg/day) increased mean diameter of the largest follicle ($P<.05$), but not estradiol-17β prior to GnRH. The proportion of cows that formed CL was not higher in cows pretreated with FSH (88%) than in controls (56%). Progesterone in plasma at 14 days after GnRH indicated that induced CL were short-lived.

In experiment III, 18 3-year-old cows were treated twice daily from day 34 to 38 postpartum as follows: (1) corn oil for 1 day followed by saline for 3.5 days, (2) corn oil for 1 day plus increasing doses of FSH for 3.5 days or (3) estradiol benzoate (75 μg) for 1 day plus FSH for 3.5 days. GnRH (300 μg) was administered 4 days after initiation of treatments. FSH or estradiol plus FSH did not affect follicular development, profiles of estrogen or LH, or occurrence or lifespan of induced CL. Sixty-one percent of the cows had CL on day 7 and progesterone in plasma of those cows averaged only 1.4 ng/ml on day 7 and 45% of these CL were short-lived.

(*Key Words*: Postpartum Anestrus, Lactating Beef Cows, LH, GnRH, Corpora Lutea, Plane of Nutrition.)

INTRODUCTION

In many beef herds a significant proportion of lactating cows have not ovulated by the beginning of the mating period (Wiltbank, 1974). Conception rates are poorer in cows mated at *first estrus*

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not responsible for the luteotrophic effect.

In conclusion, the results seem to indicate that the action of progesterone is direct, rather than mediated through E_2. The direct effect can be at ovarian level or on the hypothalamo-pituitary axis to alter the pattern of LH and or FSH secretion. This alteration could be more beneficial in priming the pre-ovulatory follicle to become a better secretor of progesterone.

References

HAMILTON, C.D., LISHMAN, A.W. & LAMB, P.A., 1979. Effect in ewes of oestrogen priming and GnRH on LH release and luteal function during early lactation in spring. *S. Afr. J. Anim. Sci.* 9(3), 197.

LIEBENBERG, G., 1983. Oestradiol control of the pre-ovulatory LH surge in ewes. M.Sc. Thesis University of Natal, South Africa.

MC LEOD, B.J., HARESIGN, W. & LAMMING, G.E., 1982. Response of seasonally anoestrus ewes to small-dose multiple injections of GnRH with and without progesterone pre-treatment. *J. Reprod. Fert.* 65(1), 223.

OLDHAM, C.M. & MARTIN, G.B., 1979. Stimulation of seasonally anovular Merino ewes by rams. II. Premature regression of ram-induced corpora lutea. *Anim. Reprod. Sci.* 1, 291.

PRATT, B.R., BERARDINELLI, J.G., STEVENS, C.P. & INSKEEP, E.K., 1982. Induced corpora lutea in the post partum beef cow. I. Comparison of gonadotrophin and effects of progestogen and oestrogen. *J. Anim. Sci.* 54(4), 822.

ROBINSON, T.J., 1950. The control of fertility in sheep. *J. agric. Sci. Camb.* 40, 275.

SHEFFEL, C.E., PRATT, B.R., FERREL, W.L. & INSKEEP, E.K., 1982. Induced corpora lutea in the post partum beef cow. II. Effects of treatment with progestogen and gonadotrophins. *J. Anim. Sci.* 54(4), 830.

WALTERS, D.L., SHORT, R.E., CONVEY, E.M., STAIGMILLAR R.B., DUNN, T.G. & KALTENBACH, C.C., 1982. Pituitary and ovarian function in post partum beef cows. III. Induction of estrus, ovulation and luteal function with intermittent small-dose injections of GnRH. *Biol. Rep.* 26(4), 655.

WEBB, R., LAMMING, G.E., HAYNES, W.B., HAFS, H.D. & MANN, J.G., 1977. Response of cyclic and post partum suckled cows to injections of synthetic LH-RH. *J. Reprod. Fert.* 50,203.

S. Afr. J. Anim. Sci. 10, 151 – 157 (1980) PAPER 32

PLASMA PROGESTERONE LEVELS IN LACTATING EWES AFTER HORMONE-INDUCED OVULATION DURING THE NON-BREEDING SEASON

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(Key words: *Progesterone, lactating ewes, non-breeding season)*
(Sleutelwoorde: *Progesteroon, lakterende ooie, nie teelseisoen)*

OPSOMMING: PLASMA PROGESTEROONPEILE BY LAKTERENDE OOIE NA OVULASIE AS GEVOLG VAN HORMOONTOEDIENING GEDURENDE DIE NIE-TEELSEISOEN

Bronstigheid, ovulasie en periferere plasma-progesteroonkonsentrasies is bepaal by 58 lakterende Suid-Afrikaanse Vleismerino-ooie wat verskillend met progestogeen, dragtige merrieserum (DMS), prostaglandien en gonadotrofiese vrystellingshormoon (GnVH) behandel is. Die ooie is by 2 post partumintervalle gedurende die nie-teelseisoen behandel. Ovulasie wat by 46 ooie plaasgevind het, het geen betekenisvolle variasie tussen die 6 hormoonbehandelings getoon nie. Slegs 7 ooie wat beide progestogeen en DMS ontvang het, is bronstig gevind. By hierdie behandeling het die plasmaprogesteroonpeile ooreengestem met dié wat vir spontane estruskringlope by nie-lakterende ooie gerapporteer is. Ooie wat progestogeen tussen 2 gespasieerde inspuitings van DMS ontvang het, het 'n normale duurte, maar 'n verlaagde piekkonsentrasie (1,40 ng/ml) van progesteroonproduksie getoon. 'n Hoe persentasie van die ooie wat met DMS alleen (60%) of GnVH alleen (70%) behandel is, het subnormale piekkonsentrasies en verkorte periodes van verhoogde plasmaprogesteroon gehad. Hierdie subnormale progesteroonproduksie is nie teegewerk deur inspuiting van DMS tweemaal per dag vir 16 dae na GnVH nie. Prostaglandien het klaarblyklik geen luteolitiese effek gehad by sekere ooie waar funksionale corpora lutea a.g.v. DMS toediening verkry is nie. Daar was geen beduidende verskille wat betref bronstigheid, ovulasie of progesteroonproduksie tussen ooie wat of 22 of 35 dae na partus behandel is nie.

SUMMARY:

Oestrus, ovulation and peripheral plasma progesterone concentrations were recorded in 58 lactating South African Mutton Merino ewes treated variously with progestagen, pregnant mare serum gonadotrophin (PMSG), prostaglandin and gonodotrophin releasing hormone (GnRH) at 2 post partum intervals (22 or 35 days post partum) during the non-breeding season.

Ovulation was induced in 46% ewes, with no significant variation between 6 different hormone treatments. Only 7 ewes (78%), all treated with progestagen and PMSG, were detected in oestrus.

Plasma progesterone levels in ewes treated with progestagen and PMS were similar to those reported for spontaneous oestrous cycles in non-lactating ewes. Ewes treated with progestagen between 2 spaced injections of PMSG showed a normal duration of progesteron production, but reduced peak concentration, viz. (1,40 ng/ml). A high proportion of ewes treated with PMSG alone (60%) or GnRH alone (70%) showed subnormal peak progesterone concentrations and shortened periods of elevated plasma progesterone. This subnormal progesterone production was not counteracted by twice-daily injections of PMSG for 16 days after GnRH injection. Prostaglandin appeared to have no luteolytic effect in some ewes in which functional corpora lutea had been induced by PMSG injection.

There were no significant differences in oestrus, ovulation or progesterone production between ewes treated 22 and 35 days post partum.

When ewes lamb in spring they usually commence sexual activity at a later stage post-partum than where lambing occurs in autumn (van Niekerk & Mulder, 1965) and attempts to induce rebreeding in lactating ewes shortly after parturition during the non-breeding season have generally been unsuccessful. Ovulation and oestrus may be induced by treatment with progestagen and pregnant mare serum gonadotrophin (PMSG), but impaired fertilization (Dawe & Fletcher, 1976) and embryo survival (Cognie, Hernandez-Barreto & Saumande, 1975) result in poor conception rates until 7 or 8 weeks post partum.

Although the causes of early post partum reproductive failure have not been clearly defined, several instances of hormonal imbalance have been reported. Luteinizing hormone (LH) release after treatment with progestagen and PMSG is lower in lactating than in non-lactating ewes (Pelletier, 1976). Prolactin is released in response to the suckling stimulus (Lamming, Moseley & McNeilly, 1972), and this may inhibit LH releasing hormone (Louw,

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Lishman, Botha, Arangie, Poultney & Gunter, 1976). Progesterone production, too, may be impaired, though the evidence is based on visually abnormal corpora lutea (Restall, Kearins, Hurdegen & Carberry, 1978) rather than on measured progesterone production. Cognie *et al.* 1975) reported that progesterone levels in pregnant ewes were higher 10-14 days after ovulation in lactating than in non-lactating ewes, but progesterone production following ovulation induced by early post partum treatment wtih progestagen and PMSG has otherwise not been noted.

The experiment reported here was designed to evaluate luteal function in lactating ewes following ovulation induced by various treatments with progestagen, PMSG, prostaglandin and gonadotrophin releasing hormone at 2 intervals after parturition. Particular attention was focussed on the possibility that PMSG treatment might induce inadequate luteal function such as has been observed where GnRH was used in attempts to initate breeding (Haresign, Foster, Haynes, Crighton & Lamming, 1975; Haresign & Lamming, 1978).

Material and Methods

Animals

Sixty lactating South African Mutton Merino ewes of mixed ages were selected on the basis of time of lambing. Thirty ewes lambed between 11 and 19 September and 30 between 27 September and 3 October 1978, so that they were on average 35 days and 22 days post partum respectively when ovulation was induced by hormone treatment on 18 October. The ewes continued to suckle their lambs and were maintained as a single flock throughout the duration of the experiment.

Treatments

Ewes in each time-of-lambing group were allotted at random to 6 treatment groups each of 5 ewes:

Group (i):
PROG / PMSG – Intravaginal sponges impregnated with 60 mg of synthetic progestagen ("Repromap", Upjohn) were inserted for an 8-day period beginning on 10 October. A single intramuscular injection of 600 iu PMSG was administered when the sponges were withdrawn on 18 October.

Group (ii):
PMSG / PROG / PMSG – "Repromap" sponges were inserted for an 8-day period beginning on 10 October. Two intramuscular injections each of 600 iu PMSG were administered, the first when sponges were inserted and the second when sponges were withdrawn on 18 October.

Group (iii):
PMSG / PG / PMSG – A single intramuscular injection of 600 iu PMSG was administered on 10 October. On 18 October, a single intramuscular injection of 125 μg of synthetic prostaglandin ("Estrumate", I.C.I.) was administered, followed immediately by a second intramuscular injection of 600 iu PMSG.

Group (iv):
PMSG – A single intramuscular injection of 600 iu PMSG was administered on 18 October.

Group (v):
GnRH – Three intramuscular injections of synthetic gonadotrophin releasing hormone (GnRH – "Cystorelin", Abbott), each of 25 μg and spaced at 1,5 hr intervals, were administered on 18 October.

Group (vi):
GnRH / PMSG – GnRH was administered as 3 intramuscular injections, each of 25 μg spaced at 1,5 hr intervals, on 18 October. Two intramuscular injections each of 30 iu PMSG were administered at 0800 hr and 1600 hr each day for 16 days between 19 October and 3 November inclusive.

Observations

Five entire rams fitted with harnesses and marking crayons were put with the ewes on 19 October. Ewes detected in oestrus were recorded and the rams removed on 25 October.

The ovaries of ewes which were not detected in oestrus were examined for evidence of recent ovulation by mid-ventral laparotomy between 25 and 27 October. Corpora lutea which were small or pale in colour were particularly noted.

Blood was taken from the jugular vein of all animals on October 18, 24, 26, 28 and 30, and on November 1, 3 and 7. Plasma was separated by centrifugation and stored at -4°C until required for progesterone assay. Progesterone was assayed by the method of Butcher, Collins & Fugo (1974). The inter-assay coefficient of variation was 16,05%.

Results

Results were taken from 58 ewes. One ewe from the PROG / PMSG (Group (i)) treatment lost its progestagen sponge and one ewe from the PMSG treatment (Group (iv)) died during the experiment.

Results from ewes treated 22 and 35 days post partum, pooled over hormone treatments, are summarised in

Table I

Effects of post-partum interval on oestrus, ovulation and plasma progesterone

	Mean post-partum interval (days ± SE)	
	22,3 ±0,37	34,6 ±0,61
Number of ewes	28	30
Ewes detected in oestrus	3	4
Ewes with corpora lutea*	19	19
Mean ovulation rate*	1,47	1,53
Progesterone production^{+} :–		
"None" :		
number of ewes	6	5
peak progesterone (ng/ml ±SE)	0.64 ±0.06	0.62 ±0.07
"Normal" :		
number of ewes	12	15
peak progesterone (ng/ml ±SE)	2.27 ±0.31	2.44 ±0.26
"Short" :		
number of ewes	8	9
peak progesterone (ng/ml ±SE)	1.89 ±0.17	1,72 ±0.15
"Early" :		
number of ewes	2	1
peak progesterone (ng/ml ±SE)	2.06 ±0.21	2,34

* Excluding the PROG / PMS treatment, in which ovulation was not recorded

\+ Defined in the text

Table 1. Post partum interval had no significant effect on oestrus, ovulation or plasma progesterone levels, and there were no significant interactions between post partum interval and hormone treatment. Therefore, results from ewes treated 22 and 35 days post partum have been pooled for subsequent presentation.

The effects of different hormone treatments on oestrus and ovulation are summarised in Table 2. Seven of 9 PROG / PMSG ewes were detected in oestrus and one of the other 2 showed evidence of recent ovulation at laparotomy. None of the 49 ewes in the other treatment groups were detected in oestrus, though 38 ovulated. There were no significant differences between treatments in the number of ewes ovulating or mean ovulation rate.

Four different patterns of progesterone production were recorded during the 20 day period following PMSG or GnRH injection on 18 October. These were defined as: "None" (Plasma progesterone did not increase significantly above the basal level of less than 1,0 ng/ml).

"Normal" (plasma progesterone increased and remained at an elevated level (i.e. above basal) through to day 16 after PMSG or GnRH injection), and

"Short" (Plasma progesterone increased, but did not remain at an elevated level through to day 16 after PMSG or GnRH injection, and

"Early" (plasma progesterone was elevated at the time of the second PMSG injection, but decreased to the basal level by day 12 and remained at this level for at least 6 days).

These patterns of progesterone production, their distribution among the 6 hormone treatment groups, and peak progesterone concentrations (the highest plasma concentration recorded during the 20 day period, usually on days 10 or 12), are presented in Table 2 and Fig. 1.

Eleven ewes, distributed without significant difference among all hormone treatments, showed the "None" pattern of progesterone production. Except for one ewe from Group 1 which exhibited oestrus and was thus not examined for evidence of ovulation these were all ewes which at laparotomy had shown no evidence of recent ovulation.

Some ewes in all treatment groups showed the "Normal" pattern of progesterone production, but the incidence was significantly higher ($P < 0,05$) in PROG / PMSG, PMSG / PROG / PMSG and PMSG / PG / PMSG treatments (72 percent) than in the other 3 treatment groups (21 percent). Peak progesterone concentration among ewes with the "Normal" pattern of production was significantly lower in the PMSG / PROG / PMSG than in other treatment groups. Plasma progesterone remained at a high level on day 20 in 5 ewes which otherwise showed a "Normal" pattern of production (Fig. 1). Three of these in the PROG / PMSG treatment group had mated with entire rams, but one each from the PMSG / PG / PMSG and PMSG groups had not been detected in oestrus.

The "Short" pattern of progesterone production was recorded only in the PMSG, GnRH and GnRH / PMSG treatment groups, but the incidence in these 3 groups was relatively high (77 percent of ewes which ovulated). Within these treatment groups "Short" patterns of progesterone production also had lower mean peak progesterone concentrations than "Normal" patterns (1,8 vs 2,95 ng/ml, $t = 3,71$, $df = 20$, $P < 0,01$).

Only 9 ewes had corpora lutea which were subjectively assessed as small or pale. Three of these had "Normal" patterns of progesterone production, but low peak levels, and the other 6 showed "Short" or "Early" patterns of progesterone production. However, 18 other

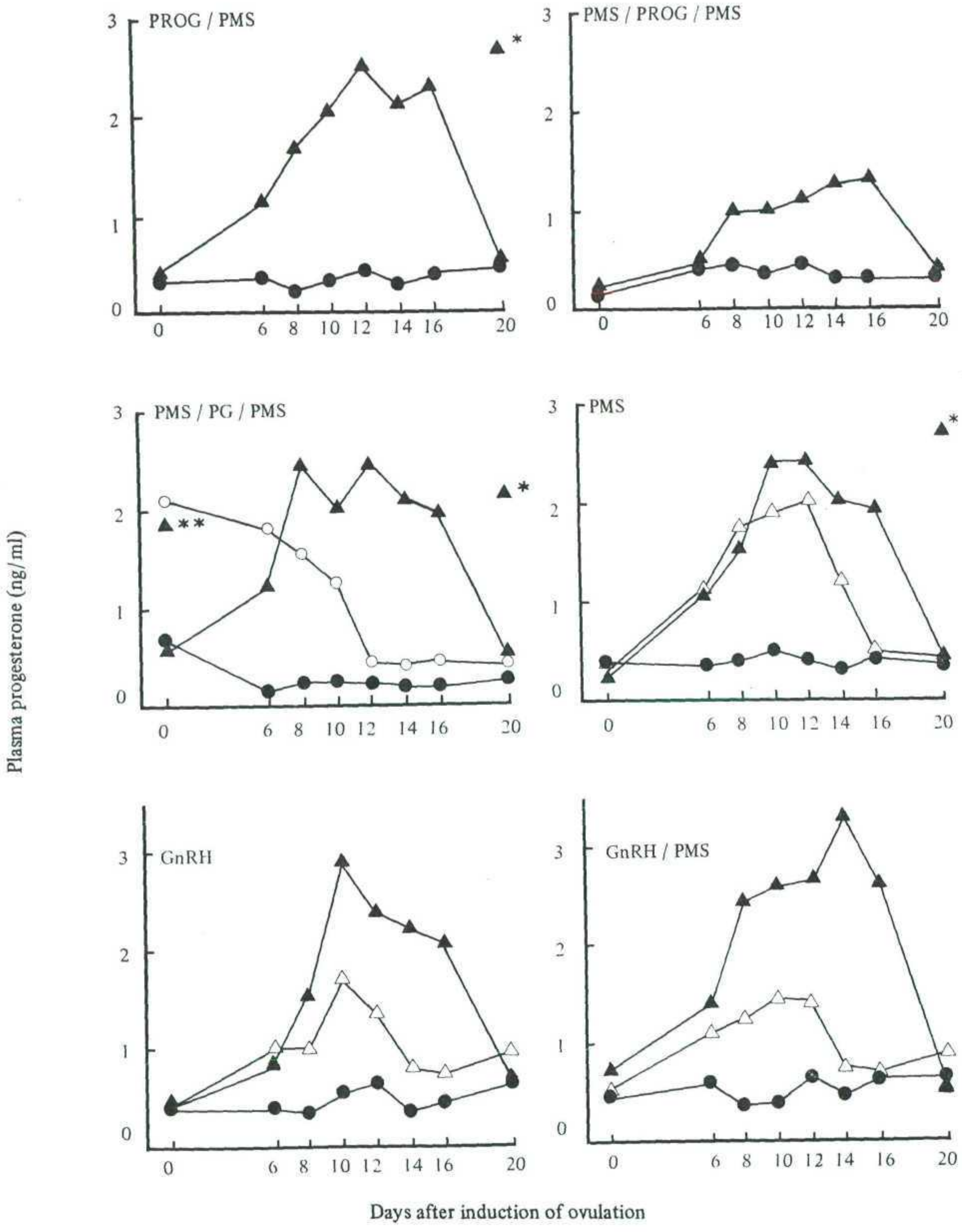

Fig. 1 *Plasma progesterone profiles in ewes after ovulation induction during lactation. Where 2 injections of PMS were given the progesterone concentrations refer to samples taken after the second PMS treatment only. The numbers of ewes represented by each curve for "None" (●–●), "Normal (▲–▲) "Short" (△–△) and "Early" (○–○) patterns of plasma progesterone are presented in Table 2.*

- ** *Two ewes which showed elevated progesterone levels at the time of second PMSG injection had "normal" patterns subsequently.*
- * *Three ewes from PROG / PMSG one from PMSG / PG / PMSG, and one from PMSG which showed "normal" patterns of progesterone except that levels remained high on day 20.*

Table 2

Effects of hormone treatment on oestrus, ovulation and plasma progesterone

	PROG/PMS	PMS/PROG/ PMS	PMS/PG/ PMS	TREATMENTS PMS	GnRH	GnRH/PMS	P
Number of ewes	9	10	10	9	10	10	
Ewes detected in oestrus	7	0	0	0	0	0	
Ewes with corpora lutea	NR	7	9	7	7	8	NS
Mean ovulation rate	NR	1,14	1,44	1,50	1,29	1,50	NS
Protesterone production :-							
"None"							
number of ewes	1	3	1	1	3	2	NS
peak progesteron (ng/ml ± SE)	0,56	0,51 ± 0,02	0,72	0,50	0,74 ± 0,12	0,75 ± 0,13	NS
"Normal":							
number of ewes	8	7	6	4	1	1	0,001
peak progesterone (ng/ml ± SE)	2,62 ± 0,35	1,40 ± 0,23	2,68 ± 0,26	2,53 ± 0,64	3,32	3,84	0,05
"Short"							
number of ewes	0	0	0	4	6	7	NS
peak progesterone (ng/ml ± SE)	–	–	–	2,05 ± 0,16	1,75 ± 0,12	1,71 ± 0,24	NS
"Early"							
number of ewes	0	0	3	0	0	0	
peak progesteron (ng/ml ± SE)	–	–	2,15 ± 0,15	–	–	–	

NR : not recorded, NS : not significant

ewes with abnormal patterns of progesterone production or low peak levels had corpora lutea of normal size and colour.

Discussion

The 2 post partum intervals at which ewes were treated in this experiment were both shorter than that at which rebreeding in spring might be expected to be successful (Hunter, 1968), and were selected to investigate changes in the response to hormone therapy within this early period. The fact that there were no significant differences in any of the parameters recorded between ewes treated 22 and 35 days post partum suggests that there may be a relatively abrupt rather than a gradual change to normal reproductive function with increasing time post partum. Although the objective was to investigate changes in response, the post partum stages were selected so that the occurrence of spontaneous ovulations would be minimal. With greater numbers of animals available, it would be desirable to include a greater number of stages so that the changes just prior to the normal onset of oestrus and ovulation can be evaluated.

Although an untreated control group was considered unnecessary under the circumstances of this experiment, obviously spontaneous ovulations could have occurred. However, the progesterone levels on day 0 provide reasonable evidence for the conclusion that ovulations did not take place prior to the initiation of hormonal treatments.

Ewes treated with progestagen and PMSG showed the typical response of lactating ewes treated early post partum – a moderately high incidence of ovulation and oestrus, but low conception. On the evidence of plasma progesterone concentrations remaining elevated instead of returning to basal levels on day 20, a maximum of 3 ewes from this treatment group may have conceived. Eight of 9 ewes showed elevated plasma progesterone concentrations, with a mean peak of 2,6 ng/ml, which were maintained through day 16 after PMSG injection. This is similar to progesterone production recorded by Thorburn, Bassett & Smith (1969) for spontaneous oestrous cycles in non-lactating ewes. Thus there was no evidence from this experiment that impaired conception following early post partum treatment with progestagen and PMSG was associatied with abnormal luteal function.

Two spaced doses of PMSG with progestagen in between (PMSG/PROG/PMSG) were included in the experiment because of preliminary success with this treatment in post partum cattle (Lishman, unpublished). Seven ewes ovulated following the second PMSG injection, but none were detected in oestrus. Further, although corpora lutea had a normal life-span, peak progesterone concentrations were significantly reduced (Fig. 1). If these results can be repeated then a study of the mechanisms involved could perhaps cast some light on the problem of sub-normal luteal function.

Treatment with prostaglandin and PMSG (PMSG/PG/PMSG) was planned on the basis that the initial PMSG injection would induce ovulation and functional corpora lutea, prostaglandin would cause rapid luteolysis, and the second PMSG injection would induce another ovulation. However, this appeared to occur in only 2 of the 10 ewes. Five ewes had basal plasma progesterone concentrations at the time of prostaglandin injection, indicating that the first PMSG injection had failed to induce either ovulation or functional corpora lutea, and there was thus no corpus luteum to be influenced by prostaglandin. The remaining 3 ewes had elevated plasma progesterone concentrations at the time of prostaglandin injection and progesterone levels remained elevated until day 12. This pattern was consistent with normal luteal function following ovulation induced by the first PMSG injection, with no luteolytic effect of prostaglandin. Previous reports on the use of prostaglandin in lactating anoestrous ewes have not been noted, and it is not clear whether the failure of prostaglandin to induce luteolysis was associated with lactation or some other factor. Since 2 of these 3 ewes each had 2 corpora lutea, the dose of prostaglandin (125 μg) may have been too low (Greyling & van der Westhuysen, 1979).

A high proportion of ewes treated with with PMSG or GnRH ovulated, but in most cases mean peak progesterone concentrations were subnormal, and elevated progesterone levels were not maintained beyond 12 or 14 days after injection. This was similar to responses to GnRH recorded in non-lactating anoestrous ewes by Haresign *et al.* (1975) and Haresign & Lamming (1978). Differences in response to PMSG and GnRH were not significant, but the GnRH treatment tended to induce a higher incidence of "Short" patterns of progesterone production, and both a lower peak concentration and shorter duration of elevated progesterone in these "Short" patterns, than the PMSG treatment.

Twice-daily injections of PMSG for 16 days after GnRH injection had no effect on either the duration or peak concentration of elevated plasma progesterone. The dose or frequency of injection of PMSG may have been insufficient to have a luteotrophic effect. Alternatively, subnormal luteal function may have been predetermined at, or soon after, ovulation and not subject to subsequent influence by luteotrophin. A third possibility lies in the evidence of Denamur, Martinet & Short (1973) that prolactin and LH are both necessary for maintenance of the ovine corpus luteum, and that LH by itself is ineffective. Since prolactin release associated with suckling declines between the second and fifth weeks post partum (McNeilly 1971), there may have been insufficient prolactin release at the post-partum intervals of this experiment to induce a luteotrophic effect with injected PMSG.

The physical appearance of corpora lutea did not provide a reliable indication of progesterone production. All 9 ewes with abnormal corpora lutea (small size or pale colour) showed either reduced peak progesterone concentration or shortened duration of progesterone production, but this also applied to a further 18 ewes in which no visible abnormalities of corpora lutea were recorded.

In spring the resumption of oestrous activity is usually preceded by silent ovulation on one or more occasions prior to the first overt oestrus (Hunter & Lishman, 1967). In the present experiment the high incidence of silent ovulations (38 out of 49 ewes) was thus at least partly due to the treatment regimes applied. However, in the 2 groups where the ewes received 2 injections of PMSG at an interval of 16 days oestrus should have been observed in at least some of the ewes. No acceptable explanation could be found although the poor luteal activity in Group (ii) and the failure to induce luteolysis amongst some ewes Group (iii) may have contributed to the result obtained. In recent years significant advances have been made in the understanding of the mechanisms involved in oestrus and ovulation in the cycling ewe, in contrast, many questions regarding the induction of early reconception in ewes which lactate during the non-breeding season remain unanswered. The findings reported here perhaps serve only to highlight some of the problems involved.

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References

BUTCHER, R.L., COLLINS, W.E. & FUGO, N.W., 1974. Plasma concentration of LH, FSH, prolactin, progesterone and oestradiol-17β throughout the 4-day estrous cycle of the rat. *Endocrinology* 94, 1704.

COGNIE, Y., HERNANDEZ-BARRETO, M. & SAUMANDE, J., 1975. Ovarian endocrine function in the lactating ewe treated for induction of out-of-season breeding. *Ann. Biol. Anim. Bioch. Biophys.*, 15, 239.

DAWE, S.T. & FLETCHER, I.C., 1976. Effect of post-partum lambing interval on fertilization in lactating ewes treated with progestagen-impregnated sponges and gonadotrophin. *Proc. Aust. Soc. Anim. Prod.*, 11, 137.

DENAMUR, R., MARTINET, J. & SHORT, R.V., 1973. Pituitary control of the ovine corpus luteum. *J. Reprod. Fert.*, 32, 207.

GREYLING, J.P.C. & VAN DER WESTHUYSEN, J.M., 1979. The synchronisation of oestrus in sheep. 2. Dose effect of prostaglandin in the double injection regime. *S. Afr. J. Anim. Sci.* 9, 193.

HARESIGN, W., FOSTER, J.P., HAYNES, N.B., CRIGHTON, D.B. & LAMMING, G.E., 1975. Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH releasing hormone. *J. Reprod. Fert.*, 43, 269.

HARESIGN, W. & LAMMING, G.E., 1978. Comparison of LH release and luteal function in cyclic and LHRH – treated oestrous ewes pretreated with PMSG or oestrogen. *J. Reprod. Fert.*, 52, 349.

HUNTER, G.L., 1968. Increasing the frequency of pregnancy in sheep. II. Artificial control of rebreeding, and problems of conception and maintenance of pregnancy during the post-partum period. *Anim. Breed. Abstr.* 36, 533.

HUNTER, G.L. & LISHMAN, A.W., 1967. Effect of the ram early in the breeding season on the incidence of ovulation and oestrus in sheep. *Proc. S. Afr. Soc. Anim. Prod.*, 6, 199.

LAMMING, G.E., MOSELEY, SANDRA & McNEILLY, JUDITH R., 1972. Prolactin release in the ewe at parturition and first suckling. *J. Endocr.*, 55, xxviii.

LOUW, B.P., LISHMAN, A.W., BOTHA, W.A., ARANGIE, P.A.R., POULTNEY, B.G. & GUNTER, M.J., 1976. Release of LH in ewes deprived of prolactin during lactation. *S. Afr. J. Anim. Sci.*, 6, 87.

McNEILLY, JUDITH R., 1971. A solid phase radioimmunoassay for ovine prolactin. *J. Endocr.*, 49, 141.

PELLETIER, J., 1976. Influence of LH-RF on LH and FSH release in domestic mammals. *Ann. Biol. Anim. Bioch. Biophys.*, 16, 213.

RESTALL, B.J., KEARINS, R.D., HURDEGEN, J. & CARBERRY, P., 1978. Induction of reproductive activity in lactating ewes. *Aust. J. agric. Res.*, 29, 181.

THORBURN, C.D., BASSETT, J.M. & SMITH, I.D., 1969. Progesterone concentration in the peripheral plasma of sheep during the oestrous cycle. *J. Endocr.*, 45, 459.

VAN NIEKERK, B.D.H. & MULDER, A.M., 1965. Duration of pregnancy and of post-partum anoestrus in autumn-mated Dorper, Döhne and Merino ewes. *Proc. S. Afr. Soc. Anim. Prod.*, 4, 205.

PAPER 33

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Attempts to Improve the Function of GnRH-Induced Corpora Lutea in Early Post-Partum Ewes through Oestradiol and/or PMSG Priming or Exogenous Luteotrophin

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ABSTRACT

Grobbelaar, J., Lishman, A.W., Botha, W.A., Els, D.L., Louw, B.P. and Van Niekerk, A., 1989. Attempts to improve the function of GnRH-induced corpora lutea in early post-partum ewes through oestradiol and/or PMSG priming or exogenous luteotrophin. *Anim. Reprod. Sci.*, 19: 245-263.

In order to investigate whether subnormal luteal function following induction of ovulation with gonadotrophin releasing hormone (GnRH) could be counteracted by appropriate stimulation before or after ovulation, S.A. Mutton Merino ewes ($n=65$) in early lactation were induced to ovulate using GnRH at a time when subnormal luteal function was likely to result. In two experiments the ewes were infused with physiological saline (SRFS), pregnant mare serum gonadotrophin (PMSG) prior to (PRFS), PMSG after (SRFP), PMSG prior to and after (PRFP) or with PMSG plus oestradiol (E_2) prior to (EPRFS) a single injection of GnRH. A group of spontaneously cycling ewes (CYC) was used to characterize normal luteal function. At laparotomy on day 15 (experiment 1) or day 11 (experiment 2) a higher incidence (83.9%) of macroscopically active corpora lutea occurred in the ewes treated with PMSG prior to GnRH (PRFS+PRFP) than where saline was infused (65.5%) at this time (SRFP+SRFS). Of the 56 ewes which completed the experiments only five showed a short cycle of approximately 6 days, while in a further 14 ewes the cycle length approached 12 days. Of the progesterone profiles 37.2% were judged to be inadequate.

In both experiments, ewes which did not receive gonadotrophin, SRFS, exhibited a progesterone profile which differed ($P<0.01$) from the pooled response of those infused with PMSG. The provision of luteotrophin after GnRH was superior to PMSG priming ($P<0.01$) in augmenting progesterone secretion. In experiment 2 PMSG priming improved luteal function so that it equalled that of spontaneously cycling ewes while infusion of oestradiol in addition to PMSG priming further improved luteal function ($P<0.05$). The pre-ovulatory LH release was reduced ($P<0.01$) by PMSG infusion before and after GnRH (not significant in experiment 1). Tonic LH levels showed that PMSG infusion raised the endogenous release of assayable LH ($P<0.05$).

INTRODUCTION

In addition to the failure to induce ovulation in a varying proportion of ewes (Reeves et al., 1972; Haresign et al., 1973; Fletcher et al., 1980) the use of gonadotrophin releasing hormone (GnRH) to initiate cyclic breeding activity in anoestrous ewes (both lactating and non-lactating) and lactating cows is severely limited by the high incidence of short-lived corpora lutea which accompany the induced ovulation (Haresign, 1975; Haresign et al., 1975). Fletcher et al. (1980) and Lewis et al. (1981) noted that the short luteal cycles were essentially of two types viz., those with a duration of approximately 6 days and those which continued for double this length of time after GnRH administration. Murdoch et al. (1983) and Troxel and Kesler (1984) describe both the abovementioned as short luteal phases while inadequate luteal phases are said to be more than 14 days, but with depressed plasma progesterone concentrations.

Arguing on the basis of results obtained from a series of experiments which were initiated in 1976, Lishman et al. (1979) proposed that the premature regression of the induced corpus luteum might be due to either quantitative deficiencies in the pre-ovulatory release of LH or to an incompetence on the part of the follicle which ovulated. The favourable results obtained with PMSG treatment prior to LHRH (Haresign and Lamming, 1978) supported the latter contention.

Seasonal anoestrus in ewes (Scaramuzzi and Baird, 1977; Walton et al., 1980) and lactation anoestrus in cows (Peters and Lamming, 1984) and ewes (Wright et al., 1981) are associated with levels of tonic LH which are lower than the levels encountered during a normal cycle. In both sheep (Kaltenbach et al., 1968; Karsch et al., 1971) and cattle (Hansel and Convey, 1983) the corpus luteum is under the luteotrophic stimulus of LH. The possibility that the induced corpus luteum is of subnormal function because tonic levels of LH in the non-cycling female are inadequate to support normal luteal function has apparently received only limited attention (Fletcher et al., 1980; Gamboni et al., 1984). Both Piper and Loucks (1974) and Piper and Wells (1974) were able to increase the mass of corpora lutea and raise plasma progesterone (P_4) concentrations by infusing ewes with LH, but these were spontaneously cycling ewes.

During early lactation (Fell et al., 1972; Lamming et al., 1972; Louw et al., 1976; Wright et al., 1981) and during anoestrus (Walton et al., 1977; Jackson and Davis, 1979) circulating levels of prolactin are high in ewes so that deficiencies in this component of the luteotrophic complex (Denamur et al., 1973) are not likely to be implicated in the function of the corpus luteum at such times.

Lishman et al. (1979) suggested also that the secretory function of corpora lutea induced by GnRH might be influenced by the interaction of steroid and pituitary hormones. This proposal is in accordance with the findings of Richards (1980) regarding the association between FSH and oestradiol and the influence of oestradiol on development of LH receptors and of Hauger et al. (1977) who demonstrated the influence of ovarian steroids on episodic LH releases. It is possible then that the lack of suitable steroid priming, be it development of receptors for LH (Schams et al., 1979) or of favourable episodic releases of LH, prior to GnRH administration may be involved in the subnormal luteal function observed after GnRH (Ainsworth et al., 1982).

This study was initiated to investigate the effect on GnRH-induced luteal function of gonadotrophin (PMSG) stimulation of the ovulatory follicle prior to ovulation, with or without oestrogen priming, and of continuous exogenous luteotrophin (PMSG) support after ovulation.

MATERIALS AND METHODS

The first of two experiments (experiment 1) was conducted during late spring (November 1979) and a second trial (experiment 2) followed during early spring (August–September 1980). These times were selected as being those during which the use of GnRH was likely to result in subnormal luteal function (Fletcher et al., 1980).

Sixty-five, multiparous S.A. Mutton Merino ewes were housed with their lambs in individual pens and fed according to NRC (1975) standards. Three days prior to the i.m. injection of 4.2 g buserelin acetate (a synthetic analogue of GnRH, Receptal, Hoechst) on day 25 ± 2 post partum (day 0) all lactating ewes were fitted with indwelling cannulae in the jugular vein according to the technique described by Grobbelaar et al. (1981). During both experiments all the ewes were infused intravenously with normal saline at the rate of 500 ml/24 h, either as a control infusion or as a carrier for exogenous hormones.

The treatments applied are described in Table 1. In three groups of ewes (PRFS, PRFP and EPRFS) PMSG (Tuco) at the rate of 200 IU per 24 h was administered in normal saline. This dose was in the range expected to induce growth and maturation of follicles, but not ovulation (Lishman et al., 1974b). In experiment 2, in addition to PMSG priming, a fourth group of ewes was infused with oestradiol-17 β (E_2) at the rate of 15 g during the first 12 h and 35 g during the second 12 h period prior to GnRH (EPRSF group).

At GnRH injection on day 0, saline infusion (500 ml/24 h) replaced the PMSG priming in the PRFS and EPRFS groups. PMSG infusion (200 IU/24 h) commenced at this time in the SRFP group and continued uninterrupted in the PRFP group. The SRFS ewes (Table 1) served as controls and they received only saline infusion, both before and after GnRH. All infusions ceased on day 15 in experiment 1 and on day 17 in experiment 2.

Blood samples were collected from the infusion cannulae into heparinized syringes and the plasma stored at −15 °C until assayed. Samples to be assayed

TABLE 1

Treatments applied and infusion schedule followed during both experiment 1 (late spring) and experiment 2 (early spring)

Group	n^3	Infusion schedule and treatment[4]
SRFS	15	Control. Saline infusion day −3 to day 15 or 17[1]
PRFS	15	PMSG infusion day −3 to day 0, and saline day 0 to day 15 or 17
EPRFS[2]	7	PMSG infusion day −3 to day 0, oestradiol infusion day −1 to day 0, and saline day 0 to day 17
SRFP	14	Saline infusion day −3 to day 0 and PMSG infusion day 0 to day 15 or 17
PRFP	14	PMSG infusion day −3 to day 15 or 17
CYC[2]	5	Non lactating cycling ewes to describe luteal function in ewes ovulating naturally

[1] Day 15 experiment 1 and day 17 experiment 2.
[2] Only during experiment 2.
[3] Some of the ewes did not complete the experiment due mainly to problems with infusion.
[4] All ewes received 4.2 μg buserelin acetate (RF) on day 0, except those in group CYC.

for P_4 were obtained daily while the pre-ovulatory LH release was measured from samples taken every 30 min for 8 h after GnRH injection. The tonic release of LH was determined in experiment 2 from samples drawn every 15 min for 2 h on day −1 and day 4.

Hormone assays

Plasma samples were assayed for progesterone using the method and antibody described by Butcher (1977). The intra- and inter-assay coefficients of variation of a pool containing 3.07 ng/ml were 7.6% and 15.7%, respectively. Mean recovery efficiency (±s.e.m.) of [^{3}H]progesterone was 93.03±0.86% ($n=28$). The sensitivity of the assay was 25 pg per tube (62 pg/ml) and the assay blank was always below this. LH was measured according to the double antibody method of Niswender et al. (1969). Rabbit anti-ovine LH serum to NIH-LH-S16 was raised in this laboratory and was diluted 1:160,000. The cross reaction with TSH was 15.9%, 18.9% with PMSG (2 IU=1.96 ng NIH-LH-S16) and negligible with FSH. Highly purified ovine LH (Papkoff, G3-256DA) was iodinated with ^{125}I by the procedure of Greenwood et al. (1963) and NIH-LH-S16 was used as standard. The second antibody against rabbit gamma globulins was raised in sheep and used at a dilution of 1:4. The intra-assay c.v. of plasma pools low and high in LH were 7.9 and 14.7%, respectively and the inter-assay c.v. was 13.1%. The sensitivity of the LH assay was 120 pg/tube (150 pg/ml) and when known amounts of LH were added to serum the recovery was linear ($y=0.90\,x;\ r^2=99.2$).

Commencing on the same day as experiment 2, non-lactating (CYC group) were observed every hour for oestrus using vasectomized rams. When oestrus commenced, jugular cannulae were inserted and blood samples were obtained every 30 min for 18 h in order to characterize the pre-ovulatory LH surge. In these ewes the pattern of progesterone secretion was established from daily samples (venipuncture) taken for 17 days after oestrus.

In order to avoid operative stress influencing progesterone secretion, ovarian examination for ovulations were delayed until day 15 (experiment 1) and day 11 (experiment 2). The laparotomy technique of Lamond and Urquhart (1961) was used and at laparotomy the number of corpora lutea, their approximate age and apparent 'activity' were noted.

Statistical analysis of results

Treatment differences in the number of ewes with 'active' corpora lutea, in the total number of corpora lutea observed and in the number of ewes not ovulating were compared by the Chi-squared test. Treatment effects on the tonic release of LH were examined by analysis of variance while differences in the GnRH induced LH release and plasma progesterone levels were analyzed as for a completely randomized design with repeated measures over time (Gill, 1978). Ewes which secreted progesterone for less than a week and those which did not ovulate following GnRH administration were omitted from the analysis of progesterone levels. The repeated measures were accommodated by conducting a split-plot analysis of variance with "animals within treatments" being considered a random effect and "time" (days or minutes) the sub-plot. The mean sum of squares of "animals within treatments" was used as error term for testing differences between treatments, whilst the residual mean square was used for testing "sub-plot" (time and time × treatment) effects. The conservative tests of significance described by Winer (1971) were applied in order to compensate for heterogeneity of error variance. In order to examine the response curves of treatments over time, separate analyses were carried out for each treatment in which the sum of squares for "time" was subdivided into polynomial effects up to the 5th degree. Preplanned orthogonal comparisons of the fitted response curves were then conducted to test for regression heterogeneity over time, i.e. parallelism of the response curves. The method can be explained as follows:

Suppose we have n animals in a group, each measured at t times. After fitting a cubic response to time we have the following skeleton Analysis of Variance:

	d.f.
Between animals	$n-1$
Cubic polynomial time	3
Residual	$(t-1)n-3$
Total	$tn-1$

Now if we have two groups of animals, one of size n_1 and the other of size n_2 we may construct 3 ANOVA tables as above, one for group 1, one for group 2 and the third for both groups pooled together, to finally form the following table:

	d.f.
Residual from group 1	$(t-1)n_1-3$
Residual from group 2	$(t-1)n_2-3$
Sum of residuals	$(t-1)(n_1+n_2)-6$
Residual from pooled groups	$(t-1)(n_1+n_2)-3$
Difference	3

The sum of squares corresponding to this difference measures the interaction between groups × cubic polynomial time. We may interpret this as a measure of "parallelism" of the curves. Notice that orthogonal contrasts between treatments can be viewed as contrasts between groups of treatments with the grouping appropriately chosen.

In experiment 2 the two additional treatments were included in order to examine the effects of oestrogen priming in addition to PMSG (EPRFS) and whether a GnRH-induced ovulation in fact resulted in inadequate luteal function when compared to spontaneously cycling, non-lactating ewes. Consequently, experiment 2 consisted of three sets of treatments. Each set was analyzed separately in the repeated measures analysis and by comparisons of the fitted response curves.

RESULTS

Ovarian examination

A decision as to the ovarian status of each ewe at laparotomy was complicated by the occurrence of luteinized follicles, regressing corpora lutea and new ovulations. Nevertheless, 75.0% of the ewes could be classified as having macroscopically active corpora lutea with a non-significantly ($X^2=3.72$, $P<0.10$) higher percentage (83.9 vs 65.5) occurring in those groups primed with PMSG (Table 2). Although the saline-treated control ewes possessed a smaller total number of corpora lutea, there was no clear evidence that PMSG treatment, either before or after GnRH, increased the ovulation rate.

Progesterone secretion

Occurrence of sub-normal luteal function. The average plasma progesterone concentration of the cycling ewes (non-lactating) for one oestrous cycle was visually compared (graphically) to the patterns shown by each ewe from the other treatment groups. The results (Table 3) revealed that of the 56 hormone-treated lactating ewes only 5 exhibited a cycle length of approximately 6 days, while a further 14 animals showed a cycle averaging 12 days in duration. There

TABLE 2

Ovulation and occurrence of apparently normal corpora lutea (CL's; based on visual examination) in ewes induced to ovulate with GnRH following stimulation with PMSG and oestradiol (experiment 1 and 2 combined)

Infusion			n[2]	Total CL's observed	[1]Ewes with active CL's (%)	Ewes apparently not ovulating
Prior to GnRH	After GnRH	Group				
Saline	Saline	SRFS	15	20	53.3	4
PMSG	Saline	PRFS	12	25	83.3	0
Saline	PMSG	SRFP	14	25	78.6	1
PMSG	PMSG	PRFP	12	24	91.7	0
Oestradiol +PMSG	Saline	EPRFS	7	13	71.4	1

[1] No significant differences.

[2] After omission of ewes eliminated for various reasons, e.g. short cycles, deaths, inoperative cannulae etc.

TABLE 3

Evaluation of corpus luteum function, as measured by progesterone secretory patterns, of hormonally treated lactating ewes using the average pattern shown by 5 cycling (non-lactating ewes) as indicative of normal function and lifespan.

Hormone infusion		Exp.	n[4]	Corpus luteum lifespan (no. of ewes)				Adequacy[3] of corpus luteum function (no. of ewes)		
Prior to GnRH	After GnRH			Normal[1]	>Normal[2]	±6 Days	±12 Days	Normal	>Normal	<Normal
Saline	Saline	1	7	2	2	0	2	1	0	5
		2	6	4	0	1	1	2	0	3
PMSG	Saline	1	7	2	2	0	3	1	2	4
		2	5	2	0	0	3	2	0	3
Saline	PMSG	1	5	2	1	1	1	1	3	0
		2	7	1	4	1	1	2	4	1
PMSG	PMSG	1	6	4	1	0	2	0	5	1
		2	6	4	3	1	1	0	4	1
Oestradiol +PMSG	Saline	2	7	3	3	1	0	2	3	1
Total			56	24	16	5	14	11	21	19

[1] Progesterone level does not decline before 12 days after GnRH.

[2] Plasma progesterone has not declined below 1.5 ng/ml by 15 days after GnRH.

[3] Peak progesterone level greater than or equal to 2 ng/ml but does not rise above 3.5 ng/ml=normal. Above 3.5 ng/ml= >Normal.

[4] Figures within the table do not sum to n due to certain ewes exhibiting no progesterone profile or having re-ovulated.

was no apparent treatment effect on the distribution of these short cycles. However, if adequate luteal function is defined as a peak progesterone level which exceeds 2 ng/ml (Fletcher et al., 1980) then a total of 33.9% of the progesterone profiles shown by the ewes appeared to be inadequate. Nine of these ewes (47.4%) occurred in the groups receiving saline prior to GnRH and a further 36.8% in the group receiving PMSG only prior to GnRH.

Plasma progesterone. Analysis of treatment effects on changes in plasma progesterone concentrations over the experimental period showed that PMSG infusion after GnRH improved luteal function in both experiments. This effect was significant ($P<0.01$) when tested by repeated measures over time (Table 4) and by comparison of the fitted response curves (Fig. 1; $P<0.01$). In the latter analysis the 3rd degree polynomial most often resulted in an acceptable fit. In experiment 1, PMSG infusion both before and after GnRH appeared to produce a response which differed from that due to PMSG only after GnRH ($P<0.05$) (Table 4). A significant interaction ($P<0.05$) was also evident in experiment 2, but in this case the former treatment (PRFP) in fact resulted in a diminished response (Fig. 1b) as supported by comparison of the response curves ($P<0.05$). In both experiments the progesterone profile of ewes which received no additional gonadotrophin (SRFS) was significantly different ($P<0.01$) to the pooled response of ewes infused with PMSG (PRFS+SRFP+PRFP).

The suggestion that plasma progesterone levels were better maintained in the spontaneously cycling ewes (CYC group, Fig. 1b) than in ewes induced to ovulate with GnRH (SRFS) without any gonadotrophin treatment was supported by non-parallelism of the response curves ($P<0.01$), but not by split-plot analysis (Table 5). Similarly, oestrogen priming in addition to PMSG (EPRFS) resulted in a response which was not parallel ($P<0.05$) to that of ewes pretreated only with PMSG (PRFS; Fig. 1b).

Luteinizing hormone

Pre-ovulatory release. From the results obtained in experiment 1 it appeared that when PMSG infusion commenced at the time of GnRH injection this suppressed (not significantly) the LH release (Table 6; Fig. 2). The results in experiment 2 differed in that all treatments which incorporated PMSG infusion resulted in a reduction in total LH release (Table 6). In ewes which received no PMSG, the preovulatory LH surge was not significantly lower than in cycling ewes (total LH for CYC = 813 ± 129 units).

These trends were supported by analysis of log LH concentration (transformed to log to reduce variation between animals) over time (Table 7) and pre-planned contrasts of estimated regression curves (97% of variance for all treatment groups in experiment 2 accounted for by 3rd degree polynomial).

TABLE 4

Comparison of PMSG infusion ($n=22$) before or after ($n=33$) GnRH utilizing a mixed model analysis of variance for plasma progesterone concentration with day after GnRH as repeated measures

Source	df	Sum of squares	Mean square	F	Probl.	Tab F
Experiment 1						
Treatment	3	51.73	17.24	4.12*	0.02	–
PMSG before	1	6.63	6.63	1.59	NS	–
PMSG after	1	44.89	44.89	10.74**	<0.01	–
Interaction	1	0.21	0.21	0.05	NS	–
Ewes: treatments	18	75.23	4.18			
Days	14	180.87	12.92	27.72**	–	4.41
Days × Treatments	42	48.25	1.15	2.46	–	3.16[1]
PMSG before × Days	14	3.75	0.27	0.57	–	4.41
PMSG after × Days	14	41.43	2.96	6.35*	–	4.41
Before × after × Days	14	3.07	0.22	0.47	–	4.41
Residual	252	117.43	0.47			
Experiment 2						
Treatment	5[3]	73.03	14.60	2.19	0.08	–
PMSG before	1	1.82	1.83	0.27	NS	–
PMSG after	1	66.25	66.25	9.96**	<0.01	–
Interaction	1	0.79	0.79	0.12	NS	–
Ewes: treatments	27	179.67	6.65			
Days	16	368.84	23.05	42.72**	–	4.21[2]
Days × Treatments	80	104.70	1.31	2.42	–	2.57
PMSG before × Days	16	10.27	0.64	1.19	–	4.21
MPSG after × Days	16	64.87	4.05	7.51*	–	4.21
Before × after × Days	16	8.07	0.54	0.93	–	4.21
Residual	432	223.12	0.54			

[1] Tabulated $F_{1,18}$ for conservative F test (Winer, 1971).
[2] Tabulated $F_{1,27}$ for conservative F test.
[3] Two additional treatment groups not included in this comparison (see Table 5).
*$P<0.05$; **$P<0.01$; NS = non-significant.

Furthermore, in experiment 2, both oestradiol in addition to PMSG (EPRFS) and PMSG both before and after GnRH (PRFP) significantly reduced the LH release ($P<0.01$) compared to the pooled response of the other groups (SRFS, PRFS and SRFP). The latter three groups did not differ significantly.

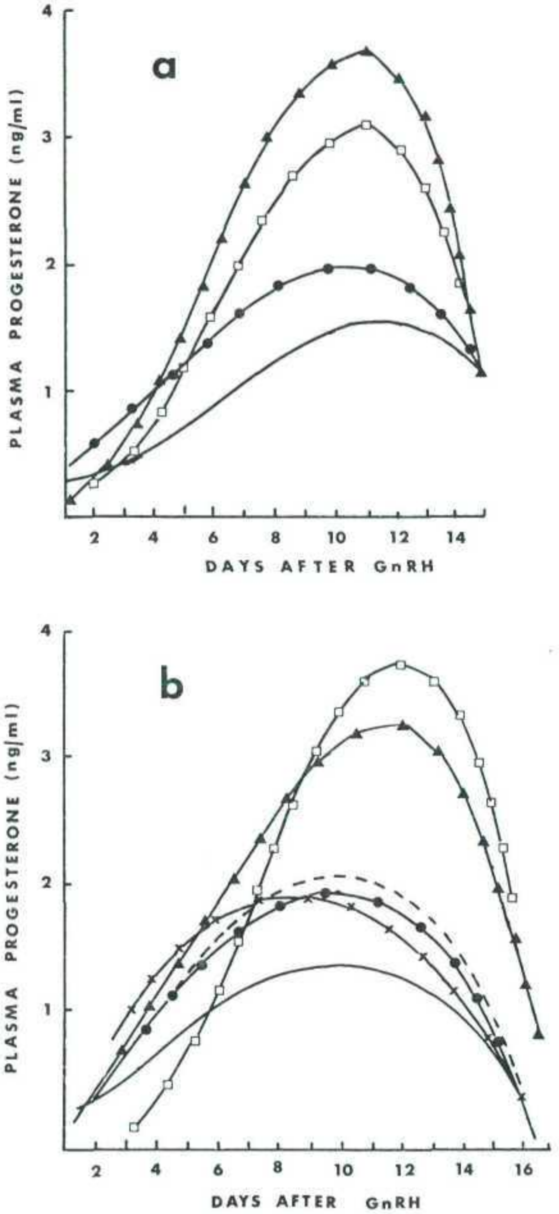

Fig. 1. a. Experiment 1. b. Experiment 2.

Estimated regression of progesterone concentration on days after GnRH injection for lactating ewes infused either with only saline (—; SRFS) with PMSG prior to (●; PRFS) after (□; SRFP), both prior to and after (▲; PRFP) or PMSG plus oestradiol prior to (×; EPRFP) GnRH when compared to spontaneously cycling, non-lactating ewes (----; CYC).

TABLE 5

Comparison of the luteal activity induced by GnRH (SRFS) with that in spontaneously cycling, non-lactating ewes (CYC) and the effect of oestrogen priming in addition to PMSG (EPRFS) prior to ovulation (experiment 2)

Source	df	Sum of squares	Mean squares	F	Prob.	Tab F^1
Treatments	5^2	73.03	14.61	2.19	0.08	
SRFS vs CYC	1	7.07	7.07	1.06	NS	
EPRFS vs PRFS	1	6.02	6.02	0.90	NS	
Ewes: treatments	27	179.67	6.65			
Days	16	368.84	23.05	42.72*		4.21
Days × Treatment	80	104.70	1.31	2.42		2.57
SRFS:CYC × Days	16	4.30	0.27	0.50		4.21
EPFRS:PRFS × Days	16	3.70	0.23	0.43		4.21
Residual	432	233.12	0.54			

[1] Tabulated $F_{1,27}$ for conservative F test.

[2] Experiment 2 consisted of six treatment groups (Table 1) with one set of orthogonal comparisons being included in Table 4.

$^*P<0.01$; NS = non-significant.

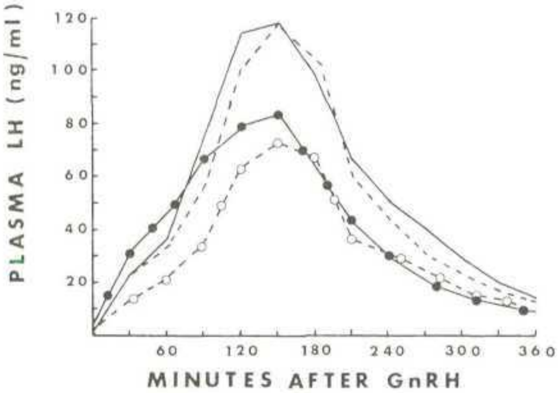

Fig. 2. Preovulatory LH release of ewes infused with PMSG (—, expt. 1; -----, expt. 2) or saline (●, expt. 1; ○, expt. 2) after GnRH injection.

The failure to show a significant inhibitory effect in experiment 1 (Table 7) is due largely to the variation between animals and the consequent high s.e.m. (Table 6).

Tonic LH levels. The mean plasma LH level as determined from nine samples

TABLE 6

The total pre-ovulatory release of LH (arbitrary units) in ewes stimulated with PMSG and oestradiol (E_2) before or after GnRH injection

Infusion		Group	n	Experiment 1		n	Experiment 2	
Prior to GnRH	After GnRH			Total LH released	As a % of SRFS		Total LH released[1]	As a % of SRFS
Saline	Saline	SRFS	7	652 ± 108	100.0	8	713 ± 95	100.0
PMSG	Saline	PRFS	7	722 ± 169	110.6	8	507 ± 85	71.2
Saline	PMSG	SRFP	6	596 ± 95	87.2	7	498 ± 79	68.6
PMSG	PMSG	PRFP	6	435 ± 83	66.6	8	337 ± 70	53.1
E_2 + PMSG	Saline	EPRFS	-	-	-	8	378 ± 98	47.3

[1] CYC > ERFS andPRFP ($P<0.01$); CYC > PRFS and SRFP ($P<0.05$); SRFS > EPRFS and SRFP ($P<0.05$).

TABLE 7

Analysis of variance of the pre-ovulatory LH concentration (transformed to log scale) with minutes after GnRH administration as repeated measure (time)

Source	df	Sum of squares	Mean square	F	Prob.	Tab F[1]
Experiment 1						
Treatment	3	23.33	7.78	1.48	NS	-
Ewes	22	115.43	5.25	28.86**	<0.001	-
Time	12	312.92	26.07	143.46**		7.94
Treatment × time	36	14.26	0.40	2.18		3.05
Residual	264	47.99	0.18			
Experiment 2						
Treatment	4	58.25	14.56	4.13*	<0.01	-
Ewes	30	105.78	3.53	38.83**	<0.001	-
Time	15	471.22	31.41	319.25**		7.56
Treatment × time	60	7.94	0.13	1.34		2.69
Residual	450	44.28	0.10			

[1] Tabulated F for conservative F tests.

*$P<0.01$; **$P<0.001$; NS = non-significant.

taken at 15 min intervals before (day −1) and after (day 4) GnRH suggested that in experiment 2 PMSG infusion resulted in an increased endogenous release of LH, measured while PMSG was being administered (Table 8). Al-

TABLE 8

The mean tonic LH levels as measured one day before (day − 1) and 4 days after (day 4) GnRH administration to groups of ewes infused with either saline, PMSG or PMSG plus oestradiol in experiment 2

Infusion		Group	Plasma LH level (ng/ml)	
Prior to GnRH	After GnRH		Day − 1 (mean)	Day 4 (mean)
Saline	Saline	SRFS	1.65 ± 0.20	1.85 ± 0.20
PMSG	Saline	PRFS	1.91 ± 0.16	1.66 ± 0.29
Saline	PMSG	SRFP	1.74 ± 0.11	1.98 ± 0.12
PMSG	PMSG	PRFP	2.13 ± 0.23	2.01 ± 0.131
Oestradiol + PMSG	Saline	EPRFS	2.45 ± 0.37	1.76 ± 0.22
SRFS + SRFP			1.69 ± 0.13^{ab}	
PRF + PRFP + EPRFS			2.14 ± 0.12^{b}	
SRFS + PRFS + EPRHS				1.75 ± 0.13^{ab}
SRFP + PRFP				1.99 ± 0.09^{ab}

^{a,b}Means within the same column with different superscripts differ ($P < 0.05$).

though no individual treatment means differed significantly the mean of those groups receiving PMSG prior to GnRH (PRFS + PRFP + EPRFS) was significantly higher ($P < 0.05$) than ewes infused only with saline at this time (SRFS + SRFP).

DISCUSSION

Luteal function

From the progesterone profiles (Fig. 1) it is clear that the ewes which received only GnRH to induce ovulation and no PMSG priming of follicles or luteotrophin after GnRH (SRFS group) exhibited a level of luteal activity which was inferior to that observed in spontaneously cycling ewes. The macroscopic appearance of the corpora lutea provided further support for this conclusion (only 53.3% ewes with "active" CL's; Table 2).

The present results suggest that stimulation of the follicle, prior to being ovulated by a GnRH-induced release of LH, improves luteal function in the early part of the cycle while gonadotrophin support after ovulation has its greatest impact on P_4 levels late in the cycle (Fig. 1). The latter finding does not contradict Gamboni et al. (1984) who were able to increase circulating P_4 levels by intravenous administration of 500 IU hCG on day 5 after ovulation in anoestrous ewes. However, Fletcher et al. (1980) could not achieve this in lactating ewes by twice daily injections of 60 IU PMSG. The level of stimulation appears to be important since Grobbelaar (1984) significantly improved luteal function by twice daily injection of 100 IU PMSG. When the pattern of P_4 secretion in spontaneously cycling ewes is used as basis then it would appear that stimulation of the follicle prior to ovulation achieves the more normal situation while PMSG which continues after ovulation results in a supraphysiological response (Fig. 1). The effect obtained by PMSG priming agrees with the results obtained by McGovern and Laing (1976), Haresign and Lamming (1978) and McNatty et al. (1982), although Haresign and Lamming (1978) noted that PMSG pre-treatment did not bring the P_4 levels up to the natural mid-cycle levels.

Mechanism of improved luteal function

Plasma LH levels in post-partum ewes are markedly lower than those associated with pre-ovulatory follicular development in cyclic ewes (Wright et al., 1983). In the present study, infusion of gonadotrophin at a low level (200 IU/24 h) appeared to result in a higher endogenous level of tonic LH (Table 8). Since PMSG cross-reacted in the LH assay, part or all of this increase might be due to PMSG and not LH. Based on a ewe mass of 54 kg and a plasma volume of 48.04 ml/kg and assuming that because of its prolonged half-life (McIntosh et al., 1975) PMSG accumulated over 2 days to reach a concentration of 0.154 IU/ml then 67.2% of the average increase of 0.45 ng LH (Table 8) would be accounted for in terms of PMSG. The response observed may also be the result of both exogenous and endogenous stimulation, but the endogenous LH levels were still only about 50% of those established for cycling ewes (Lishman et al., 1974a).

Similarly, PMSG infusion at the same low level after GnRH administration also increased (0.24 ng/ml; Table 8) tonic LH levels and because of cross-reaction in the LH assay PMSG could have accounted for all of this increase (assuming accumulation over 3 days). Spilman et al. (1973) also observed a sharp increase in plasma LH levels the day after PMSG injection in prepuberal calves where PMSG did not cross-react in the LH assay. The primary cause of the improved luteal activity is not clear. The levels of P_4 observed, when compared to those in cycling ewes, suggest that exogenous luteotrophin was the major stimulus (Fig. 1).

Grobbelaar (1984) demonstrated that PMSG infusion after GnRH results in a larger CL than GnRH alone. This CL had a lower secretory activity (in vitro) per unit mass than where no PMSG was given, but was more sensitive to LH stimulation in terms of progesterone release. Therefore, the higher P_4 level in ewes infused with PMSG after GnRH is the result of a larger CL being exposed to higher levels of gonadotrophin. Our results and those of McNeilly et al. (1981) suggest that the inferior CL induced by GnRH is not only smaller

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Pituitary LH response

Exogenous hormone treatment depressed the pituitary release of LH in response to GnRH by as much as 62.7% in this study (Table 6). In contrast, Haresign and Lamming (1978) reported that PMSG priming significantly increased the response to GnRH, although the doses, routes of administration and timing of treatments differed from those reported here. In beef cattle, Ford and Stormshak (1975) noted a reduced pituitary response to GnRH following PMSG injections, but Lishman et al. (1979) did not demonstrate an effect of small doses of FSH given for 3 days prior to GnRH.

According to Restall et al. (1977) the quantity of LH released by the pituitary depends on a change in pituitary sensitivity to GnRH, a change in pituitary LH content and/or altered synthesis of LH. Some support can be found for a densitization of the pituitary by PMSG in those ewes where PMSG infusion commenced at the time of GnRH administration (SRFP groups). The effect was manifested so rapidly that it is unlikely that the rate of LH synthesis or pituitary content could have been influenced in so short a time.

The apparent increase in endogenous tonic LH levels (Table 8) due to PMSG priming cannot be ignored in attempting to account for the suppressed pre-ovulatory LH release. Unfortunately, cross-reaction of PMSG in the RIA for LH confounds the issue. In the presence of depleted pituitary stores during early lactation (Jenkin et al., 1977; Crowder et al., 1982; Clarke et al., 1984) the release of LH in response to infusion, to achieve higher basal levels of LH, may have further depleted the pituitary LH content. Consequently, the release in response to GnRH is reduced. A similar reduction in pre-ovulatory LH release in response to GnRH has been noted in Brangus cows and was attributed to a greater secretion of LH before GnRH challenge (Mason and Randel, 1983). However, the abovementioned suggestion does not account for the effect of PMSG at the time of GnRH injection.

The results obtained here demonstrate that exogenous gonadotrophin can enhance luteal function with supplementation after GnRH injection achieving the highest plasma P_4 levels. An important question that remains is whether the improved CL can support pregnancy as suggested by Kittok et al. (1983) and Gamboni et al. (1984) although Grobbelaar (1984) was not able to confirm this.

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REFERENCES

Ainsworth, L., Lachance, R. and Labrie, F., 1982. Effect of GnRH-induced endogenous luteinizing hormone release and exogenous progestogen treatment on ovarian activity in the post partum ewe. J. Anim. Sci., 54: 998–1004.

Alila, H.W. and Hansel, W., 1984. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol. Reprod., 31: 1015–1026.

Butcher, R.L., 1977. Changes in gonadotropins and steroids associated with unilateral ovariectomy of the rat. Endocrinology, 101: 830–840.

Clarke, I.J., Wright, P.J., Chamley, W.A. and Burman, K., 1984. Differences in the reproductive endocrine status of ewes in the early post-partum period and during seasonal anoestrus. J. Reprod. Fertil., 70: 591–597.

Crowder, M.E., Gilles, P.A., Tamanini, C., Moss, G.E. and Nett, T.M., 1982. Pituitary content of gonadotrophins and GnRH-receptors in pregnant, postpartum and steroid-treated OVX ewes. J. Anim. Sci., 54: 1235–1242.

Denamur, R., Martinet, J. and Short, R.V., 1973. Pituitary control of the ovine corpus luteum. J. Reprod. Fertil., 32: 207–220.

Donaldson, L. and Hansel, W., 1965. Histological study of bovine corpora lutea. J. Dairy Sci., 48: 905–909.

Farin, C.E., Schwall, R.H., Gamboni, F., Sawyer, H.R. and Niswender, G.D., 1985. Effect of LH and hCG on size distributions of luteal cells in the cycling ewe. Biol. Reprod., 32 (Suppl. 1): 44.

Fell, L.R., Beck, C., Brown, J.M., Catt, K.J., Cummings, I.A. and Goding, J.R., 1972. Solid-phase radioimmunoassay of ovine prolactin in antibody-coated tubes. Prolactin secretion during estradiol treatment at parturition, and during milking. Endocrinology, 91: 1329–1336.

Fitz, T.A., Mayan, M.H., Sawyer, H.R. and Niswender, G.D., 1982. Characterization of two steroidogenic cell types in the ovine corpus luteum. Biol. Reprod., 27: 703–711.

Fletcher, I.C., Lishman, A.W., Thring, B. and Holmes, J.A., 1980. Plasma progesterone levels in

following parturition than in those bred after several estrous cycles (Perkins and Kidder, 1963; Casida *et al.*, 1968; Short *et al.*, 1972; Whitmore *et al.*, 1974). There is increasing evidence that luteal function may be deficient during early estrous cycles improving only gradually during subsequent cycles (Short *et al.*, 1972; Edgerton and Hafs, 1973; Dickey *et al.*, 1975).

Inadequate steroid secretion by the ovary during early lactation could contribute to the high incidence of ovulation without overt estrus during this time (Casida *et al.*, 1968; Whitmore *et al.*, 1974; Castenson *et al.*, 1976). After injection of gonadotropin releasing hormone (GnRH) between 20 and 40 days postpartum, corpora lutea (CL) were detected in a limited number of anestrous lactating beef cows (Britt *et al.*, 1975; Webb *et al.*, 1975). Many induced CL were short-lived (<14 days) and peripheral concentrations of progesterone were low (<1 ng/ml) relative to those observed during a normal estrous cycle. Priming with pregnant mare serum gonadotropin (PMSG) has improved the ovulatory response to GnRH in prepuberal rats (Steger *et al.*, 1975) and gilts (Baker *et al.*, 1973).

Follicular development may be deficient in postpartum anestrous beef cows. Antral formation in ovarian follicles requires follicle-stimulating hormone (FSH). Increased binding of FSH to granulosal cells after treatment of rats with estrogen (Goldenberg *et al.*, 1972) follows the increase in mitosis of granulosal cells due to estrogen (Louvet and Vaitukaitis, 1976). While FSH alone increased receptors for LH in granulosal cells (Richards *et al.*, 1976), the increase was greater in immature hypophysectomized rats treated with estradiol plus FSH. Both LH and FSH are necessary for synthesis of estradiol-17β (Fortune and Armstrong, 1978). Increased numbers of receptors for gonadotropins in preovulatory follicles brought about by interactions of steroid and protein hormones might improve ovulatory response to GnRH and/or the performance of CL induced by GnRH.

Underfeeding, either pre- or postpartum, can delay onset of estrous cycles following calving (Dunn *et al.*, 1969; Whitman *et al.*, 1975). Research into the mechanisms whereby undernutrition reduces fertility has produced conflicting results regarding the release of LH and luteal activity (Dunn *et al.*, 1974; Agpar *et al.*, 1975; Beal *et al.*, 1975; Spitzer *et al.*, 1975). It therefore appeared pertinent to test the suggestion of Gombe and Hansel (1973) that the ability of the ovary to respond to gonadotropins may be reduced by inadequate feeding.

Objectives of these studies were to examine: 1) effects of energy restriction during late gestation and early lactation on pituitary and ovarian responses to treatment with FSH and GnRH, and 2) the effects of exogenous FSH or estradiol on follicular growth and on the formation and life span of CL following injections of GnRH.

MATERIALS AND METHODS

Experiment I. Thirty days prior to expected calving, 43 2-year-old Hereford heifers (bred at a synchronized estrus) were weighed and divided randomly into groups of 20 and 23 which were fed 115% (high) or 60% (low) of the recommended requirements for energy, respectively (NRC, 1970). All animals were fed alfalfa hay and corn silage; those on the high plane received corn grain in addition. After parturition the low level yielded 50% of NRC, while the high level was increased to maintain 115%. After approximately 48 days postpartum, all cows were fed at the high level. The cows were weighed again shortly before and after calving and at 30 and 48 days postpartum.

At 29 days postpartum ovarian activity was assessed by palpation *per rectum*. Commencing on day-30 postpartum, the cows were divided randomly within feeding groups and injected (s.c.) twice daily (6:00 am and 5:00 pm) for 3 days with either 0, .125 or .25 mg FSH[5] in saline. These doses were in a range expected to induce growth of follicles and estrogen syntheses, but not ovulation. Thus, the treatment was expected to 1) provide a measure of the sensitivity of the ovary in terms of steroid secretion and 2) induce follicular growth prior to a GnRH-induced release of LH. Blood samples (20 ml) were drawn (venipuncture) daily from 29 through 33 days postpartum to quantify estradiol-17β. The morning after the last injection of FSH (day 0; 34 days postpartum) each cow received (i.m.) 300 μg GnRH[6] and during the ensuing 7 hr blood was collected every 30 min by jugular cannulae for measurement of LH. Blood was drawn every 3 or 4 days for 18 days after GnRH for quantification of progesterone. Rectal palpations were performed on day 7 and again on day 14 after GnRH to detect CL.

Experiment II. In view of the possibility that doses of FSH in experiment I had been insufficient to stimulate the ovaries, a second experiment was conducted utilizing only those cows which apparently had not ovulated following treatment

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[5] FSH-P, Armour-Baldwin Laboratories, Omaha, NE.

[6] GnRH, Abbott Laboratories.

lactating ewes after hormone induced ovulation during the non breeding season. S. Afr. J. Anim. Sci., 10: 1'51-158.

Ford, S.P. and Stormshak, F., 1975. Effect of PMS and GnRH on serum LH and P4 in heifers. J. Anim. Sci., 41: 353.

Gamboni, F., Fitz, T.A., Hoyer, P.B., Wise, M.E., Mayan, M.H. and Niswender, G.D., 1984. Effect of human chorionic gonadotrophin on induced ovine corpora lutea during the anestrous season. Domest. Anim. Endocrinol., 1: 79-88.

Gill, J.L., 1978. Design and Analysis of Experiments in the Animal and Medical Sciences, Vol. 2. Iowa State University Press, Ames, IA, pp. 203-217.

Greenwood, F.C., Hunter, W.N.M. and Glover, J.S., 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem. J., 89: 114-123.

Grobbelaar, J., 1984. Luteal function during early post partum in the ewe. Ph.D. Thesis, University of Natal, Pietermaritzburg, South Africa.

Grobbelaar, J., Lishman, A.W., Botha, W.A., Millar, D.J. and Lesch, S.F., 1981. A simple technique for continuous intravenous infusion of adult sheep. S. Afr. J. Anim. Sci., 11: 55-56.

Hansel, W. and Convey, E.M., 1983. Physiology of the estrous cycle. J. Anim. Sci., 57 (Suppl. 2): 404-424.

Haresign, W., 1975. Ovarian response to synthetic LH-RH in anoestrous ewes. J. Reprod. Fertil., 44: 127-129.

Haresign, W. and Lamming, G.E., 1978. Comparison of LH release and luteal function in cyclic and LHRH treated anoestrous ewes pre-treated with PMSG or oestrogen. J. Reprod. Fertil., 52: 349-353.

Haresign, W., Haynes, N.B. and Lamming, G.E., 1973. The effects of synthetic gonadotropic-releasing factor on ovarian function in anoestrous ewes. J. Reprod. Fertil. 35: 600 (abstr.).

Haresign, W., Foster, J.P., Haynes, N.B., Crighton, D.B. and Lamming, G.E., 1975. Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. J. Reprod. Fertil., 43: 269-278.

Hauger, R.L., Karsch, F.J. and Foster, D.L., 1977. A new concept for control of the estrous cycle of the ewe based on the temporal relationship between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. Endocrinology, 101: 807-817.

Jackson, G.L. and Davis, S.L., 1979. Comparison of luteinizing hormone and prolactin levels in cycling and anoestrous ewes. Neuroendocrinology, 28: 256-263.

Jenkin, G., Heap, R.B. and Symons, D.B.A., 1977. Pituitary responsiveness to synthetic LH-RH and pituitary LH content at various reproductive stages in sheep. J. Reprod. Fertil., 49: 207-214.

Kaltenbach, C.L., Craber, J.W., Niswender, G.D. and Nalbandov, A.V., 1968. Effect of hypophysectomy on formation and maintenance of corpora lutea in the ewe. Endocrinology, 82: 753-759.

Karsch, F.J., Cook, B., Ellincott, A.R., Foster, D.L., Jackson, G.L. and Nalbandov, A.V., 1971. Failure of infused prolactin to prolong the life-span of the corpus luteum in the ewe. Endocrinology, 89: 272-275.

Kittok, R.J., Stellflug, J.N. and Lowry, S.R., 1983. Enhanced progesterone and pregnancy rate after gonadotropin administration in lactating ewes. J. Anim. Sci., 56: 652-655.

Lamming, G.E., Mosley, S. and McNeilly, J.R., 1972. Prolactin release in the ewe at parturition and first suckling. J. Endocrinol., 55: XXVII.

Lamond, D.R. and Urquhart, E.J., 1961. Sheep laparotomy craddle. Aust. Vet. J., 37: 430-431.

Lewis, G.S., Lishman, A.W., Butcher, R.L., Dailey, R.A. and Inskeep, E.K., 1981. Factors affecting function of induced corpora lutea in postpartum anestrous ewes. J. Anim. Sci., 52: 1122-1129.

Lishman, A.W., Stielau, W.J., Dreosti, I.E., Stewart, A.M. and Botha, W.A., 1974a. Plasma luteinizing hormone levels in ewes failing to exhibit oestrus during lactation and in ewes isolated from rams. S. Afr. J. Anim. Sci., 4: 45–49.

Lishman, A.W., Stielau, W.J., Swart, C.E. and Botha, W.A., 1974b. Nutrition of the ewe and the ovarian sensitivity to gonadotrophins. Agroanimalia, 6: 7–12.

Lishman, A.W., Allison, S.M.J., Fogwell, R.L. and Inskeep, E.K., 1979. Follicular development and function of induced corpora lutea in underfed postpartum anestrous beef cows. J. Anim. Sci., 4: 867–875.

Louw, B.P., Lishman, A.W., Botha, W.A., Arangie, P.A.R., Poultney, B.G. and Gunter, M.J., 1976. Release of luteinizing hormone (LH) in ewes deprived of prolactin during lactation. S. Afr. J. Anim. Sci., 6: 87–91.

Mason, G.L. and Randel, R.D., 1983. Effect of monensin and suckling on the GnRH induced luteinizing hormone surge and the effect of monensin on the postpartum interval in Brangus cows. Theriogenology, 19: 331–342.

McGovern, P.T. and Laing, J.A., 1976. Ovulation rate in ewes treated with pregnant mares' serum gonadotrophin and luteinizing hormone releasing factor. J. Agric. Sci., 86: 127–129.

McIntosh, J.E.A., Moor, R.M. and Allen, W.R., 1975. Pregnant mare serum gonadotrophin: rate of clearance from the circulation of sheep. J. Reprod. Fertil., 44: 95–100.

McNatty, K.P., Ball, K., Gibb, M., Hudson, N. and Thurley, D.C., 1982. Induction of cyclic ovarian activity in seasonally anoestrous ewes with exogenous GnRH. J. Reprod. Fertil., 64: 93–96.

McNeilly, A.S., Hunter, M., Land, R.B. and Frazer, H.M., 1981. Inadequate corpus luteum function after the induction of ovulation in anoestrus ewes by LH-RH or an LH-RH agonist. J. Reprod. Fertil., 63: 137–144.

Murdoch, W.J., De Silva, M. and Dunn, T.G., 1983. Luteal phase insufficiency in the ewe as a consequence of premature induction of ovulation by intrafollicular injection of gonadotropins. J. Anim. Sci., 57: 1507–1511.

National Research Council, 1975. Nutrient requirements of sheep. No. 5. National Academy of Sciences, Washington, DC, p. 2.

Niswender, G.D., Reichert, L.E., Midgley, A.R. and Nalbandov, A.V., 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology, 84: 1166–1173.

Peters, A.R. and Lamming, G.E., 1984. Reproductive activity of the cow in the post-partum period. II. Endocrine patterns and induction of ovulation. Br. Vet. J., 140: 269–280.

Piper, E.L. and Loucks, G.C., 1974. The result of a sustained release of LH in the cyclic ewe. J. Anim. Sci., 38: 226 (abstr.).

Piper, E.L. and Wells, G.L., 1974. Effect of infusion of LH and prolactin in the cyclic ewe. J. Anim. Sci., 38: 226 (abstr.).

Reeves, J.J., Arimura, A., Schally, A.V., Kragt, C.L., Beck, T.W. and Casey, J.M., 1972. Effects of synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH) on serum LH, serum FSH and ovulation in anestrous ewes. J. Anim. Sci., 35: 84–89.

Restall, B.J., Kearins, R.D. and Starr, B.G., 1977. Studies of the pituitary function in lactating ewes. J. Reprod. Fertil., 49: 291–296.

Richards, J.S., 1980. Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol. Rev., 60: 51–89.

Scaramuzzi, R.J. and Baird, D.T., 1977. Pulsatile release of luteinizing hormone and the secretion of ovarian steroids in sheep during anestrus. Endocrinology, 101: 1801–1806.

Schams, D., Schallenberger, E., Menzer, C.L., Stangl, J., Zottmeier, K., Hoffman, B. and Karg, H., 1979. Profiles of LH, FSH and progesterone in post-partum dairy cows and their relationship to the commencement of cyclic functions. Theriogenology, 10: 453–468.

Spilman, C.H., Seidel, G.E., Larson, L.L., Vukman, G.R. and Foote, R.H., 1973. Progesterone, 20β-hydroxypregn-4-en-3-one, and luteinizing hormone levels in superovulated prepuberal and postpuberal cattle. Biol. Reprod., 9: 116–24.

Troxel, T.R. and Kesler, D.J., 1984. The effect of progestin and GnRH treatments on ovarian function and reproductive hormone secretion of anestrous postpartum suckled beef cows. Theriogenology, 21: 699–711.

Walton, J.S., McNeilly, J.R., McNeilly, A.S. and Cunningham, F.J., 1977. Changes in concentrations of follicle-stimulating hormone, luteinizing hormone, prolactin and progesterone in the plasma of ewes during the transition from anoestrus to breeding activity. J. Endocrinol., 75: 127–137.

Walton, J.S., Evins, J.D., Fitzgerald, B.P. and Cunningham, F.J., 1980. Abrupt decrease in day length and short-term changes in the plasma concentrations of FSH, LH and prolactin in anoestrous ewes. J. Reprod. Fertil., 59: 163–171.

Winer, B.J., 1971. Statistical Principles in Experimental Design (2nd Edn.). McGraw-Hill Book Company, New York, NY, 256 pp.

Wright, P.J., Jenkin, G. and Heap, R.B., 1981. Prolactin and LH release in response to LH-RH and TRH in ewes during di-oestrus, pregnancy and post partum. J. Reprod. Fertil., 62: 447–453.

Wright, P.J., Geytenbeek, P.E., Clarke, I.J. and Findlay, J.K., 1983. LH release and luteal function in post-partum acyclic ewes after pulsatile administration of LH-RH. J. Reprod. Fertil., 67: 257–262.

Augmentation of luteal function in the lactating ewe after induction of ovulation with a gonadropin releasing hormone

PAPER 34

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Numerous studies have shown that in the acyclic ewe, the first corpus luteum (CL) after administering gonadotropin releasing hormone (GnRH) will lead to inadequate progesterone (P_4) concentrations or will be short-lived. An attempt was made to promote the function of the GnRH-induced CL in lactating ewes (n = 70) either by increasing endogenous LH release through administration of naloxone (4 injections at 1 mg/kg body mass every 2 hrs over 3 days) or via an exogenous supply of gonadotropin (PMSG, 100 I.U., twice daily for 3 days). Treatments were applied in an effort to promote maturation of the preovulatory follicle or to stimulate the CL. Naloxone, either before or after, and PMSG prior to GnRH (Day 0) did not increase plasma P_4 concentrations. PMSG on Days 3–5 after GnRH was more effective in promoting luteal function (compared to untreated controls) than similar treatment on Days 6–8 or 9–11. This was evidenced by a greater ($P < 0,05$) total area under the P_4 curve (34,2 ± 2,9 vs 25,7 ± 2,9 units), an increased ($P < 0,05$) number of days for which P_4 concentrations exceeded 2,0 ng/ml (6,6 ± 1,5 vs 2,5 ± 1,1 days) and a higher ($P < 0,05$) P_4 level on Days 8–12. Only the P_4 concentrations on Days 10 and 11 were improved ($P < 0,05$) by PMSG on Days 6–8, while PMSG on Days 9–11 was without significant effect. Luteotropic support of the CL for a short period prior to mid-cycle was thus more beneficial to luteal function than stimulation at any other stage.

Verskeie studies met anestrus ooie het getoon dat die eerste corpus luteum (CL) na toediening van gonadotropiese vrystellingshormoon (GnVH) onvoldoende progesteroon (P_4) afskei, of 'n kort lewensduur het. 'n Poging is aangewend om die funksionering van 'n GnVH-verwekte CL by 70 lakterende ooie te verbeter. Endogene vrystelling van LH is bevorder deur middel van naloxonetoediening (4 inspuitings elke 2 uur vir 3 dae). Hierdie behandeling is met 'n eksogene bron van gonadotrofien (DMSG twee keer per dag vir 3 dae) vergelyk. Die behandelings is toegepas om, of volwassendheid van die follikel te bevorder of die CL na ovulasie te stimuleer. Naloxone, voor of na, en DMSG voor GnVH-toediening het nie die plasma-progesteroon waardes verhoog nie. Waar DMSG-behandeling op die derde dag na GnVH-inspuiting begin is, was die af skeiding van P_4 höer as waar toediening op of die sesde of negende dag begin is. Hierdie gevolgtrekking word gestaaf deur die groter ($P < 0,05$) area onder die P_4-kurwe (34,2 ± 2,9 vs 25,7 ± 2,9 eenhede), 'n verhoogde ($P < 0,05$) aantal dae waarop plasma P_4–konsentrasies 2,0 ng/ml oorskry het (6,6 ± 1,5 vs 2,5 ± 1,1 dae) en 'n höer ($P < 0,05$) P_4-waarde 8–12 dae na GnVH-toediening, in vergelyking met die kontrole. Slegs die DMSG-toedienings op Dae 6 tot 9 het die P_4 konsentrasies op Dae 10 en 11 verhoog ($P < 0,05$) terwyl DMSG-toediening op Dae 9 tot 11 geen effek gehad het nie. Die mees voordelige stadium om die funksie van die CL te bevorder, is dus kort na ovulasie.

Keywords: Lactating ewes, luteal function, opioids, progesterone, PMSG.

Introduction

Ovulation can be induced in the majority of postpartum or seasonally anoestrous ewes by treatment with gonadotropin releasing hormone (GnRH). However, the ensuing luteal function is subnormal, being characterized by reduced blood levels of progesterone (Hunter *et al.*, 1987) or a corpus luteum (CL) which regresses prematurely (Legan *et al.*, 1985).

Luteinizing hormone (LH) is required for preovulatory maturation of follicles (Haresign & Lamming, 1978) and this hormone may be needed to maintain CL function (Hansel & Convey, 1983). Consequently, inadequacies of the GnRH–induced CL could be attributed to deficiencies in the release of LH which is suppressed during the postpartum anoestrous period (Wright *et al.*, 1983). The low frequency of episodic LH release during the early postpartum period has been attributed to a prolongation in the time interval between GnRH releases from the hypothalamus (Wright *et al.*, 1981).

Where fertilization is successful, but the CL does not function normally, pregnancy is unlikely to continue. It has even been suggested that the embryo will not develop unless progesterone concentrations exceed a threshold level (Staples & Hansel, 1961). This is supported by the finding that administration of gonadotropins within a few days after mating, in order to stimulate the CL (Gamboni *et al.*, 1984), greatly improved pregnancy rates (Kittock *et al.*, 1983).

Endogenous opioid peptides (EOP) inhibit GnRH release from the hypothalamus of cattle (MacDonald *et al.*, 1986) and sheep (Matthews & Murdoch, 1985; Stansfield *et al.*, 1987). However, direct effects of EOP on the pituitary of these species have also been demonstrated (Matteri & Moberg, 1985; Chao *et al.*, 1986). LH secretion in the cycling (Brooks *et al.*, 1986) and postpartum ewe (Gregg *et al.*, 1986) thus appears to be modulated by EOP.

In view of the foregoing this study focussed on two main aspects: 1) When the lactating ewe is induced to ovulate by the administration of GnRH, will an increase in endogenous

LH levels (tonic LH) via administration of an opioid antagonist such as naloxone (NAL) promote maturation of follicles destined to ovulate. This may then result in improved luteal function as has been achieved by administering PMSG (Haresign & Lamming, 1978; Grobbelaar *et al.*, 1989). When naloxone is applied after ovulation, the increase in frequency of LH release can be expected to improve the secretory function of the newly formed CL. 2) When exogenous luteotropic support is provided in the form of PMSG, at what stage postovulation should administration occur in order to maximize the beneficial effects on luteal function?

Materials and methods

Experimental design

Seventy, lactating, South African Merino ewes, ranging in age from two to five years were used in the spring lambing season. Ewes lambed over a period of three weeks and in order to reduce seasonal effects, were assigned to one of three blocks so that all ewes that lambed within a period of a week were blocked together. Numbers within a block were thus dictated by the date of parturition. Within a block, ewes were assigned randomly to seven different treatment groups, irrespective of the age of the ewe or the number of lambs born (single or twin lambs). The layout of the treatment schedule is listed in Figure 1. At 35 ± 4 days postpartum (Day 0) all ewes received 4,2 g buserelin acetate (GnRH, Receptal, Hoechst) in a single im. injection. PMSG (UpJohn) treatments were administered twice daily for 3 consecutive days. Ewes received im. injections of 100 IU PMSG at 12–hourly intervals at 06:00 h and 18:00 h. Naloxone (Sigma International) was dissolved in 0,9% saline to a concentration of 0,05 g naloxone/2ml saline. NAL was injected by jugular venepuncture every 2 h at 0800 h, 1000 h, 1200 h and 1400 h for 3 consecutive days, either before (NAL–) or after (NAL+) GnRH. Ewes received 0,2 g Naloxone/day (approximately 1 mg naloxone/kg mass of the ewe per injection) during the experiment. Progesterone levels were measured in jugular blood samples collected every two days and drawn prior to the first administration of naloxone or PMSG for that day. Sampling continued for 18 days after the GnRH injection. Ewes which had cycled spontaneously prior to the onset of the experiment were identified by measuring the progesterone concentration of a sample taken five days prior to GnRH administration.

Table 1 Number of ewes which cycled spontaneously prior to treatment or which reovulated shortly after ovulation was induced by GnRH on Day 0

Treatment relative to GnRH	Group	Cycled spontaneously	Reovulated	Were anoestrus
Control	Control	1	2	7
Before ovulation:				
Naloxone Days –3 to –1	NAL-	1	4^a	5
PMSG Days –3 to –1	PMSG-	2	4^a	4
After ovulation:				
PMSG Days 3 to 5	PMSG3	0	2^b	8
PMSG Days 6 to 8	PMSG6	1	2^b	7
PMSG Days 9 to 11	PMSG9	0	3^b	7
Naloxone Days 2 to 4	NAL +	0	1^b	9

Numbers with different superscripts differ significantly ($P < 0,05$)

Hormone determination

Serum concentrations of P_4 were determined by radioimmunoassay (Butcher, 1977). Intra- and inter-assay co-efficients of variation were 13,56% (Pool 1), 19,07% (Pool 2) and 9,35% (Pool 1), and 7,8% (Pool 2), respectively. Recovery of ^{3}H-progesterone added to the serum before extraction was 89,64 ± 3,95% with a co-efficient of variation of 4,4%.

Statistical analysis

Initially, hormonal data were analysed as a split-plot design with 'treatment effects' as the whole plot and 'time' as the subplot stratum. Homogeneity of the variance-covariance structures for each treatment and pooled treatments were

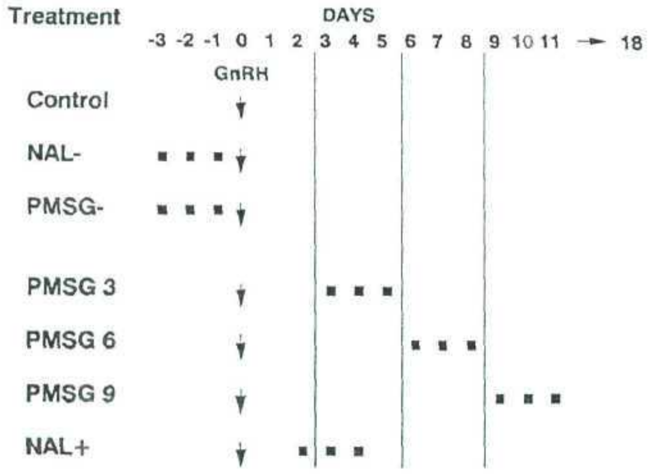

Figure 1 Diagrammatic representation of treatments applied and the time of administration in relation to GnRH injection (Day 0).

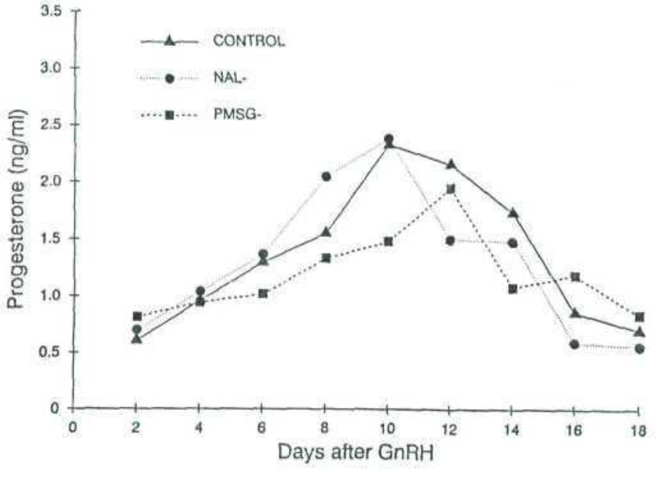

Figure 2 Effect of treatment with naloxone or PMSG prior to ovulation on subsequent luteal function in ewes.

assessed using the repeated measures macro of the Genstat Package. A correction factor was estimated and applied to the degrees of freedom (DF) in the F test when compound symmetry was not present. However, when homogeniety of the error variance fails to hold, the univariate splitplot tests of treatment means are inconclusive (Gill & Hafs, 1971). The analysis then proceeded with a combination of orthogonal comparisons of nth order regression curves for each treatment (Clarke, personal communication) and by partitioning of the treatment x period interaction to permit sensitive comparisons of treatments. These were evaluated with Bonferroni t statistics (Gill, 1986). Treatment effects on the proportion of ewes which exhibited a short-lived CL were tested by chi-square.

Results

On examination of the individual plasma P_4 profiles it became evident that some ewes had ovulated prior to the commencement of treatments ($P_4 > 0{,}5$ ng/ml) whereas others had undergone a short cycle and had re-ovulated approximately 6–8 days after the GnRH injection. In many such ewes the P_4 concentration on day 18 had not declined below 2 ng/ml. These ewes (Table 1) were eliminated for further analysis of the results. Definitions of normal (McLeod *et al.*, 1983; Brown *et al.*, 1988) and abnormal luteal function (Hunter *et al.*, 1988) were applied in eliminating animals. A greater proportion ($P < 0{,}05$) of ewes exhibited a short-lived CL in those groups treated before GnRH (PMSG–, NAL–) than where treatments commenced after ovulation (Table 1). The mean P_4 concentration, for ewes within each treatment based on samples drawn every two days, is depicted in Figures 2 and 3. It is clear (Figure 2) that neither PMSG nor naloxone, when administered prior to ovulation, resulted in an improved luteal function (compared to the untreated controls). In contrast, provision of luteotropin on Days 3–5 after ovulation markedly stimulated the secretory activity of the CL, as evidenced by the significant ($P < 0{,}05$) increase in the total area under the P_4 curve, from Day 0 to 18 after GnRH (Table 2). No significant response to the immediate stimulatory effect of PMSG from Days 3–5 of the induced cycle (measured by the area under the P_4 curve from Days 4 to 6) was detected (Table 2). However, by Days 7–9 the effect of PMSG early in the cycle (PMSG 3) was stimulatory (Table 2). In contrast, PMSG administration either on Days 6–8 or 9–11 did not alter significantly, either the area under the P_4 curve for the whole cycle or shortly after such treatments commenced (Table 2). Although ewes treated with PMSG after GnRH administration exhibited higher peak P_4 concentrations than untreated controls or those given PMSG prior to ovulation (Table 3), the differences were not significant. The augmentation of luteal function by PMSG administration on Days 3–5 is illustrated further by the significantly ($P < 0{,}05$) greater number of days during which P_4 levels remained above 2 ng/ml than in Control ewes and those treated before GnRH administration (Table 3). Application of the repeated measures analysis indicated a highly significant effect of both 'time' and of the interaction between treatment and time. The epsilon value used to reduce DF for the F test was 0,6972. A significant time × treatment interaction occurred and this suggested that treatment differences might exist. Preplanned orthogonal contrasts of the fitted response curves, using 'time' to the 4th degree and 'ewes within each treatment', supported the earlier conclusion that neither provision of naloxone nor PMSG (CONTR vs PMSG– and NAL–; PMSG– vs NAL–) before ovulation were beneficial as regards luteal function. This analysis also suggested that exogenous luteotropic support was stimulatory (CONTR *vs* PMSG 3, PMSG 6 and PMSG 9; $P < 0{,}01$), but that stage of the cycle during which it was provided was perhaps of lesser importance (PMSG 3 *vs* PMSG 6 and PMSG 9: not significant, PMSG 6 *vs* PMSG 9: not significant). Preplanned non-orthogonal comparisons between treatment means (Bonferroni's test) for specific days indicated that PMSG on Days 3–5 and Days 6–8 significantly ($P < 0{,}05$) increased plasma P_4 levels on Days 8–12 and 10–11, respectively (Figure 4), whereas when injected on Days 9–11 no significant effect occurred.

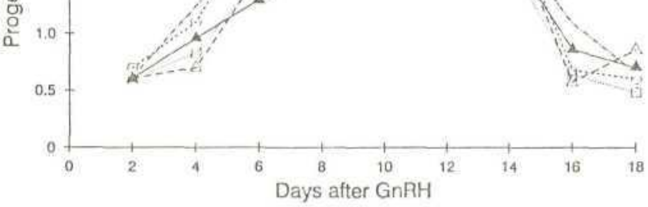

Figure 3 Influence of exogenous or endogenous luteotropic support on progesterone production in ewes.

Table 2 Area under the progesterone curve after GnRH-induced ovulation in ewes treated to stimulate luteal function

Treatment group		Mean area ± SEM			
		Days			
	n	0–18	4–6	7–9	10–12
Control	7	25,7 ± 2,4^{a}	3,0 ± 0,3^{a}	4,4 ± 0,5^{a}	6,6 ± 1,0^{ab}
NAL-	5	27,4 ± 3,7^{ab}	3,3 ± 0,6^{a}	5,4 ± 1,1^{ab}	6,2 ± 0,7^{a}
PMSG-	4	24,9 ± 2,9^{ab}	2,9 ± 0,4^{a}	3,7 ± 0,8^{a}	5,5 ± 1,2^{a}
PMSG3	8	34,2 ± 2,9^{b}	4,3 ± 0,6^{a}	7,6 ± 0,9^{b}	9,4 ± 0,9^{b}
PMSG6	7	29,8 ± 4,1^{ab}	3,1 ± 0,5^{a}	6,4 ± 0,9^{ab}	9,2 ± 1,3^{ab}
PMSG9	7	28,6 ± 3,9^{ab}	2,9 ± 0,4^{a}	5,4 ± 0,8^{ab}	8,2 ± 1,5^{ab}
NAL +	9	27,4 ± 3,7^{ab}	3,7 ± 0,8^{a}	5,5 ± 1,2^{ab}	6,6 ± 0,9^{ab}

$^{a b}$ Means within the same column without common superscripts differ ($P < 0{,}05$).

Table 3 Peak P_4 concentrations and number of days P_4 exceeded 2 ng/ml for ewes treated with PMSG or Naloxone before or after a GnRH-induced ovulation

Treatment	Peak P_4 level (ng/ml)±SEM	No of days P_4>2ng/ml ±SEM
Control	2,04 ± 0,44^a	2,5 ± 1,1^a
NAL-	2,10 ± 1,30^a	2,4 ± 1,5^a
PMSG-	2,02 ± 1,10^a	2,5 ± 1,2^a
PMSG3	3,15 ± 0,33^a	6,6 ± 1,5^b
PMSG6	3,11 ± 0,44^a	5,3 ± 1,2^{ab}
PMSG9	2,77 ± 0,52^a	3,8 ± 1,3^{ab}
NAL+	2,20 ± 1,27^a	4,6 ± 1,5^{ab}

$^{a b}$ Means within the same column with different superscripts differ ($P < 0,05$).

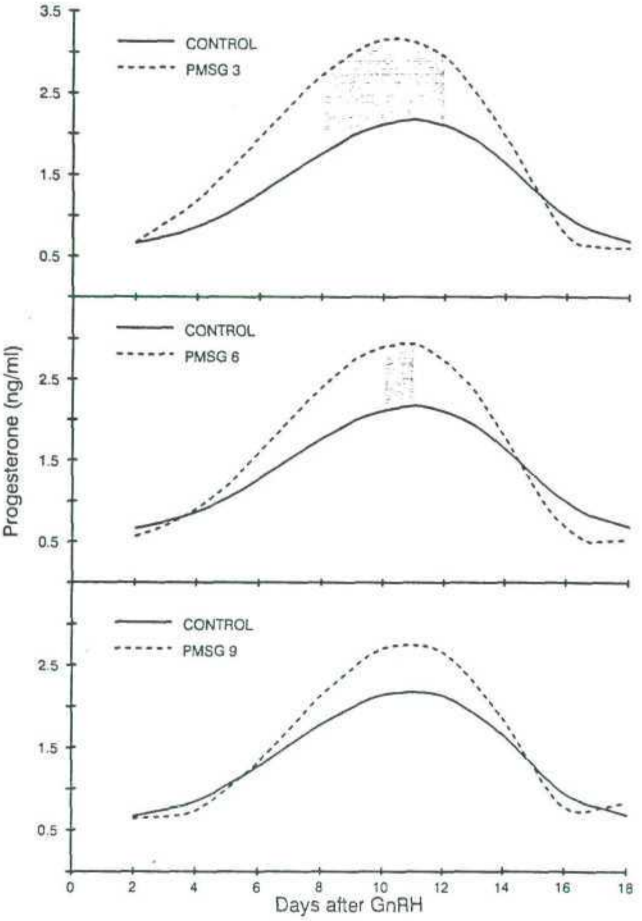

Figure 4 Estimated regression curves of progesterone concentration for ewes receiving PMSG for 3 days beginning on Days 3, 6 or 9 after GnRH administration. The shaded area indicates significant differences ($P < 0,05$) in mean daily concentration.

Discussion

Conclusions from both the present study and that of Grobbelaar *et al.* (1989) suffer from one important handicap. This was the failure to induce a high incidence of short-lived CL when the ewes (Controls) received GnRH and no other hormone treatment. This contrasts with studies in which more than 60% of the anoestrous ewes given GnRH as a single, large injection (Haresign *et al.*, 1975) or repeated small doses, with or without a bolus injection (McLeod *et al.*, 1982b; Hunter *et al.*, 1986; Hunter *et al.*, 1988) exhibited premature demise of the CL.

The data in Table 1 show that of all the ewes treated, only 36% exhibited a short-lived CL. Contrary to expectation, the highest incidence was in the groups treated with PMSG or naloxone prior to GnRH to stimulate follicle maturation (Table 1). This does not conform with reports where either PMSG (Haresign & Lamming, 1978) or repeated injections of LH (McNeilly *et al.*, 1982) or GnRH (McLeod *et al.*, 1982b) were administered prior to ovulation. When the GnRH dose was as large as 1 000 ng and administered every 2 h for 8 days, the luteal function was judged to be normal (McLeod *et al.*, 1982a) although the rise in P_4 was delayed until after the cessation of GnRH administration. The P_4 profiles described by McLeod *et al.* (1982a) are similar to many of those seen in the present study, particularly where PMSG was administered prior to GnRH. It appears that such secretory patterns are worthy of closer scrutiny since Legan *et al.* (1985), Southee *et al.* 1988) and Hunter *et al.* (1988) have recently demonstrated that the postovulatory progesterone rise may only slightly exceed 0,5 ng/ml and be of transient duration.

Except for the difference in season (spring *vs* autumn), there is no obvious reason why the results of the present study should differ notably from those reported by Grobbelaar *et al.* (1989). In the latter study, PMSG pretreatment improved the quality of luteal function to equal that of spontaneously cycling, non-lactating ewes. Continuous exposure to elevated levels of LH is suspected of causing desensitization of ovarian receptors (Clayton *et al.*, 1979). If this occurs, infusion of LH (Grobbelaar *et al.*, 1989) is likely to have been more detrimental than twice daily injections (present study). However, the results reported here do not support such an expectation.

A further possibility worth considering is that in cycling ewes the preovulatory follicle is exposed to episodic LH pulses. In contrast, ewes receiving twice daily injections of PMSG would be exposed to fairly constant levels of gonadotropin because of the extended half-life of this preparation (McIntosh *et al.*, 1975). The question as to whether or nor LH must be delivered in an episodic fashion in order to sustain maturation of follicles remains unanswered (McNatty *et al.*, 1982).

The foregoing should be viewed in the light of the dramatic effect, on plasma P_4 levels, of luteotropic support reported here (Figure 3) and also observed by Kittok *et al.* (1983) and Grobbelaar *et al.* (1989). It appears that the mechanism which results in a short-lived CL is not the same as that responsible for subnormal progesterone secretion during a cycle of approximately normal duration. It has been suggested that in ewes destined to have a short-lived CL the premature regression of the CL is due to an early release of $PGF_2\alpha$ from the uterus (Legan *et al.*, 1985; Hunter *et al.*, 1989). However, Rahmanian & Murdoch (1987) have shown that in the cycling ewe the CL can have an inherent limited lifespan that is not dependent on the presence of the uterus.

The suggestion that short-lived and inadequate CL are the result of different mechanisms is supported by Rahmanian & Murdoch (1987). They proposed that factors which influence the quality of CL function reside within the follicle. The follicle can thus be capable of ovulating before it acquires the capacity to form a normal CL (deZerega & Hodgen 1981; Murdoch *et al.*, 1983).

The possibility remains that inadequate luteal function could be due to deficiencies in maturation of the follicle prior to ovulation or the consequence of insufficient luteotropic support of the developing CL. Evidence favouring the latter is provided by the stimulatory effect of PMSG when administered after ovulation (Figure 3). However, just how inadequate luteal function is defined can modify the interpretation. Grobbelaar *et al.* (1989) suggested that P_4 levels need to rise above 2 ng/ml, whereas McLeod *et al.* (1983) have set a limit of $> 1,5$ ng/ml, while Southee *et al.* (1988) proposed that such a level needs to be exceeded for at least 8 days. If the latter criteria were to be applied to the present study then only one ewe would have been judged to have shown normal luteal function.

Gamboni *et al.* (1984) injected anoestrous ewes with 500 i.u. hCG on Day 5 after induced ovulation and recorded an increase in plasma P_4 on Day 10. This agrees with the response obtained from PMSG on Days 3–5 (Figures 3 and 4). The stimulatory effect of PMSG, when delayed until Day 9 after GnRH (Figure 2) is similar to that obtained where hCG was administered on Days 11–13 in lactating ewes (Kittok *et al.*, 1983).

Although there is little doubt that the administration of PMSG in the early part of an induced cycle will improve the function of the CL, the effect on pregnancy rates of ewes remains to be determined.

The failure of naloxone treatment (either before or after GnRH) to stimulate luteal function might be explained on the basis that endogenous LH levels were not increased by the treatment regime followed. This contention is based on the finding that when naloxone was given to rams on subsequent days the responsiveness of LH secretion changed (Ebling & Lincoln, 1985). Similarly, Currie & Rawlings (1989) observed a transient effect on LH pulse amplitude in cycling ewes. However, the enhancement of pulse frequency by naloxone continued over a 26-h infusion period (Currie & Rawlings, 1989). Seasonal effects were also possible since Ebling & Lincoln (1985) proposed that opioid inhibition of LH release may be highest when sheep are expected to be sexually active. The experiment reported here was conducted at the start of the breeding season.

Although Brooks *et al.* (1986) suggested that naloxone will increase LH levels only in the presence of raised P_4 concentrations, this is not supported by Currie & Rawlings (1989). Furthermore, naloxone has been shown to be capable of raising tonic LH concentration in lactating ewes (Gregg *et al.*, 1986; Newton *et al.*, 1988). In addition, constant infusion of 0,5 mg naloxone/kg/h for 24 h resulted in a slight increase in progesterone levels in cycling ewes (Currie & Rawlings, 1989). Accordingly, the sampling schedule employed in the present study was not designed to measure the effect on LH levels. However, Whisnant *et al.* (1986) noted a relationship between days postpartum and the dose of naloxone that was needed to improve tonic LH levels in lactating beef cows. The possibility thus exists that the dose injected and the duration of naloxone administration may have been inappropriate in the present study. Future studies will need to address this question as well as the possibility that enkephalins produced by the CL (Cupo *et al.*, 1987) may also exert a role.

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References

BROOKS, A.N., LAMMING, G.E., LEES, P.D. & HAYNES, N.B., 1986. Opioid modulation of LH secretion in the ewe. *J. Reprod, Fert,* 76, 693.

BROWN, B.W., COGNIE, Y., CHEMINEAU, P., POULIN, N. & SALAMA, O.A., 1988. Ovarian capillary blood flow in seasonally anoestrous ewes induced to ovulate by treatment with GnRH. *J. Reprod. Fert.* 84, 653.

BUTCHER, R.L., 1977. Changes in gonadotropins and steroids associated with unilateral ovariectomy of the rat. *Endocrinology* 101,930.

CHAO, C.C., MOSS, G.M. & MALVEN, P.V., 1986. Direct opioid regulation of pituitary release of bovine luteinizing hormone. *Life Sci.* 39, 527.

CLAYTON, R.N., HARWOOD, J.P. & CATT, S.K., 1979. Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. *Nature* 282, 90.

CUPO, A., MENEZO, Y. & BUCNO, L., 1987. Enkephalin production by the corpus luteum. *Neuropeptides* 9, 237.

CURRIE, W.D. & RAWLINGS, N.C., 1989. Fluctuation in responsiveness of LH and lack of responsiveness of FSH to prolonged infusion of morphine and naloxone in the ewe. *J. Anim. Sci.* 86, 359.

DE ZEREGA, G.S. & HODGEN, G.D., 1981. Luteal phase dysfunction infertility: A sequel to aberrant folliculogenesis. *Fertil.Steril.*35,489.

EBLING F.J.P. & LINCOLN, G.A., 1985. Endogenous opioids and the control of seasonal LH secretion in Soay rams. *J. Endocr.* 107, 341.

GAMBONI, F., FITZ, T.A., HOYER, P.B., WISE, M.E., MAYAN, M.H. & NISWENDER, G.D., 1984. Effect of human chorionic gonadotropin on induced ovine corpora lutea during the anestrous season. *Dom. Anim. Endocr.* 1, 79.

GILL, J.L., 1986. Repeated measurement: sensitive tests for experiments with few animals. *J. Anim. Sci.* 63, 943.

GILL, J.L. & HAFS, H.D., 1971. Analysis of repeated measurements of animals. *J. Anim. Sci.*332,331.

GREGG, D.W., MOSS, G.E., HUDGENS, R.E. & MALVEN, P.W., 1986. Endogenous opioid modulation of luteinizing hormone and prolactin secretion in postpartum ewes and cows. *J. Anim. Sci.* 63, 838.

GROBBELAAR, J., LISHMAN, A.W., BOTHA, W.A., ELS, D.L., LOUW, B.P. & VAN NIEKERK A., 1989. Attempts to improve the function of GnRH-induced corpora lutea in early postpartum ewes through oestradiol and/or PMSG priming or exogenous luteotrophin. *Anim. Reprod. Sci,* 19, 245.

HANSEL, W. & CONVEY, E.M., 1983. Physiology of the oestrous cycle. *J. Anim. Sci,* 57 (Suppl. 2), 404.

HARESIGN, W. & LAMMING, G.E., 1978. Comparison of LH release and luteal function in cyclic and LH-RH treated anoestrus ewes pretreated with PMSG or oestrogen. *J. Reprod. Fert.* 52,349.

HARESIGN, W., FOSTER, J.P., HAYNES, N.B., CRICHTON, D.B. & LAMMING, G.E., 1975. Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. *J. Reprod, Fert.* 43, 269.

HUNTER, M.G., AYAD, V.J., GILBERT, C.L., SOUTHEE, J.A. & WATHES, D.C., 1989. Role of prostaglandin $F_{2\alpha}$ and oxytocin in the regression of GnRH-induced abnormal corpora lutea in anoestrous ewes. *J. Reprod. Fert.* 85, 551.

HUNTER, M.G., SOUTHEE, J.A. & HARESIGN, W., 1987. The effect of progesterone on follicular function in anoestrous ewes. In: *Follicular growth and ovulation rate in farm animals*. Ed.J.F. Roche and D.O. Callaghan. Martinus Nijhoff Publ., Dordrecht.

HUNTER, M.G., SOUTHEE, J.A. & LAMMING, G.E., 1988. Function of abnormal corpora lutea *in vitro* after GnRH-induced ovulation in the anoestrous ewe. *J. Reprod. Fert.* 84,139.

HUNTER, M.G., SOUTHEE, J.A., McLEOD, B.J. & HARESIGN, W., 1986. Progesterone pretreatment has a direct effect on GnRH-induced preovulatory follicles to determine their ability to develop into normal corpora lutea in anoestrous ewes. *J. Reprod. Fert.* 76, 349.

KITTOCK, R.J., STELLFLUG, J.N. & LOWRY, S.R., 1983. Enhanced progesterone and pregnancy rate after gonadotropin administration in lactating ewes. *J. Anim. Sci*, 56, 652.

KITTOK, R.J., STELLFLUG, J.N. & LOWRY, S.R., 1983. Enhanced progesterone and pregnancy rate after gonadotropin administration in lactating ewes. *J. Anim.Sci.* 56, 652.

LEGAN, S.J., I'ANSON, H., FITZGERALD, B.P. & AKAYDIN, M.S., 1985. Importance of short luteal phases in the endocrine mechanism controlling initiation of oestrous cycles in anoestrous ewes. *Endocrinology* 1174, 1530.

MACDONALD, R.D., PETERS, J.L. & DEAVER, D.R., 1986. Naloxone induced LH secretion in prepubertal Holstein bulls. *J. Anim Sci.* 63 (Suppl. 1), 372.

MATHEWS, M.V. & MURDOCH, W.J., 1984. Increased secretion of luteinizing hormone in prepubertal ewes treated with an antagonist of opiates. *Dom. Anim. Endocr.* 1, 167.

MATTERI, R.L. & MOBERG, G.P., 1985. The effect of opioid peptides on ovine pituitary gonadotropin secretion *in vitro*. *Peptides* 6, 957.

MCINTOSH, J.E.A., MOOR, R.M. & ALLEN, W.R., 1975. Pregnant mare serum gonadotrophin: rate of clearance from the circulation of sheep. *J. Reprod. Fert* 44, 95.

MCLEOD, B.J., HARESIGN, W. & LAMMING, G.E., 1982a. The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injections of GnRH. *J. Reprod. Fert.* 65, 215.

MCLEOD, B.J., HARESIGN, W. & LAMMING, G.E., 1982b. Response of seasonally anoestrous ewes to small-dose multiple injections of GnRH with and without progesterone pretreatment. *J. Reprod. Fert.* 65, 223.

MCLEOD, B.J., HARESIGN, W. & LAMMING, G.E., 1983. Induction of ovulation in seasonally anoestrous ewes by continuous infusion of low doses of GnRH. *J. Reprod. Fert.* 68, 489.

MCNATTY, J.P., BALL, K., GIBBS, M.N., HUDSON, N. & THURLEY, D.C., 1982. Induction of cyclic ovarian activity in seasonally anoestrous ewes with exogenous GnRH. *J. Reprod. Fert.* 64, 93.

MCNEILLY, A.S., 1980. Paradoxical prolactin. *Nature*, Lond. 184, 212.

MCNEILLY, A.S., O'CONNELL, M. & BAIRD, D.T., 1982. Induction of ovulation and normal luteal function by pulsed injection of luteinizing hormone in anoestrous ewes. *Endocrinology* 110, 1292.

MURDOCH, W.J., DE SILVA & DUNN, T.G., 1983. Luteal phase insufficiency in the ewe as a consequence of premature induction of ovulation by intrafollicular injection of gonadotropins. *J. Anim. Sci.* 57, 1507.

NEWTON, G.R., SCHILLO, K.K. & EDGERTON, L.A., 1988. Effects of weaning and naloxone on luteinizing hormone secretion in postpartunm ewes. *Biol. Reprod.* 39, 532.

O'SHEA, J.D., RODGERS, R.J. & WRIGHT, P.J., 1984. Morphometric analysis and function *in vivo* and *in vitro* of corpora lutea from ewes treated with LHRH during seasonal anoestrus. *J. Reprod. Fert.* 72, 75.

RAHMANIAN, M.S. & MURDOCH, W.J., 1987. Function of ovine corpora lutea after administration of luteinising hormone-releasing hormone. *J. Anim. Sci.* 64, 648.

SOUTHEE, J.A., HUNTER, M.G. & HARESIGN, N., 1988. Function of abnormal corpora lutea *in vivo* after GnRH-induced ovulation in the anoestrous ewe. *J. Reprod. Fert.* 84, 131.

SOUTHEE, J.A., HUNTER, M.G., LAW, A.S. & HARESIGN, W.,1988. Effect of hysterectomy on the short life-cycle corpus luteum produced after GnRH-induced ovulation in the anoestrous ewe. *J. Reprod. Fert.* 84, 149.

STANSFIELD, S.C., KNIGHT, P.G., HOWLES, C.M. & CUNNINGHAM, F.J., 1987. Endogenous opioid peptide modulation of LH secretion in the ewe lamb: possible involvement of 5-hydroxytryptamine. *J. Endocr.* 116, 403.

STAPLES, R.E. & HANSEL, W., 1961. Luteal function and embryo survival in the bovine. *J. Dairy Sci.* 44, 2040.

WHISNANT, C.S., KISER, J.E., THOMPSON, F.N. & BARB, C.R., 1986. Opioid inhibition of luteinizing hormone secretion during the postpartum period in suckled beef cows. *J. Anim. Sci.* 63,1445.

WHISNANT, C.S., THOMPSON, F.N., KISER, J.E. & BARB, C.R., 1986. Effect of Naloxone on serum luteinizing hormone, cortisol and prolactin concentrations in anoestrous beef cows. *J. Anim. Sci.* 62, 1340.

WRIGHT, P.J., GEYTENBECK, P.E., CLARKE, I.J. & FINDLEY, J.K., 1981. Evidence for a change in oestradiol negative feedback and LH pulse frequency in postpartum ewes. *J. Reprod. Fert.* 61, 97.

WRIGHT, P.F., GEYTENBACH, P.E., CLARKE, I.J. & FINDLEY, J.K., 1983. LH release and luteal function in postpartum acyclic ewes after pulsatile administration of LH-RH. *J. Reprod. Fert* 67,257.

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eview

eficiencies in luteal function during re-initiation of cyclic breeding activity in beef cows and in ewes

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Mechanisms that have been proposed to account for (i) premature regression or (ii) subnormal secretory activity of corpora lutea (CL) in cows or in ewes are reviewed. Processes which occur prior to, at the time of, or following, ovulation are considered. Infrequent pulsatile release of LH, particularly during early lactation, may result in ovulation of an immature follicle with the resulting CL exhibiting normal secretory activity. Such incompetent follicles possess reduced numbers of LH receptors and they secrete lower levels of oestradiol-17β. The evidence involving deficiencies, in (i) the preovulatory LH surge (ii) luteotropin support of the CL or (iii) LH receptors within the CL, is equivocal. A premature release of prostaglandin $F_2\alpha$ from the uterus apparently results in early demise of the first CL in cows and in ewes.

Keywords: Cattle, deficient luteal function, mechanisms, post-anoestrus, postpartum, progesterone, sheep.

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roduction

is generally recognized that calving rate and lambing
centage are major contributors to the profitability of beef
ds and ewe-flocks. Often, the reproductive performance
beef breeding herds and sheep flocks is so low that
ling rates have to be reduced severely in order to
intain herd/flock numbers. To produce a beef calf every
5 days, or to increase the frequency of lambing to more
n once a year, requires that breeding females become
gnant during the early lactational period. This is
ticularly true when it is beneficial to advance the calving
e of cows which calve late. Although much research
rt has been expended in devising procedures, which
our early breeding in sheep and cows and out-of-season
eding in ewes, to date the success rate has been below
ectations. Apparently, those mechanisms which favour
ception need to be re-established in the early postpartum
noestrous female.

he problem of poor luteal function, that occurs when
ding cycles are induced, is reviewed in this paper. The
ntion is also to describe how an understanding of the
ject has evolved during the last decade. The main
hasis is on the beef cow but, since dairy cows and ewes
ear to exhibit similar problems, information from these
es of livestock is included in an attempt to clarify and
erstand the processes involved.

ificial induction of breeding

eviewing attempts at precipitating the onset of breeding
vity, particularly those which have gained prominence
ing the last decade, it becomes apparent that whenever
ding was initiated, either naturally (Kiracofe *et al.*,
9; Corah *et al.*, 1974; Yuthasatrakosol *et al.*, 1977;
nphrey *et al.*, 1976; Walton *et al.*, 1977; Bulman &
ming, 1978; Lamming, 1978; Prybil & Butler, 1978; Schams *et al.*, 1979; LaVoie *et al.*, 1981; Peters & Riley, 1982; Manns *et al.*, 1983; Meisterling & Dailey, 1987) or artificially (Britt *et al.*, 1975; Crighton *et al.*, 1975; Haresign *et al.*, 1975; Fonesca *et al.*, 1977; Smith *et al.*, 1979; Carter *et al.*, 1980) the luteal function consequent to the first ovulation tended to be inferior in duration and/or quality. Even when pregnancy was allowed to continue for only 50 days the lifespan of the first luteal structure was reduced upon re-initiation of sexual cycles in a large proportion of heifers (Wright *et al.*, 1984; Copelin *et al.*, 1989a).

This phenomenon was highlighted when gonadotropin releasing hormone (GnRH) was first used to induce ovulation in lactating beef cows (Britt *et al.*, 1975; Webb *et al.*, 1977; Lishman *et al.*, 1979) and in lactating (Ainsworth *et al.*, 1982) or seasonally anoestrous ewes (Crighton *et al.*, 1975; Haresign *et al.*, 1975; Shareha *et al.*, 1976). However, luteal function which ceased prematurely has subsequently been demonstrated to occur even when ovulation was induced by injecting PMSG in lactating ewes (Fletcher *et al.*, 1980), by introduction of rams near the end of seasonal anoestrus (Oldham & Martin, 1979; Knight *et al.*, 1981), or when the suckling stimulus was manipulated to precipitate ovulation in beef cows (Flood *et al.*, 1979; Ward *et al.*, 1979; Odde *et al.*, 1980; Ramirez-Godinez *et al.*, 1980; Ramirez-Godinez *et al.*, 1981; Ramirez-Godinez *et al.*, 1982a). The foregoing discussion implies that, when exogenous hormones are utilized to precipitate the onset of breeding, the frequent occurrence of corpora lutea (CL) with a shortened lifespan, does not represent as great a failure of the technique as commonly believed. Apparently, exogenous hormones only mimic that which happens naturally in the cow or ewe when ovarian cycles commence spontaneously. Exposure of the reproductive system to progesterone (P4) for a short period of time could therefore be a vital prerequisite to the restoration of full reproductive function. Although Peters & Lamming (1984) believed that P4 priming is not essential, evidence will be presented here to illustrate the beneficial consequences of prior exposure to this gonadal steroid.

The problem of luteal insufficiency may not end after the first short luteal cycle (Lishman *et al.*, 1979). Evidence continues to accumulate which suggests that the secretory activity of the CL improves over several cycles postpartum (Stevenson & Britt, 1979; Webb *et al.*, 1980; Duby *et al.*, 1985; Butterfield, 1986). This is supported by the finding that only 12% of first postpartum ovulations in beef cows were fertile (Holness *et al.*, 1980), particularly when they occurred before 50 days postpartum in *Bos indicus* cows (Wells *et al.*, 1985). There appeared to be an association between concentrations of P4 in the cycle preceding that in which mating occurred and the success of conception (Henricks *et al.*, 1971; Folman *et al.*, 1973; Corah *et al.*, 1974; Holness *et.al.*, 1977; Meisterling & Dailey, 1987). Further support for the belief that luteal incompetence may have been exerting an important influence on conception rates during the early postpartum period has been obtained for beef cows by Rutter & Randel (1984) and for sheep by Rhind *et al.* (1980).

Types of abnormal luteal function

Two distinct types of abnormal luteal function occurred in ewes (Fletcher *et al.*, 1980; Inskeep & Murdoch, 1980; diZerega & Hodgen, 1981; Lewis *et al.*, 1983) and in beef cows (Troxel & Kesler, 1984b). According to Murdoch *et al.* (1983) and Troxel & Kesler (1984b), one type incorporated a luteal lifespan of 6 to 12 days (the short luteal phase), while the other was characterized by a luteal phase of more than 14 days, but with depressed plasma P4 (the inadequate luteal phase).

Although short-lived CL occur almost exclusively when breeding is initiated for the first time or after a period of sexual rest, inadequate CL seem to occur at any stage during the reproductive life of cattle or sheep. Apparently, the short life-cycle CL functioned for a longer period in the ewe when large, single (Haresign, 1976; McNeilly *et al.*, 1981; O'Shea *et al.*, 1984) rather than multiple, low-dose treatment with GnRH was applied (Hunter *et al.*, 1988; Southee *et al.*, 1988). In the latter case only a transient rise in plasma concentrations of P4, which lasted for about four days, was seen (Hunter *et al.*, 1988; Southee *et al.*, 1988), resembling that which occurred prior to puberty in the ewe (Berardinelli *et al.*, 1979) and after ram introduction in anoestrous ewes (Knight *et al.*, 1981).

The possibility that inadequate and short-lived CL may be the result of different mechanisms will be examined in this review. In order to reduce the postpartum interval to conception, particularly in cows, the problem of luteal insufficiency in the early phases of reinitiation of sexual activity should be eliminated (Ramirez-Godinez *et al.*, 1981; 1982b). Elimination of short-lived luteal phases is also advantageous when reduction of the time interval between successive parturitions in cows that calve late is required. In demonstrating the relationship amongst factors contributing to infertility, Short *et al.* (1990) have illustrated the importance of short oestrous cycles in increasing postpartum infertility.

Cellular composition of the CL

Follicular origin

In attempting to account for deficiencies in luteal function, attention needs to be focused on the origin of the cells that differentiate to form the CL. Any changes in the final cellular composition of the CL also warrant consideration.

In many domestic animals the CL contain two distinct steroidogenic cell-types (Niswender *et al.*, 1985a). These cell-types, which have different morphological and biochemical properties (O'Shea *et al.*, 1979; 1980; Fitz *et al.*, 1982; Glass *et al.*, 1984; Hoyer & Niswender, 1985) have been termed small or large luteal cells. The follicular origin of the small luteal cells appears to be the theca interna of the preovulatory follicle in cattle (Donaldson & Hansel, 1965; Priedkalns *et al.*, 1968) and in the ewe (Deane *et al.*, 1966; McClellan *et al.*, 1975), whereas the large luteal cells originate from the granulosal cells (Donaldson & Hansel, 1965; McClellan *et al.*, 1977; O'Shea *et al.*, 1980). The question as to whether large and small luteal cells persist as discrete and closed populations throughout the lifespan of the CL is more controversial (O'Shea, 1987). Several lines of evidence (Donaldson & Hansel, 1965; Alila & Hansel, 1984; Farin *et al.*, 1985; Niswender *et al.*, 1986; Farin *et al.*, 1988) support the suggestion that small luteal cells may be able to differentiate into large luteal cells as the cycle progresses (Hansel & Dowd, 1986). However, Farin *et al.* (1988) postulated that such conversion to large luteal cells occurs only during the early part of the oestrous cycle. There may even be stem cells in the CL which differentiate into small steroidogenic cells. These, in turn, may become large luteal cells (Niswender *et al.*, 1985).

Contribution of different cell types to circulating P4 concentrations

LH is the major luteotropin in domestic ruminants (Niswender *et al.*, 1985) and in both sheep (Fitz *et al.*, 1982; Rodgers & O'Shea, 1982; Rodgers *et al.*, 1983) and cattle (Ursely & Leymarie, 1979; Koos & Hansel, 1981) a marked difference in the response to LH by large and small luteal cells is exhibited *in vitro* (Koos & Hansel, 1981; Harrison *et al.*, 1897; O'Shea, 1987). Because of much higher numbers of LH receptors on small than on large luteal cells (Fitz *et al.*, 1982; Harrison *et al.*, 1987), the P4 secretory response to LH is probably confined almost entirely to these cells (O'Shea, 1987). Furthermore, Niswender *et al.* (1985) have calculated that during mid-cycle approximately 20% of the P4 in the ovarian vein is secreted by the small luteal cells, while nearly 80% appears to be secreted by large cells, which have only a few functional receptors for LH (Harrison *et al.*, 1987). On a per cell basis the large luteal cells produce substantially more P4 than small luteal cells (O'Shea, 1987).

Mechanisms contributing to reduced luteal function

The foregoing brief description of the cellular components of the normally functioning CL, their reputed follicular origins and the contribution each type of cell could be making to circulating P4, facilitates an evaluation of the mechanisms which may be resulting in impaired function of induced CL (Figure 1). The mechanisms can be classified into three main categories: (A) deficiencies in the maturational process within the preovulatory follicle and/or inadequacies of the ovulatory stimulus; (B) shortcomings in the support of the CL once they have formed; or (C) a premature activation of the luteolytic process.

Theoretically, the CL could be influenced by at least two different types of tropic stimuli, the one increasing its secretory activity and the other prolonging its lifespan (Schomberg *et al*., 1967). Such considerations are important when evaluating possible imperfections in the normal process of CL formation and continued function. The proposal (Troxel & Kesler, 1984a) that inadequate luteal function could be composed of two distinct aspects, namely, luteal lifespan and secretory activity, agrees with the foregoing.

The possibility that deficiencies of the follicle may be reflected in subnormal concentrations of P4 during the course of a cycle of approximately normal duration, whereas short-lived CL could more probably be the consequence of premature activation of luteolysis, is examined in this article. An attempt will thus be made to answer the question posed by Short *et al*. (1990), namely, 'Why is the first oestrous cycle short — is it because the CL are not capable of functioning normally, or is it because signals are being given prematurely for regression to occur?' The former would imply that although the luteotropic signal (Figure 1, Component 12) is normal, either the CL do not recognize the luteotropin because of a lack of LH receptors (Component 13) or the cellular make-up may be altered in some way. Premature regression could involve release of $PGF_2\alpha$ at an inappropriate stage of the development of the CL (Component 20) and this could in turn be the consequence of lack of P4 priming of the uterus (Component 22). The reader should refer also to the papers by Lauderdale (1986) and by Garverick & Smith (1986).

The short-lived CL formed following spontaneous ovulation after parturition appears to be due to mechanisms similar to those resulting in short-lived CL after an artificially induced ovulation. This is supported by the results obtained when (1) the first CL formed spontaneously after parturition was compared with the CL of normally cycling cows (Smith *et al*., 1986; Braden *et al*., 1989a) or ewes (Braden *et al*., 1989b) and (2) CL induced following a period of pretreatment with P4 or without P4 were compared (Rutter *et al*., 1985) However, care should be exercised in assuming that the deficiencies of the former are necessarily the same as those of the latter. Kesler *et al*. (1981) have made a similar conclusion.

Deficiencies prior to ovulation

The need for proper maturation of the preovulatory follicle

In 1975, it was suggested by one of us (EKI) that to induce ovulation in lactating beef cows, using GnRH, at least one ovarian follicle should have matured sufficiently to be palpable on the ovarian surface prior to administration of releasing hormone. This contention was subsequently substantiated (Lishman *et al*., 1979) and has found support elsewhere (Kesler *et al*., 1978; Garverick *et al*., 1980; Smith *et al*., 1983; Peters & Lamming, 1984). Furthermore, Haresign & Lamming (1978) have demonstrated that in anoestrous ewes, stimulation of the pre-ovulatory follicle with PMSG, prior to administration of GnRH, significantly increased the incidence of ovulation. The foregoing discussion relates to the success with which ovulation can be induced. This event usually precedes formation of the CL, and as pointed out by Garverick *et al*. (1988), formation of the CL is a continuation of follicular maturation. It has been suggested (Odde *et al*., 1980) that inadequate preparation of the follicle (Component 9) might also be responsible for poor luteal function in the cow. In fact, gonadotropin treatment prior to the induction of ovulation in sheep (Haresign & Lamming, 1978; Grobbelaar *et al*., 1989) and cows (Lishman *et al*., 1979; Sheffel *et al*., 1982) has resulted in improved function of the resulting CL.

Changes within the maturing follicle that may influence the function of the resulting CL

In the rat as follicles mature during the pre-ovulatory stage, under the influence of a sustained increase (Figure 1, Components 6 and 8) in LH or FSH (Richards, 1980), the number of LH receptors in the thecal and granulosal cells increases (Uilenbroek & Richards, 1979). The appearance of LH receptors in the granulosal cells denotes the follicle destined to ovulate (Webb & England, 1982). These granulosal cells also possess receptors to FSH (see Richards, 1980 for review).

With this information as background, it is possible to consider what deficiencies of the follicle destined to ovulate could result in an inadequate CL in a cow or ewe. The cellular composition and source of the large and of the small luteal cells should be borne in mind here. One possibility is a deficiency in cell numbers, cell sizes, or proportions of large to small luteal cells (O'Shea *et al*., 1984) while another is a deficiency of receptors (Component 13) to luteotropin could result in the CL failing to recognize or respond to luteotropin (Kesler *et al*., 1981; Rutter & Randel, 1984). The latter assumes that quantities of luteotropin are adequate at such times.

O'Shea *et al*. (1984) believed that the defect of the follicle which ovulates following treatment with releasing hormone may in fact encompass sub-optimal numbers of granulosal and/or thecal cells (Component 9). A deficiency of granulosal cells is likely to be more significant, as their capacity for mitosis, after ovulation, is probably non-existent (McClellan *et al*, 1975; O'Shea *et al*., 1980); and stimulation of the follicle before ovulation possibly improves P4 production (by the CL) by favouring development (numbers, sizes, or receptors) of granulosal cells from which the large luteal cells develop. On the other hand, the LH-sensitive thecal cells continued to divide for at least 5 days after ovulation during the normal oestrous cycle (O'Shea *et al*., 1980) while the GnRH-induced CL did not persist beyond this time (Kesler *et al*., 1981). This agrees with the findings of Lishman *et al*. (1979).

If it is accepted that large luteal cells can develop from

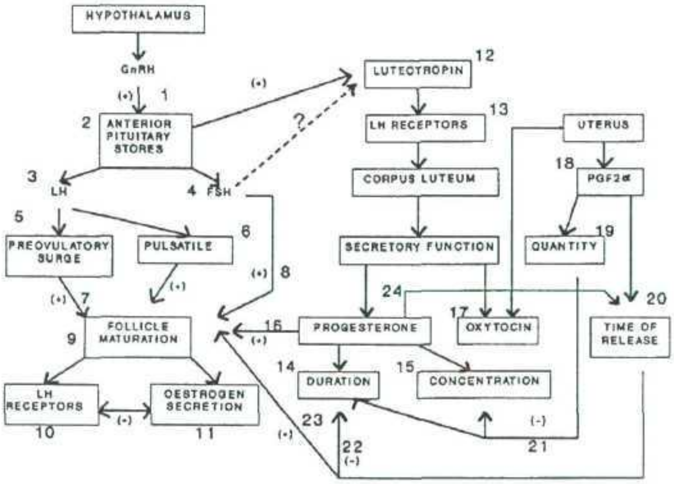

Figure 1 Possible deficiencies of the hypothalamo-pituitary-ovarian complex which could lead to a short-lived or subnormal corpus luteum and the role of the uterus in reducing luteal competence.

small luteal cells, then the statement by Gamboni *et al.* (1984) that large luteal cells increase in abundance about the middle of the normal oestrous cycle implies that the number of thecal cells within the developing follicle is of cardinal importance.

Based on morphometric examination, O'Shea *et al.* (1984) demonstrated that the reduced luteal volume of the GnRH-induced CL, in anoestrous ewes, was associated with reduced numbers of luteal cells per CL. However, no evidence was found of a fall in the proportion of large to small luteal cells which could explain the lowered basal P4 production observed in induced CL.

These findings have been confirmed in spontaneously ovulating, lactating ewes (Braden *et al.*, 1989b) and cows (Duby *et al.*, 1985), and also in cows induced to ovulate with GnRH (Rutter *et al.*, 1985). The former authors concluded that although the number of granulosal cells was almost doubled in preovulatory follicles expected to form short-lived CL in cows (Braden *et al.*, 1989a) this does not result in CL containing a greater proportion of large luteal cells.

Even though thecal cells appear to give rise to the luteal cells which respond to luteotropin, the granulosal layer of the preovulaytory follicle may play a role in the response to post ovulatory LH. This is supported by the suggestion (Kesler *et al.*, 1981) that non-proliferating granulosal cells could acquire the ability to respond to LH and thereby increase concentrations of P4 between days 5 and 7 in cycling ewes. Fritz *et al.* (1982) and Gamboni *et al.* (1984) maintained, however, that the sources of the small and large luteal cells are unclear; they supported the conclusion that the large cells lack receptors for LH.

It thus appears possible that increased concentrations of postovulatory LH could promote secretion of P4 via cells of granulosal or thecal origin (Kesler *et al.*, 1981; Fitz *et al.* 1982), with the evidence favouring an effect of LH on the small luteal cells (Fitz *et al.*, 1982).

Why are mature follicles scarce during times of sexual rest?

Research into the reasons why mature follicles are rare in the ovaries during periods of anoestrus has been based upon the observed low concentrations of LH in plasma at such times (see Peters & Lamming, 1984 and Nett, 1987 for review). This situation was at least partly the result of low pituitary reserves of LH (Component 2) during the early postpartum period (Jenkin *et al.*, 1977; Crowder *et al.*, 1982; Clarke *et al.*, 1984; Moss *et al.*, 1985). Consequently, in attempting to account for the poor response to releasing hormone in the postpartum cow, the deficiencies were considered to possibly include inadequate release of LH, either for several days prior to ovulation (Component 6), or in shortcomings (duration and/or peak concentrations; Component 5) of the preovulatory surge of LH (Kesler *et al.*, 1978; Lishman *et al.*, 1979; Troxel *et al.*, 1980).

In order to determine the deficiencies of the follicle which ovulates in response to GnRH and which cellular components are being affected, a close study of follicle maturation in spontaneously ovulating ewes or cows and an evaluation of the hormonal mechanisms which support this maturation are necessary.

Hormonal mechanisms which drive the follicle to maturation

Since the work of Haresign & Lamming (1978), suggesting that exogenous gonadotropin priming of the ovary can promote induction of normal luteal function when GnRH is used to induce ovulation, considerable research has focused on this aspect.

The final stages of follicular development (Component 9), in both the spontaneously cycling ewe and cow, were associated with a marked increase in the frequency of LH episodes (Yuthasastraskosol *et al.*, 1977; Baird, 1978; Rahe *et al.*, 1980; Karsch *et al.*, 1983). Following regression of the CL, the frequency of the LH episodes increased from one per 3 to 12 h interval to one every 1 to 2 h immediately before the LH peak (see McLeod *et al.*, 1982a, for review). A similar mechanism has been proposed for the prepubertal ewe (Foster & Olster, 1985). In contrast, periods of ovarian acyclicity were associated with a low frequency of LH episodes (Lamming *et al.*, 1981; Peters *et al.*, 1981; McLeod *et al.*, 1982a; 1982b; Wright *et al.*, 1983; Clarke, 1988; Savio *et al.*, 1990). Thus, during seasonal anoestrus in the ewe (Hunter & Southee, 1987) and during the early postpartum period in dairy cows (Savio *et al.*, 1990), the pulsatile release of LH was insufficient to promote the final maturation of follicles (Hunter & Southee, 1987).

LH (McNeilly *et al.*, 1982) or GnRH (McLeod *et al.*, 1982a; 1982b; McNatty *et al.*, 1982; Guajardo *et al.*, 1988) was administered repeatedly in anoestrous ewes and postpartum cows (Riley *et al.*, 1981), so as to increase the LH pulse frequency to that seen during the follicular phase of the normal oestrous cycle. This induced ovulation, as it did in prepubertal ewes (Keisler *et al.*, 1985; Pirl & Adams, 1987). However, CL function was still subnormal (McNeilly *et al.*, 1981; McLeod *et al.*, 1982a; 1982b; Hunter *et al.*, 1986).

These findings agree with the observation that although weaning induced short-lived CL after the first oestrus in beef cows, the pattern of gonadotropins did not differ for hree days prior to to the first or second oestrus (Garverick t al., 1988). The second oestrus was followed by normal uteal phases in that study.

The formation of properly functional CL apparently equires a duration (at least 20 h), and possibly a pattern, of LH stimulation of the ovary similar to that occurring in yclic females from the time of luteolysis to the start of the preovulatory surge (Wright *et al.*, 1984). While it may be obligatory for the pituitary to release LH in a pulsatile ashion (Wang *et al.*, 1976; Jewelewicz *et al.*, 1977), it is not clear whether the pattern of episodic delivery of LH to he ovary is critical for promoting the final stages of ollicular maturation (McNatty *et al.*, 1982). To support ollicular maturation a pulsatile delivery of LH to the ovary nay be preferable to constant exposure (McNatty *et al.*, 981). However, widely differing patterns of LH stimulation luring the pre-ovulatory period have resulted in normal ollicular maturation and some CL function (Keisler *et al.*, 985). Such considerations have important practical mplications should it become necessary to supply exogenous gonadotropin. Obviously, it would be much impler to achieve a relatively continuous delivery, whereas pulsatile patterns are difficult to duplicate on any reasonable cale.

Recently, it has been suggested that $PGF_2\alpha$ may also be nvolved in follicular development (Component 23) in early postpartum cows (Guilbault *et al.*, 1987); and evidence to upport this hypothesis has been obtained in cows Villeneuve *et al.*, 1988) and in ewes (Villeneuve *et al.*, 989). Accordingly, either the quantity or pattern of $PGF_2\alpha$ elease may be inappropriate prior to ovulation resulting in ubnormal CL function.

Why are LH pulses infrequent in early postpartum?

Pituitary reserves of LH and patterns of GNRH secretion

Nett (1987) has reviewed possible deficiencies of the hypothalamic-hypophysial axis that could result in a reduced ecretion of LH during the early part of the postpartum period, while Clarke (1988) has discussed the seasonally anoestrous ewe. Moss *et al.*, (1985) suggested that lowered pituitary concentrations of LH (Component 2) was more ikely to be one of the initial limitations to the re-establishment of oestrous cycles rather than alterations in he sensitivity of this organ to GnRH.

Cognisance should be taken of the fact that in the najority of studies on pituitary LH content and release patterns, LH was measured by radio-immunoassy. Moss *et al.*, (1988) have pointed out that only a fraction of the mmunoactive LH in the bovine pituitary is biologically active. Furthermore, Weesner *et al.*, (1987) concluded that ow biological activity of LH may contribute to postpartum nfertility in cows.

Evaluation of the GnRH content of hypothalamic neurons Leshin *et al.*, 1988) suggested a reduced synthesis of GnRH luring early postpartum (up to about day 40 postpartum). With reduced synthesis of GnRH, there could be a lack of timulus (Component 1) for secretion of LH and for its elease in an appropriate manner. This conclusion is not upported by the findings in anoestrous ewes (Clarke, 1988). GnRH pulses were seen to be half as frequent as during the luteal phase, suggesting reduced hypothalamic secretion of GnRH. However, injecting oestradiol benzoate did not cause a surge in GnRH secretion within 12 h. Consequently, deficiencies in secretion of LH (frequency and/or pulse amplitude) are possibly not due to a lack of hypothalamic GnRH. Nett (1987) concluded that during the postpartum period the hypothalamus in fact contained sufficient stores of GnRH to stimulate the anterior pituitary. He maintained that the critical question which needed to be answered was whether GnRH is secreted in a manner which will stimulate ovarian cyclicity. Once the pituitary stores of LH had been replenished, then pulses in the secretion of LH could increase in frequency to culminate in the first postpartum oestrus (Humphrey *et al.*, 1983) with the LH pulse amplitude reflecting the releasable pituitary reserves (Clarke & Cummins, 1985). Ovarian acyclicity thus appeared to be the result of a failure of follicle development, possibly due to an inadequate frequency of LH pulses (Component 6). This, in turn, reflected inadequate pulsatile release of GnRH (Wright *et al.*, 1981; 1983; Wise *et al.*, 1989).

Feedback response to E2

Infrequent pulses of LH may not be the only deficiency during early postpartum. Peters (1984) has proposed that although the positive feedback mechanism of E2 in triggering the pre-ovulatory LH release may be functional, recovery of maximal activity may continue over an extended period. Therefore, the hypothalamo-pituitary axis would need to recover responsiveness to increasing plasma concentrations of E2 before the first ovulation can occur. The results of Nancarrow *et al.* (1977), Wright & Findlay (1977), Zaied *et al.* (1980) and Irvin *et al.* (1981) support the conclusion that pituitary responsiveness to GnRH was restored before that of the hypothalamus to E2. Wise *et al.* (1986) concluded that the resumption of reproductive cycles in postpartum ewes occurred about the time that the concentration of receptors for E2 in the anterior pituitary and hypothalamus increased.

Parfet *et al.* (1986) stated that the absence of oestrous cycles in suckled beef cows near 30 days postpartum was not due to deficiencies in (1) ovarian follicular development, (2) anterior pituitary concentrations of LH and FSH or *in vitro* releasability of LH or (3) pituitary receptors for GnRH.

Apparently, in the properly fed cow, at about one month after calving, the hypothalamo-pituitary axis is fully able to support resumption of ovarian cycles, but the suckling stimulus inhibits pulsatile release of LH (see Williams, 1989 for review).

Is FSH deficient during early postpartum period?

FSH is considered to play only a permissive role in the onset of ovarian cycles postpartum. Nevertheless, the existence of a threshold which needs to be exceeded, so as to enable LH to stimulate ovarian activity, has not been excluded (Peters & Lamming, 1984; Garcia-Winder *et al.*, 1986).

Fortune & Quirke (1988) proposed that FSH is critical to support growth and development of bovine preovulatory follicles during and after luteolysis. Consequently, a deficiency of FSH during the late luteal to early follicular phase could perhaps inhibit the development and function of the preovulatory follicle (Fortune & Quirke, 1988). If FSH is deficient during the early lactational period then maturation of the pre-ovulatory follicle (Component 9) might be impeded. This contention is supported by the observation that P4 priming increased the lifespan of induced CL only in beef cows in which the plasma concentrations of FSH were relatively high at the time of P4 implantation (Garcia-Winder *et al.*, 1986). This agrees with the observation that suppression of FSH release by administration of follicular fluid, while Norgestomet was implanted, reduced the incidence of ovulation in cycling ewes (Larson *et al.*, 1987) and tended to shorten luteal lifespan in postpartum cows (Larson, 1987). Furthermore, FSH concentrations were lower over the last four days before the first preovulatory LH surge induced by weaning in beef cows. FSH concentrations were also lower compared to the same period prior to the second preovulatory LH surge (Ramirez-Godinez *et al.*, 1985b). The former preceded short-lived CL, while the latter preceded normal luteal function. However, Garverick *et al.* (1988) reported no difference in FSH concentrations prior to the first induced (weaning) oestrus as compared to the subsequent oestrus. This is supported by Lewis & Bolt (1987) who found that suckling did not reduce GnRH-induced release of FSH in postpartum ewes.

The proposal (Driancourt & Fry, 1988) that follicles must first be primed (i.e. sensitized) by FSH before they are able to respond to LH is in accordance with the foregoing suggested role for FSH. The magnitude of the FSH changes that are required is, however, unknown (Driancourt & Fry, 1988).

Lack of follicular maturation in the postpartum cow

A model to explain inadequate maturation of follicles in the postpartum, anoestrous cow has been derived by Nett (1987). To account for low concentrations of LH during early lactation, he suggested that during pregnancy the high concentrations of P4 and E2 resulted in a prolonged negative feedback on the hypothalamic-hypophysial axis. Accordingly, the synthesis of LH was inhibited and pituitary stores became depleted so that basal release of LH was reduced. Weesner *et al.* (1987) have produced results which supported this conclusion. After parturition, a two-phase recovery of the hypothalamic-pituitary-gonadal axis occurred with the first phase (lasting 2 – 5 weeks) perhaps characterized by infrequent releases of GnRH (one pulse / 4 – 8h). Once pituitary stores of LH had been replenished then the amplitude of the LH pulses was sufficient to stimulate follicular growth. This denoted the start of the second phase of the recovery process, during which the increased circulating concentrations stimulated growth of ovarian follicles, which in turn produced E2. At this point in time, the frequency of release of GnRH also increased with a consequent increase in the frequency of LH pulses. The final stages of follicular development ensued and culminated in the first ovulation.

Intrafollicular receptors for LH and the role of E2

Development of LH receptors

Even if luteotropin secretion is sufficient to support normal CL function (discussed later), then short-lived or inadequate CL may not recognize LH. This raises the question (of sufficient LH receptors, possibly within the maturing follicle and subsequently in the CL (Component 10 and 13).

According to Fortune & Quirke (1988), E2, in addition to its role as the major steroid messenger produced by mature follicles, also acted within the follicle to regulate its development and function. They proposed that as the preovulatory follicle matured, the initial action of E2 was that of positive feedback on its own production, via increased androgen synthesis.

During pre-ovulatory growth, follicles become more responsive to LH and acquire an increased ability to synthesize E2 (see Webb & England, 1982, for review). Improved responsiveness to basal levels of LH was related to an increase in LH receptors in the granulosal cells (Webb & England, 1982); the induction of these vitally important receptors, in turn, may have been influenced by E2 (Richards, 1980; Webb & England, 1982). This suggested that increased follicular concentrations of E2 may have played a leading role in the induction of increased numbers of granulosal LH receptors (Component 10) during the preovulatory period (Uilenbroek *et al.*, 1979). The E2 may have been derived via small changes in basal LH not detected by the usual assays (Richards, 1980) and may be important in the function of the resulting CL. Both E2 and FSH are necessary for production of LH receptors before luteinization of granulosal cells (McNatty, 1979; Richards, 1980). Also, numbers of thecal and granulosal receptors for LH within the maturing follicle were related to subsequent luteal function in rats (Richards & Midgley, 1976) and in humans (McNatty, 1979). However, Spicer *et al.* (1986) observed that despite increased E2 in the fluid of large follicles between days 14 and 28 postpartum, neither the receptors for LH nor FSH increased in number. The results obtained by Braden *et al.* (1989a) support these findings with regard to E2 and LH receptors in the first preovulatory follicle formed (by weaning) during the postpartum period as compared to preovulatory follicles in cycling cows.

E2 and luteal lifespan

A positive role of E2 is supported by the finding (Larson, 1987) that in postpartum beef cows, administration of hCG (to induce ovulation) more often led to CL with a normal lifespan in cows with high plasma E2 than in cows with low E2. Similarly, the largest preovulatory follicle possessed decreased numbers of receptors for LH in the granulosa and theca in cows which were predicted to have short luteal phases than in Norgestomet pretreated cows which were expected to show normal luteal lifespans (Inskeep *et al.*, 1988). Garcia-Winder *et al.* (1987) and Inskeep *et al.* (1988) found a higher concentration of E2 in the fluid of the largest follicle of cows which had been implanted with synthetic progestogen (Norgestomet). Such implanted cows commonly had CL with a normal lifespan. Similarly, implanting E2 in addition to the intermittent injection of GnRH (Wright et al., 1982), implanting E2 shortly after calving (Dyer *et al.*, 1988; Day *et al.*, 1990) or the infusion of catechol estradiol (a metabolite of oestradiol) into the uterine horn Nephew *et al.*, 1989) promoted normal luteal lifespan in actating beef cows.

ntrafollicular E2

The improved responsiveness of the follicle may reflect an mproved number of granulosal cells per follicle or even an effect of intrafollicular E2 (Richards, 1980). If the latter is mportant then it could explain why raising the systemic evels of this steroid, through exogenous sources, has not necessarily been beneficial to CL function (Lishman *et al.*, 1979; Grobbelaar *et al.*, 1989), i.e. concentration of E2 may not have been increased within the follicle. Accordingly, Garverick & Smith (1986) have concluded that high concentrations of E2 and adequate thecal vascularization may be mportant determinants of subsequent luteal function. Hunter *et al.* (1988) supported this, stating that inadequate vascularization of the CL may lead to premature regression of this structure in anoestrous ewes because the CL were then more vulnerable to the vasconstrictive effects of $PGF_2\alpha$. The ability of vasodilatory drugs to negate the cycle shortening effect of oxytocin administered early in the cycle n cows, provides additional supportive evidence (Kotiwica, 1988).

The conclusion that E2 may be required, within the mature follicle, at a level which is not achieved by the usual m. dose of E2 administered, seems justified, particularly since microgram quantities of E2 are known to occur in the follicular fluid (England *et al.*, 1973). Further support is provided by the demonstration that, in the cow, concentrations of E2 were higher and more pulsatile in the vena cava than in the jugular (Walters *et al.*, 1984). In addition, the stimulation of normal luteal activity in anoestrous ewes, by repeated low doses of GnRH, was probably dependent on ovulation being induced via E2 from the developing follicle and not due to a fairly immediate release of LH in response to the exogenous GnRH (McLeod & Haresign, 1984a; 1984b). A similar situation may hold with regard to the ram-nduced ovulation at the onset of the breeding season. This ovulation reputedly is not preceded by a preovulatory rise in circulating concentrations of E2 (Pearce *et al.*, 1985; Knight *et al.*, 1978).

Exposure of the granulosal cells to LH may promote luteinization within the follicle (Channing, 1970) and this could occur during the preovulatory surge (Moor *et al.*, 1973) when E2 production by the thecal cells is terminated (Moor, 1974). It has been demonstrated (Moor, 1973) that the preovulatory follicle produced significant amounts of P4. This was supported by the finding of an increased blood level of P4, particularly in the vena cava, before ovulation in the cow (Walters & Schallenberger, 1984). It is perhaps at this critical stage that inadequacies of the follicle that will be ovulated by GnRH first become evident and this is supported by the association between a small elevation in plasma P4 on the day of GnRH injection or calf removal and subsequent function of the induced CL (Williams, 1989).

Evidence from a number of sources supports the conclusion that E2 within the preovulatory follicle is associated with the function of the resulting CL. Whether follicular concentrations of E2 are directly involved in fully maturing the follicle destined to ovulate or whether they simply reflect the degree of maturation, remains to be clarified.

Deficiencies at the time of ovulation or post ovulation

Could abnormalities of the preovulatory LH surge result in a short-lived or defective CL?

The preovulatory LH surge induced by GnRH was smaller in anoestrous ewes (Foster & Crighton, 1974) and of shorter duration in postpartum cows (Lishman *et al.*, 1979) than that observed at spontaneous oestrus. Nevertheless, it was concluded (Crighton *et al.*, 1975; Haresign *et al.*, 1975) that deficiencies of the preovulatory LH release (Component 7) could not be implicated in the ability of GnRH-induced CL to function normally. A similar conclusion has been made concerning the preovulatory release induced by weaning in beef cows (Ramirez-Godinez *et al.*, 1982b). In contrast, McNatty *et al.* (1981) maintained that an inappropriate preovulatory LH surge was involved. They proposed that to realise ovulation and normal CL in anoestrous ewes, the LH surge should persist for about four hours and the peak concentration should rise above 30 ng/ml. Troxel & Kesler (1984b) were in agreement, stating that the magnitude and duration of the GnRH-induced LH surge appeared to be associated with enhanced CL function and lifespan. Similarly, Cruz & Kesler (1988) reported that cows with normal luteal function had a greater GnRH-induced LH release than cows with short luteal phases. Shirar *et al.* (1989) supported this viewpoint, but emphasized the importance of duration of the preovulatory LH release. They observed that progestogen treatment, prior to GnRH, changed the pattern of LH release and possibly thereby improved luteal function. Lewis *et al.* (1983) confirmed the effect on LH release, but not on luteal function, in postpartum, anoestrous ewes.

Can the reduced lifespan or lowered secretory activity of the first CL be attributed to sub-optimal luteotropic support?

Prolongation of luteal lifespan and stimulation of P4 secretion

In discussing the problem of luteal insufficiency during the postpartum period, little distinction has been made between luteal secretion and lifespan of the CL, although it has been suggested that different mechanisms might be involved. Schomberg *et al.* (1967) believed that from a biological viewpoint, prolongation of the functional life of the CL may be more important than increasing its secretory activity. Until recently, both the luteotropic stimulus responsible for increased secretory activity and that prolonging lifespan have been assumed to be vested in one hormone (Schomberg *et al.*, 1967). There is an extensive body of evidence that the bovine CL is dependent primarily on LH to maintain secretion of P4 (Hansel & Echternkamp, 1972). Whether CL showing weak secretory activity are more likely to be overcome by marginal levels of luteolytic agents or fail to recognise LH, as could occur during lactation, has not been elucidated.

Niswender *et al.* (1986) have reviewed the roles of LH and prolactin in regulating luteal function in ewes; their research led them to conclude that LH is the primary luteotropic hormone with no role for prolactin being detectable. FSH receptors have been detected in luteal cells of cows Manns *et al.* (1984); and Walters *et al.* (1984) suggested that since 97% of separate FSH pulses during the mid-luteal phase in the cow were associated with P4 pulses, it is possible that FSH could be the principal hormone that stimulates P4 secretion. They maintained that this did not exclude a luteotropic action of LH (Hoffmann *et al.*, 1974) in addition to a stimulatory action of FSH on release of P4 in the cow. Baird *et al.* (1976) believed that a certain minimal basal level of LH is necessary for P4 secretion.

There was even the possibility of an interaction of oxytocin (from the CL) and LH in regulating P4 synthesis, with oxytocin increasing the reponse to LH (Pekala *et al.*, 1983). In ewes it appeared that disruption of luteotropic support from the pituitary during formation of the CL (± day 1 of the cycle) resulted in premature luteal regression (Kaltenbach *et al.*, 1968; Mallory *et al.*, 1986) or reduced CL function (Mallory *et al.*, 1986). However, once the CL had formed, alterations to the luteotropic signal appeared to have had little effect on P4 secretion, providing LH was not removed completely (Niswender *et al.*, 1986). The foregoing was supported by the demonstration of large numbers of LH receptors on steroidogenically active luteal cells, which fell precipitously at the time of luteolysis (Dickman *et al.*, 1978; Rao *et al.*, 1979; Spicer *et al.*, 1981).

Deficiencies in luteotropic support

The short-lived CL

The possibility that the luteotropic stimulus (Component 12) may be insufficient during early postpartum to maintain the CL for its normal lifespan has been the subject of several studies. Peters & Lamming (1984) and Rutter & Randel (1984) supported the conclusion that concentrations of LH were in fact adequate during the short P4 rise that often preceded the first oestrus, and Copelin *et al.* (1987) and Garverick *et al.* (1988) obtained results to confirm this. In contrast, O'Shea *et al.* (1984) have suggested that besides defects of the follicle which ovulated in response to GnRH, sub-optimal luteotropic support in the post-ovulatory period could have accounted for subnormal luteal-cell numbers and sizes which were characteristic of the CL induced by GnRH. Atkinson & Williamson (1985) have concluded that lack of luteotropic support may be implicated in the short-lived CL induced by ram introduction. Kesler *et al.* (1981) do not agree that luteotropin could be lacking at such times, and Duby *et al.* (1985) favour Kesler's viewpoint. Similarly, in cattle, mean concentrations, as well as frequency and amplitude of pulses of LH were similar for short-lived and normal CL (Ramirez-Godinez *et al.*, 1982b; Rutter *et al.*, 1985; Garcia-Winder *et al.*, 1986; Garverick *et al.*, 1988), while a chronic increase in secretion of LH via continuous infusion of GnRH did not sustain the activity of GnRH-induced CL (D'Occhio *et al.*, 1989). However, elevation of LH concentrations by injection of microencapsulated GnRH did promote normal luteal function (Roberts *et al.*, 1989). When Carruthers *et al.* (1986) injected hCG after treatment with GnRH, the incidence of short cycles was in fact increased.

The inadequate CL

From the foregoing presentation, it appears reasonable to conclude that short-lived CL are not the result of a deficiency in luteotropic support. The question then arises as to whether a similar conclusion would be valid for the inadequate CL (Component 15). Grobbelaar *et al.*, (1989) attempted to determine whether additional luteotropic support would improve the secretory activity of GnRH-induced CL. They administered PMSG for up to 17 days, commencing at the time of GnRH infusion, and this appeared to elicit a supraphysiological response with plasma concentrations of P4 rising to almost twice the peak concentrations seen in normally cycling ewes. These results are somewhat similar to those obtained when GnRH was administered in small doses over eight days (McLeod *et al.*, 1982a); and O'Shea *et al.* (1984) concluded that this could be taken as demonstration of a lack of luteotropic support at such times.

If it is accepted that luteotropin may not be sufficient to realise normal luteal function (Component 12), then the problem becomes one of just when endogenous supplies need to be augmented. In cyclic cows, Walters & Schallenberger (1984) could detect no pulsatile LH for 6 to 12 h after the LH surge with basal levels falling even below those recorded before the preovulatory LH release. In contrast, FSH showed a distinct increase in basal concentrations 4 to 12 h after the LH surge. By limiting the administration of PMSG to only three days, Pearson & Lishman (1989) demonstrated that a luteotropic stimulus provided early in the induced cycle (days 3–5) improved luteal function to a greater extent than when PMSG was provided after day 5.

Improving pregnancy rates through stimulation of CL function

When the possibility of inadequate luteal function, shortly after mating, has been related to pregnancy rates, the results have at times been encouraging, but often inconsistent. Thus, injection of hCG after ovulation improved luteal function in ewes (Gamboni *et al.*, 1984) and cows (Breuel *et al.*, 1989), but the pregnancy rate was not always improved (Breuel *et al.*, 1990).

The possibility remains that inadequate luteal function, particularly, soon after resumption of cyclic ovarian activity, could reduce pregnancy rates.

Provision of additional luteotropin to inhibit luteolysis?

The possibility that luteal lifespan may be extended by negating the luteolytic action of $PGF_2\alpha$ (to be reviewed later) via provision of extra luteotropin also has been investigated.

It was proposed that the short-lived CL might also be particularly sensitive to $PGF_2\alpha$. This hypothesis was based partly on the observation that in the normal cycling ewe large luteal cells possessed a higher number of receptors for this luteolysin than small luteal cells did (Fitz *et al.*, 1982). Similarly, in cows it has been shown that the large luteal cells are the target of the luteolytic effect of $PGF_2\alpha$ (Alila *et al.*, 1988). An increased sensitivity to $PGF_2\alpha$ would presuppose a greater proportion of large luteal cells in the short-lived CL. This was supported by the demonstration that in ewes expected to exhibit short cycles, the CL had a higher number of large luteal cells having greater numbers of receptors for $PGF_2\alpha$ than CL anticipated to have a normal lifespan (Braden & Niswender, 1985). However, the proportion of large luteal cells was apparently not increased when the CL were short-lived in other studies in ewes (O'Shea *et al.*, 1984; Braden *et al.*, 1989b) and in cows (Rutter *et al.*, 1985). From their research, Garcia-Winder *et al.* (1986), Garverick *et al.* (1988), and Copelin *et al.* (1986; 1988) concluded that hypersensitivity to uterine luteolysin was not a cause of short-lived CL in cows. In contrast, Hunter *et al.* (1988) maintained that the short life-cycle CL in prepuberal and in anoestrous ewes was due to an increased sensitivity to tonic release of $PGF_2\alpha$. Hunter *et al.* (1988) based their opinion on the finding that although GnRH-induced CL were maintained after removal of the uterus, the secretory activity appeared limited. They suggested that inadequate vascularization of the CL could increase the vulnerability to the vasoconstrictive effects of $PGF_2\alpha$ released on about day 4 after ovulation (Hunter *et al.*, 1988).

Schirar *et al.* (1989) concluded that the first luteal tissue formed in postpartum ewes possessed reduced functional capacity (Component 15), and Lewis & Bolt (1987) showed that the uterus suppressed function of GnRH-induced CL in postpartum ewes. In attempting to overcome the luteolytic action of $PGF_2\alpha$, Bolt (1979) demonstrated that multiple injections of hCG could inhibit the luteolytic effect of $PGF_2\alpha$ and thereby postpone luteal regression in the cycling ewe. Similar effects of hCG (Donaldson & Hansel, 1960; Wiltbank *et al.*, 1961; Seguin *et al.*, 1977) and of LH (Karsch *et al.*, 1971) in counteracting natural luteolysis have been shown in the cow. Although administration of hCG on days 9 and 10 of the cycle did increase plasma concentrations of P4, this did not overcome the regressive action of a subminimal dose (10 mg) of $PGF_2\alpha$ (Copelin *et al.*, 1986). These results agree with those of Sasser *et al.* (1977) for the ewe and of Gonzalez-Mencio *et al.* (1977) for the cow, where constant infusion of LH did not inhibit the luteolytic effect of $PGF_2\alpha$. The possibility that the CL was desensitized by the hCG treatment was excluded (Litch & Condon, 1988).

LH receptors within the CL and luteal function

It is possible that the induced CL may be unable to respond to the luteotropin (Component 13) even though concentrations of LH may be adequate to support normal luteal function. Copelin *et al.* (1987) have evaluated the evidence that has been obtained in favour of this proposal. This hypothesis is in accordance with the suggestion (McNeilly *et al.*, 1981) that the increased numbers of receptors for LH, on the granulosal cells of the bovine CL, could be a major factor in regulation of secretion of P4 by the ensuing CL (Dickman *et al.*, 1978).

Kesler *et al.* (1981) have shown that the GnRH-induced ovulations which did not develop beyond day five post-ovulation also did not respond to LH *in vitro*. McNeilly *et al.* (1981) have confirmed this by demonstrating that in ewes the GnRH-induced, subnormal CL were less responsive to LH *in vitro*. McNeilly *et al.* (1981) and O'Shea *et al.* (1984) concluded that such CL were not less responsive to LH than normal CL. The latter implies that LH receptors are not deficient in the inadequate CL, and Rutter *et al.* (1985), Smith *et al.* (1986) and Braden *et al.* (1989b) have provided evidence to support this contention.

Both McNeilly *et al.* (1981) and Hunter *et al.* (1988) concluded that lack of gonadotropin receptors was probably not a fundamental cause of premature regression of the short-lived CL. In contrast, there is evidence for a lack of LH receptors. This may be deduced from the finding that where postpartum cows were primed with Norgestomet, the concentrations of LH receptors were increased in both the granulosa and thecal cells of the largest follicle compared to the largest follicle of non-primed cows (Inskeep *et al.*, 1988). Such acquisition of increased numbers of LH receptors and greater secretion of E2 were critical to preovulatory dominance of a single follicle (Spicer *et al.*, 1986b). However, McNeilly *et al.* (1981) believed that there may not be a simple relationship between the binding of LH to luteal cells and the secretion of P4. Consequently, the failure of the short-lived CL to recognise LH was not a factor that caused early luteal regression (Rutter *et al.*, 1985). Whether such a mechanism could account for subnormal CL function is not clear.

The role of the uterus and of $PGF_2\alpha$ in subnormal luteal function

Presence of $PGF_2\alpha$ during early lactation

The possibility that premature regression of CL, which appeared to function normally for approximately 7 days after induced ovulation, was due to the luteolytic effect of $PGF_2\alpha$, (Components 19 and 20; Odde *et al.*, 1980; 1981) needs consideration. Lewis *et al.* (1981) investigated this aspect but measured $PGF_2\alpha$ only on day 7 after administration, and also excluded those ewes which exhibited short cycles. Nevertheless, they recorded lower concentrations of $PGF_2\alpha$ in ewes treated with P4, and such ewes commonly show normal luteal function. Subsequently, increased jugular plasma concentrations of prostaglandin metabolite (PGFM) during the early postpartum interval have been demonstrated in milked dairy cows (Thatcher *et al.*, 1980) and suckled beef cows (Troxel *et al.*, 1984).

Oxytocin and the release of $PGF_2\alpha$

Oxytocin administration during the early part of the cycle has reduced luteal lifespan in cycling cows (Armstrong & Hansel, 1959; Hansel & Wagner, 1960; Milvae & Hansel, 1980) and it was suggested that oxytocin functioned via elevated levels of uterine $PGF_2\alpha$ (Schomberg *et al.*, 1967; Newcomb *et al.*, 1977; Milvae & Hansel, 1980) in a manner similar to that of an IUD inserted early in the cycle in ewes (Pexton *et al.*, 1975). This would account for the effect of suckling via the pituitary release of oxytocin (Troxel & Kesler, 1984a). Suckling has not been clearly shown to affect luteal lifespan, although increasing the number of lambs suckled did reduce plasma concentrations of P4

(Lewis *et al.*, 1981) and also the proportion of ewes that formed CL in response to GnRH (Lewis & Bolt, 1987).

When the CL releases oxytocin (Component 17), it may cause $PGF_2\alpha$ release (Component 18) which in turn, regresses the CL (McCracken *et al.*, 1984; Schams *et al.*, 1985). An imbalance in luteal levels of oxytocin or a change in the response of the uterus to basal levels of oxytocin was proposed (Hunter *et al.*, 1988) as a cause of the eventual demise of the CL. Schirar *et al.* (1989) did not agree with such a simple explanation and proposed that early release of $PGF_2\alpha$ may have been only one of several possible factors which may control the lifespan of the first CL after lambing. This is supported by the finding that in anoestrous ewes, when the uterine horn adjacent to the induced CL was removed 3 days before ovulation, this induced more normal synthesis of P4 than removal of an adjacent horn 2 days after the bolus injection of GnRH (Day *et al.*, 1989).

Cooper & Inskeep (1989), using blood from the posterior vena cava, could not demonstrate a close association between pulses of oxytocin and $PGF_2\alpha$ in lactating beef cows induced to ovulate with hCG, but concentrations of PGFM were highly correlated with oxytocin concentrations, both at the same hour ($r = 0{,}69$) and one hour earlier ($r = 0{,}83$). Peter *et al.* (1989) were able to detect parallelism between concentrations of PGFM and oxytocin in early postpartum dairy cows.

Negating the effect of $PGF_2\alpha$

The possibility of involvement of $PGF_2\alpha$ in premature regression of the CL (Components 19 and 20) is supported by the observation that treatment with substances which block the action of $PGF_2\alpha$ increased the functional lifespan of CL destined to be short-lived in beef cows (Troxel & Kesler, 1984a), in goats (Battye *et al.*, 1988) and in dairy cows (Dobson *et al.*, 1987). Further evidence for the involvement of $PGF_2\alpha$ comes from the demonstration that active immunization of early-weaned, anoestrous beef cows against $PGF_2\alpha$ extended the lifespan of the first CL (Copelin *et al.*, 1989b).

Removing the source of $PGF_2\alpha$

Strong evidence that a premature release of $PGF_2\alpha$ from the uterus (Component 20) could be reponsible for the early demise of the first induced CL in beef cows has been provided by Copelin *et al.* (1988). They demonstrated that CL formed after early weaning of calves did not possess an inherently short lifespan because removal of the uterus did not result in premature luteolysis. Furthermore, Wright *et al.* (1988) showed that the previously gravid uterus had to be present to induce premature regression of the first CL, subsequent to induced abortion in heifers. Similar results had been reported following formation of the first luteal tissue in prepuberal lambs (Keisler, 1983) and following GnRH-induced ovulation in anoestrous (Southee *et al.*, 1985; Hunter *et al.*, 1988) and lactating ewes (Lewis & Bolt, 1987).

Demonstrating the release of $PGF_2\alpha$

Neither Troxel & Kesler (1984b) nor Garcia-Winder *et al.* (1986) could show that the metabolite of $PGF_2\alpha$ (15keto–13,14 dihydro $PGF_2\alpha$) increased in cows having short-lived CL, but this has been demonstrated in superovulated goats (Battye *et al.*, 1988) and in dairy cows (Peter *et al.*, 1989).

An important piece of new evidence is the finding that in postpartum beef cows induced to ovulate with hCG and in which the CL were short-lived, an early release of $PGF_2\alpha$ could be demonstrated in plasma from the inferior vena cava (Cooper & Inskeep, 1989). In the same cows, PGFM and oxytocin did not show early rises. Similarly, in dairy cows which ovulated spontaneously, increased concentrations of PGFM were detected early in the cycle when the CL were short-lived (Peter *et al.*, 1989).

How does progesterone priming result in CL with a normal lifespan?

In seeking an explanation for the frequent failure of corpora lutea, when ovulation is induced through the use of exogenous hormones, it may be helpful to study the changes that occur when cycles commenced naturally. Both at puberty in cattle (Berardinelli *et al.*, 1979) and sheep (Berardinelli *et al.*, 1980) and following parturition in both dairy (Morrow *et al.*, 1966) and beef cows (Short *et al.*, 1972; Ward *et al.*, 1979), the first full-length oestrous cycle that occurred spontaneously was often preceded by a period of several days during which plasma P4 levels were increased (see Lauderdale, 1986 for a more extensive review). Furthermore, when exogenous P4 was administered for a number of days, this promoted the restoration of full luteal activity in postpartum cows induced to ovulate with GnRH or hCG (Pratt *et al.*, 1982; Sheffel *et al.*, 1982).

Such results support the generally accepted hypothesis that the short-lived CL provides a minimum critical concentration of P4 to ensure normal function of the subsequent CL in ewes (Pearce, 1985) and cows (Lamming *et al.*, 1981).

Lishman *et al.* (1979) proposed that future work should consider the mechanisms by which P4, prior to the first oestrus, affected follicular development. McLeod & Haresign (1984b) have discussed how P4 priming achieved normal luteal function in the seasonally anoestrous ewe given GnRH, and Smith *et al.* (1983) have considered the postpartum cow.

Delaying the LH surge

Although P4 priming has been observed to delay the LH surge (Martin *et al.*, 1980; McLeod *et al.*, 1982b; Pearce *et al.*, 1985; Pearce *et al.*, 1987; Southee *et al.*, 1988) the prolongation of exposure of follicles to gonadotropin is probably not the reason why P4 priming promotes subsequent luteal function (McLeod & Haresign, 1984). Wright *et al.* (1984) agreed with this conclusion, suggesting that P4 has effects on the hypothalamo-pituitary axis not mediated simply by delaying the LH surge. In the opinion of McLeod & Haresign (1984) the most likely explanation is that P4 had a direct effect on the ovulatory follicle (Component 16), perhaps having altered its ability to respond to changes in tonic gonadotropins or in some way changed the pattern of growth, thereby ensuring that all antral follicles were at the appropriate stage of development o respond to the increase in LH episodes (McLeod *et al.*, 982b; Hunter & Southee, 1987).

direct effect of P4 on the follicle

he possibility that P4 priming may have been acting via a irect effect on the follicle, as proposed by McLeod & Iaresign (1984) and Hunter *et al.* (1986), was supported by he finding that as little as two days of exposure to P4 acilitated full luteal function. In contrast, the final stages of ollicular growth were said to require 4 – 5 days Wodzicka-Tomaszewska *et al.*, 1974; Legan & Karsch, 979). Similarly, Pearce *et al.* (1987) suggested that retreatment with P4 may have induced changes within the eveloping follicle to convert short-lived CL induced by am introduction to CL having a normal lifespan. The eneficial effects of P4 were produced by a minimum of 30 priming and were found to persist for at least 5 days after ermination of the P4 administration (Pearce *et al.*, 1985; 987).

Where multiple low doses of GnRH (250 ng every 2 h for 8 h) were used to induce ovulation in anoestrous ewes, nly a transient rise in plasma P4 (levels rose to a peak of ± 0,5 ng/ml over about 4 days) was recorded where P4 riming had not been applied. In contrast, P4 priming esulted in a normal luteal lifespan (Southee *et al.*, 1988). A imilar positive effect of pretreatment with P4 on lifespan of L, induced by weaning of the calf (Ramirez-Godinez *et l.*, 1981) or by exogenous gonadotropin (Pratt *et al.*, 1982; heffel *et al.*, 1982) on level of function of the CL in noestrous postpartum cows, has been reported.

The hypothesis incorporating a direct effect of P4 on the ollicle was supported by Hunter & Southee (1987). They oted a significant increase in the concentration of P4 in ollicles < 4 mm in diameter; during *in vitro* incubation the irgest follicle from P4-primed anoestrous ewes secreted ess E2, but bound more hCG to the thecal cells. Their esults supported the hypothesis that P4 retarded the rate of ollicle development and thereby secretion rate of E2. This in accordance with the finding that the LH surge is elayed in P4-primed anoestrous ewes treated with GnRH McLeod *et al.*, 1982; Hunter *et al.*, 1986). It appeared that 4 priming synchronized the random anoestrous pattern of ollicular development (Legan *et al.*, 1985), so that the ersistence of a single follicle was encouraged (Garcia-Vinder *et al.*, 1987). Interestingly, both the size and osition of the largest follicle were altered, so that fewer F1 ollicles were imbedded within the ovary in Norgestomet-retreated cows (Garcia-Winder *et al.*, 1987).

ncreased LH receptors

arcia-Winder *et al.* (1987) speculated that P4 may increase eceptors for LH in the largest follicle (Component 10), and nskeep *et al.* (1988) have confirmed this; whereas Jones *et l.* (1989) proposed that P4 may be involved in the egulation of LH receptors during development of the CL. upport for an effect on the responsiveness of the follicle Martin *et al.*, 1984; McLeod & Haresign, 1984; Hunter *et l.*, 1986) can be obtained from Walters & Schallenberger 1984). They accounted for the increased E2 pulse mplitude before the LH surge in cows (Chenault *et al.*, 1975; Schams *et al.*, 1977) as having been due to an increase in the number of LH receptors in the pre-ovulatory follicle at that time (Walters *et al.*, 1982; Staigmiller *et al.*, 1982).

Increased blood flow

Brown & Mattner (1984) have proposed a further mechanism by which P4 priming, prior to introduction of rams or treatment with GnRH, might have led to normal luteal function. They argued that P4 could have promoted the response of ovarian vasculature to endogenous E2. The marked increase in blood flow to the ovary could then have augmented the supply of nutrients to the developing CL. Continuation of this work has shown that administration of P4 before onset of two-hourly GnRH pulse injections, while inducing normal oestrous cycles in anoestrous ewes, also significantly increased the mean capillary blood flow in the ovaries (Brown *et al.*, 1988). They proposed that rate of capillary blood flow in the ovaries, near the time of ovulation, may have been a critical factor in normal development and maturation of ovulatory follicles which then became fully functional CL.

Preventing premature release of $PGF_{2}\alpha$

Recently, a further mechanism by which P4 pretreatment ensures a normal lifespan of the CL, induced in an anoestrous ewe, has been proposed. This suggestion incorporated a direct action on the uterus to lower concentration of oxytocin and to prevent premature release of $PGF_{2}\alpha$ (Hunter *et al.*, 1989). In postpartum cows induced to ovulate via injection of hCG, Cooper & Inskeep (1989) demonstrated that pretreatment with Norgestomet implants reduced the concentrations of $PGF_{2}\alpha$ from days 4 through 10 postovulation (Component 24). The latter appeared to prematurely regress the CL in those cows not primed with P4.

Although Cooper & Inskeep (1989) found no effect of Norgestomet on concentrations of oxytocin or of PGFM, their results for $PGF_{2}\alpha$ are supported by the demonstration of higher *in vitro* production of $PGF_{2}\alpha$ by day 5 endometria obtained from cows expected to have short-lived CL compared to cows pretreated with Norgestomet (Zollers *et al.*, 1989). Using this experimental model, Hu *et al.* (1989) found that the CL destined to be short-lived secreted higher levels of $PGF_{2}\alpha$ than CL expected to function normally. Furthermore, the first postpartum CL in ewes was found to have 2,4 times as many receptors for $PGF_{2}\alpha$ as normal CL (Braden & Niswender, 1986).

A question which still needs to be answered is what role, if any, increased concentrations of E2 play, within the preovulatory follicle, in preventing premature release of $PGF_{2}\alpha$.

General comments

The research which has been conducted to investigate the possible mechanisms by which luteal lifespan or function could be reduced has yielded results which point to only a few deficiencies. These are summarized in Figure 2.

Based on available findings it would seem that premature demise of the first CL (formed at puberty, after parturition

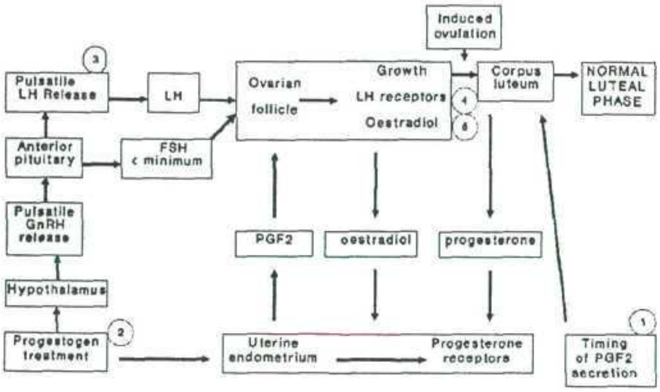

Figure 2 Mechanisms which have been demonstrated to contribute to deficient luteal function in lactating ewes and cows.

or at the onset of the new breeding season) is the result of early release of $PGF_2\alpha$ by the uterus (Figure 2, Component 1). Although pretreatment with P4 is an effective countermeasure (Component 2), the processes involved remain obscure.

In contrast, the explanation for subnormal luteal function of approximately normal duration appears more complex. Improper or incomplete maturation of the preovulatory follicle remains implicated in both the formation of the CL and the preparation of the uterus for its normal role. Although an increased frequency of LH pulses (Component 3) appears to be vital in promoting follicular maturation, the interrelationship between the interval between parturition and first oestrus in beef cows maintained under ranching conditions, varies considerably. There is also much individual variation in response to stimuli aimed at reducing this open period. Consequently, it would be advantageous if some objective indication of just when a cow is ready to respond to stimuli could be obtained. From the published research it would appear the the pulsatile release of LH (Nett *et al.*, 1987) is the indicator sought after. However, this is completely impractical to measure on the farm, and because of the interdependence of the pituitary and the follicles within the ovary (Kesler *et al.*, 1977; Lishman *et al.*, 1979; Troxel *et al.*, 1980; Irvin *et al.*, 1981; Smith *et al.*, 1983), ovarian examination per rectum appears to be the only practical tool presently available. However, more widespread application of ovarian examination by ultrasonography holds considerable promise for the future.

References

AINSWORTH, L., LACHANCE, R. & LABRIE, F., 1980. Effect of GnRH-induced endogenous luteinizing hormone release and exogenous progestogen treatment on ovarian activity in the post-partum ewe. *J. Anim. Sci.* 54, 998.

ALILA, H.W., DOWD, J.P., CORRADINO, R.A., HARRIS, W.V. & HANSEL, W., 1988. Control of progesterone production in small and large bovine luteal cells separated by flow cytometry. *J. Reprod. Fert.* 82, 645.

ALILA, H.W. & HANSEL, W., 1984. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. *Biol. Reprod.* 31, 1015.

ARMSTRONG, D.T. & HANSEL, W., 1959. Alteration of the bovine estrous cycle with oxytocin. *J. Dairy Sci.* 42, 533.

ATKINSON, S. & WILLIAMSON, P., 1985. Ram-induced growth of ovarian follicles and gonadotrophin inhibition in anoestrous ewes. *J. Reprod. Fert.* 73, 185.

BAIRD, D.T., 1978. Pulsatile secretion of LH and ovarian estradiol during the follicular phase of the sheep estrous cycle. *Biol. Reprod.* 18, 359.

BAIRD, D.T., SWANSTON, I., & SCARRAMUZZI, R.J., 1976. Pulsatile release of LH and secretion of ovarian steroids in sheep during the luteal phase of the estrous cycle. *Endocrinology* 98, 1490.

BATTYE, K.M., FAIRCLOUGH, R.J., CAMERON, A.W. & TROUNSON, A.O., 1988. Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goat (*Capra hircus*). *J. Reprod. Fert.* 84, 425.

BERARDINELLI, J.G., DAILEY, R.A., BUTCHER, R.L. & INSKEEP, E.K., 1980. Source of circulating progesterone in prepubertal ewes. *Biol. Reprod.* 22, 233.

BOLT, D.J., 1979. Reduction by human chorionic gonadotropin of the luteolytic effect of prostaglandin $F_2\alpha$ in ewes. *Prostaglandins* 18, 387.

BRADEN, T.D., KING, M.E., ODDE, K.G. & NISWENDER, G.D., 1989. Development of preovulatory follicles expected to form shortlived corpora lutea in beef cows. *J. Reprod. Fert.* 85, 97.

BRADEN, T.D. & NISWENDER, G.D., 1985. Differential loss of the two steroidogenic cell types in the bovine corpus luteum following prostaglandin $(PG)F_2\alpha$. *Biol. Reprod.* 32 (Suppl. 1), 44.

BRADEN, T.D. & NISWENDER, G.D., 1986. Progesterone production, LH-receptors and $PGF_2\alpha$-receptors from first postpartum corpora lutea of ewes. *J. Anim. Sci.* 63 (Suppl. 1), 350.

BRADEN, T.D., SAWYER, H.R. & NISWENDER, G.D., 1989. Functional and morphological characteristics of the first corpus luteum formed after parturition in ewes. *J. Reprod. Fert.* 86, 525.

BREUEL, K.F., SPITZER, J.C. & HENRICKS, D.M., 1989. Systemic progesterone concentration following human chorionic gonadotropin administration at various times during the estrous cycle in beef heifers. *J. Anim. Sci.* 67, 1564.

BREUEL, K.F., SPITZER, J.C., THOMPSON, C.E. & BREUEL, J.F., 1990. First-service pregnancy rate in beef heifers as influenced by human chorionic gonadotropin administration before and/or after breeding. *Theriogenology* 34, 139.

BRITT, J.H., KISER, T.E., SEGUIN, B.E., HAFS, H.D., OXENDER, W.D. & RITCHIE, B.D., 1975. Fertility after GnRH and $PGF_2\alpha$ in suckling cows. *J. Anim. Sci.* 43, 345 (Abstr.).

BROWN, B.W. & MATTNER, P.E., 1984. Effects of ovarian hormones on ovarian capillary blood flow in anoestrous ewes. *Aust. J. Biol. Sci.* 37, 389.

BROWN, B.W., COGNIE, Y., CHEMINEAU, P., POULIN, N. & SALAMA, O.A., 1988. Ovarian capillary blood flow in seasonally anoestrous ewes induced to ovulate by treatment with GnRH. *J. Reprod. Fert.* 84, 653.

BULMANN, D.C. & LAMMING, G.E., 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. *J. Reprod, Fert.* 54, 447.

BUTTERFIELD, W.M., 1986. The progesterone assay of milk as an indicator of the reproductive status of the dairy herd. M.Sc.(Agric) thesis. University of Natal.

CARRUTHERS, T.D., MANNS, J.G. & RUTTER, L.M., 1986. Failure of human chorionic gonadotropin injection to sustain gonadotropin-releasing hormone-induced corpora lutea in postpartum beef cows. *Biol. Reprod.* 35, 846.

CARTER, M.L., DIERSCHKE, D.L., RUTLEDGE, J.J. & HAUSER, R.E., 1980. Effect of gonadotropin-releasing hormone and calf removal on pituitary-ovarian function and reproduction performance in postpartum beef cows. *J. Anim. Sci.* 51, 903.

CHANNING, C.P., 1970. Influence of the *in vivo* and *in vitro* hormonal environment upon luteinization of granulosa cells in tissue culture. *Recent Progr. Horm. Res.* 26, 589.

CHENAULT, J.R., THATCHER, W.W., KALRA, P.S., ABRAMS, R.M. & WILCOX, C.J., 1975. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. *J. Dairy Sci.* 58, 709.

CLARKE, I.J. & CUMMINS, J.T., 1985. GnRH pulse frequency determines LH pulse amplitude by altering the amount of releasable LH in the pituitary gland of ewes. *J. Reprod. Fert.* 73, 425.

CLARKE, I.J., WRIGHT, P.J., CHAMLEY, W.A. & BURMAN, K., 1984. Differences in the reproductive endocrine status of ewes in the early post-partum period and during seasonal anoestrus. *J. Reprod. Fert.* 70, 591.

COOPER, D.A. & INSKEEP, E.K., 1989. Prostaglandin $F_2\alpha$ (PGF) and oxytocin during premature regression of corpora lutea in the postpartum beef cow. *Anim. Sci.* 67 (Suppl. 1), 337 (Abstr.).

COPELIN, J.P., SMITH, M.F., GARVERICK, H.A. & YOUNGQUIST, R.S., 1987. Effect of the uterus on subnormal luteal function in anoestrous beef cows. *J. Anim. Sci.* 64, 1506.

COPELIN, J.P., SMITH, M.F., GAVERICK, H.A. & YOUNGQUIST, R.S., 1989a. Effect of termination of pregnancy or long-term progesterone exposure on subsequent estrous cycle length and concentration of progesterone in plasma of heifers. *J. Anim. Sci.* 67, 1552.

COPELIN, J.P., SMITH, M.F., KEISLER, D.H. & GARVERICK, H.A., 1989b. Active immunization of prepartum and postpartum cows against prostaglandin $F_2\alpha$ ($PGF_2\alpha$): Effect on lifespan and progesterone secretion of short-lived corpora lutea. *Anim. Sci.* 67 (Suppl. 2), 140 (Abstr.).

COPELIN, J.P., SMITH, M.F., GARVERICK, H.A., YOUNQUIST, R.S., McVEY, W.R. & INSKEEP, E.K., 1988. Responsiveness of bovine corpora lutea to prostaglandin $F_2\alpha$: Comparison of corpora lutea anticipated to have short or normal lifespans. *J. Anim. Sci.* 66, 1236.

CORAH, L.R., QUEALY, A.P., DUNN, T.G. & KALTENBACH, C.C., 1974. Prepartum and postpartum levels of progesterone and estradiol in beef heifers fed two levels of energy. *J. Anim. Sci.* 39, 380.

CRIGHTON, D.B., FOSTER, J.P., HARESIGN, W. & SCOTT, S.A., 1975. Plasma LH and progesterone levels after single or multiple injections of synthetic LH-RH in anoestrous ewes and comparison with levels during the oestrous cycle. *J. Reprod. Fert.* 44, 121.

CROWDER, M.E., GILLES, P.A., TAMANINI, C., MOSS, G.E. & NETT, T.M., 1982. Pituitary content of gonadotropins and GnRH-receptors in pregnant, postpartum and steroid-treated OVX ewes. *J. Anim. Sci.* 54, 1235.

CRUZ, L.C. & KESLER, D.J., 1988. Relationship of GnRH-induced LH and FSH profiles on the ovulation response and corpus luteum function of postpartum suckled beef cows. *11th Int. Congr. Reprod. A.I.* Dublin, Ireland, 18.

DAY, M.L., DYER, R.M., WILSON, G.W. & POPE, W.F., 1990. Influence of estradiol on duration of anestrus and incidence of short estrous cycles in postpartum cows. *Dom. Anim. Endocr.* 7, 19.

DAY, M.L., HU, Y., NEPHEW, K.P. & POPE, W.P., 1989. Role of the uterus in formation of the short-lived corpus luteum in anestrous ewes. *J. Anim. Sci.* 67 (Suppl. 1), 336 (Abstr.).

DEAN, H.W., HAY, M.F., MOOR, R.M., ROWSON, L.E.A. & SHORT, R.V., 1966. The corpus luteum of the sheep: relationships between morphology and function during the oestrous cycle. *Acta. Endocrinol.* 51, 245.

DIEKMAN, M.A., O'CALLAGHAN, P., NETT, T.M. & NISWENDER, G.D., 1978. Effect of prostaglandin $F_2\alpha$ on the number of LH receptors in ovine corpora lutea. *Biol. Reprod.* 19, 1010.

iZEREGA, G.S. & HODGEN, G.D., 1981. Luteal phase dysfunction infertility: A sequel to aberrant folliculogenesis. *Fertil. Steril.* 35, 489.

D'OCCHIO, M.J.D., GIFFORD, D.R., EARL, C.R., WEATHERLY, T. & VON RECHENBERG, W., 1989. Pituitary and ovarian responses of post-partum acyclic beef cows to continuous long-term GnRH and GnRH agonist treatment. *J. Reprod. Fert.* 85, 495.

DOBSON, H., ALAM, M.G.S. & KANCHEV, L.N., 1987. Effect of betamethasone treatment on luteal lifespan and the LH response to GnRH in dairy cows. *J. Reprod. Fert.* 80, 25.

DONALDSON, L.E. & HANSEL, W., 1965. Prolongation of life span of the bovine corpus luteum by single injection of bovine luteinizing hormone. *J. Dairy Sci.* 48, 903.

DRIANCOURT, M.A. & FRY, R.C., 1988. Differentiation of ovulatory follicles in sheep. 18th Biennial. Symp. Anim. Reprod. *J. Anim. Sci.* 66 (Suppl. 2), 9.

DUBY, R.T., BROWNING, T., CAREY, D. & BLACK, D.L., 1985. Progesterone synthesis and histology of postpartum corpora lutea. *Theriogenology* 23, 619.

DYER, R.M., WILSON, G.W. & DAY, M.L., 1988. Effect of chronic administration of estradiol on the interval from calving to first heat in beef cows. In *Beef cattle. Research Report, Ohio Agric Res. Center.* Wooster, Ohio State Univ., 55.

NGLAND, B.G., KARAVULAS, H.J., HAUSER, E.R & CASIDA, L.E., 1973. Ovarian follicular estrogens in Angus heifers. *J. Anim. Sci.* 37, 1176.

FARIN, C.E., SCHWALL, R.H., GAMBONI, F., SAWYER, H.R. & NISWENDER, G.D., 1985. Effect of LH and hCG on size distributions of luteal cells in the cycling ewe. *Biol. Reprod.* 32 (Suppl. 1), 13 (Abstr.).

FARIN, C.E., MOELLER, C.L., MAYAN, H., GAMBONI, F., SAWYER, H.R. & NISWENDER, G.D., 1988. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the bovine corpus luteum. *Biol. Reprod.* 38, 413.

FITZ, T.A., MAYAN, M.H., SAWYER, H.R. & NISWENDER, G.D., 1982. Characterization of two steroidogenic cell types in the bovine corpus luteum. *Biol. Reprod.* 27, 703.

FLETCHER, I.C., LISHMAN, A.W., THRING, B. & HOLMES, J.A., 1980. Plasma progesterone levels in lactating ewes after hormone induced ovulation during the non-breeding season. *S. Afr. J. Anim. Sci,* 10, 151.

FLOOD, P.F., MANNS, J.G., HUMPHREY, W.D. & MAPLETOFT, R.J., 1979. The first corpus luteum of the post-partum beef cow. *J. Reprod. Fert.* 59, 252.

FOLMAN, Y., ROSENBERG, M., HERZ, Z. & DAVIDSON, M., 1973. The relationship between plasma progesterone concentration and conception in postpartum dairy cows maintained on two levels of nutrition. *J. Reprod. Fert.* 34, 267.

FONESCA, F.A., KOSUGIYAMA, M., BRITT, J.M. & RITCHIE, H.D., 1977. Ovulation, estrous cycles and endocrine responses after GnRH in suckled cows. *J. Anim. Sci.* 45 (Suppl. 1), 158.

FORTUNE, J.E. & QUIRKE, S.M. , 1988. Regulation of steroidogenesis in bovine preovulatory follicles. 18th Biennial Symp. Anim. Reprod. *J. Anim. Sci.* 66 (Suppl. 2), 1.

FOSTER, J.P. & CRIGHTON, D.B., 1974. Luteinizing hormone (LH) release after single injections of a synthetic LH-releasing hormone (LH-RH) in the ewe at three different reproductive stages and comparison with natural LH release at oestrus. *Theringenology* 2, 87.

FOSTER, D.L. & OLSTER, D.H., 1985. Effect of restricted nutrition on puberty in the lamb: Patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. *Endocrinology* 116, 375.

GAMBONI, F., FITZ, T.A., HOYER, P.B., WISE, M.E., MAYAN, M.H. & NISWENDER, G.D., 1984. Effect of human chorionic gonadotropin on induced bovine corpora lutea during the anestrous season. *Dom. Anim. Endocr.* 1, 79.

GARCIA-WINDER, M., LEWIS, P.E., DEAVER, D.R., SMITH, V.G., LEWIS, G.S. & INSKEEP, E.K., 1986. Endocrine profiles associated with life span of induced corpora lutea in postpartum beef: cows. *J. Anim. Sci* 62, 1353.

GARCIA-WINDER, M., LEWIS, P.E., TOWNSEND, E.C. & INSKEEP, E.K., 1987. Effects of norgestomet on follicular development in postpartum beef cows. *J. Anim. Sci.* 64, 1099.

GARVERICK, H.A., ELMORE, R.G., VALLAINCOURT, D.H. & SHARP, A.J., 1980. Ovarian response to gonadotropin-releasing hormone in postpartum dairy cows. *Am. J. Vet. Res.* 41, 1582.

GARVERICK, H.A. & SMITH, M.F., 1986. Mechanisms associated with subnormal luteal function. *J. Anim. Sci.* 62 (Suppl. 2), 92.

GARVERICK, H.A., PARFET, J.R., LEE, C.N., COPELIN, J.P., YOUNGQUIST, R.S. & SMITH, M.F., 1988. Relationship of pre- and post-ovulatory gonadotropin concentrations to subnormal luteal function in postpartum beef cattle. *J. Anim. Sci.* 66, 104.

GLASS, J.D., FITZ, T.A. & NISWENDER, G.D., 1984. Cytosolic receptor for estradiol in the corpus luteum of the ewe: variation throughout the estrous cycle and distribution between large and small steroidogenic cell types. *Biol. Reprod.* 31, 967.

GROBBELAAR, J., LISHMAN, A.W., BOTHA, W.A., ELS, D.L., LOUW, B.P. & VAN NIEKERK, A., 1989. Attempts to improve the function of GnRH-induced corpora lutea in early postpartum ewes through oestradiol and/or PMSG priming or exogenous luteotrophin. *Anim. Reprod. Sci.* 19, 245.

GUAJARDO, I., MOLINA, V., GOMEZ, G., GALLEGOS, J. & GARCIA-WINDER, J., 1986. Effects of low doses of GnRH on lifespan of corpora lutea (CL) induced with hCG during the anestrous season in Suffolk ewes. *J. Anim. Sci.* 66 (Suppl. 1), 382 (Abstr.).

GUILBAULT, L.A., THATCHER, W.W., DROST, M. & HALBEL, G.K., 1987. Influence of physiological infusion of prostaglandin $F_2\alpha$

into postpartum cows with partially suppressed endogenous production of prostaglandins. 1. Uterine and ovarian morphological responses. *Theriogenology* 27, 931.

HANSEL, W. & DOWD, J.P., 1986. New concepts of the control of corpus luteum function. *J. Reprod. Fert.* 78, 755.

HANSEL, W. & ECHTERNKAMP, S.E., 1972. Control of ovarian function in domestic animals. *Amer. Zool.* 12, 225.

HANSEL, W. & WAGNER, W.C., 1960. Luteal inhibition in the bovine as a result of oxytocin injections, uterine dilation, and intrauterine infusions of seminal and preputial fluids. *Dairy Sci.* 43, 796.

HARESIGN, W. & LAMMING, G.E., 1978. Comparison of LH release in cyclic and LH-RH treated anoestrous ewes pretreated with PMSG or oestrogen. *J. Reprod. Fert.* 52, 349.

HARESIGN, W., FOSTER, J.P., HAYNES, N.B., CRIGHTON, D.B. & LAMMING, G.E., 1975. Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. *J. Reprod. Fert.* 43, 269.

HARRISON, L.M., KENNY, N. & NISWENDER, G.D., 1987. Progesterone production, LH receptors, and oxytocin secretion by ovine luteal cell types on day 6, 10 and 15 of the oestrous cycle and day 25 of pregnancy. *J. Reprod. Fert.* 79, 539.

HELMER, S.D. & BRITT, J.H., 1986. Fertility of dairy cattle treated with human chorionic gonadotropin (hCG) to stimulate progesterone secretion. *Theriogenology* 26, 683.

HENRICKS, D.M., LAMOND, D.R., HILL, J.R. & DICKEY, J.F., 1971. Plasma preogesterone concentrations before mating and in early pregnancy in the beef heifer. *J. Anim. Sci.* 33, 450.

HOFFMANN, B., SCHAMS, D., BOPP, R., ENDER, M.L., GIMENEZ, T. & KARG, H., 1974. Luteotrophic factors in the cow: evidence for LH rather than prolactin. *J. Reprod. Fert.* 40, 77.

HOLNESS, D.H., ELLISON, J.A., SPROWSON, G.W. & DE CARALHO, A., 1977. Aspects of fertility in Friesland dairy cows with particular reference to the concentration of progesterone in peripheral plasma. *Rhod. J. Agric. Res.* 15, 109.

HOLNESS, D.H., HALE, D.H. & HOPLEY, J.D.H., 1980. Ovarian activity and conception during the post-partum period in Afrikaner and Mashona cows. *Zimbabwe J. Agric. Res.* 18, 3.

HOYER, P.B. & NISWENDER, G.D., 1985. The regulation of steroidogenesis is different in the two types of bovine luteal cells. *Can. J. Physiol. Pharmacol.* 63, 240.

HU, Y., SANDERS, J.D.H., KURZ, S.G., OTTOBRE, J.S. & DAY, M.L., 1989. Effects of calcium ionophore on *in vitro* prostaglandin production by bovine corpora lutea destined to be normal or short-lived. *J. Anim. Sci.* 67 (Suppl. 1), 411 (Abstr.).

HUMPHREY, W.D., KALTENBACH, C.C., DUNN, T.G., KORITNIK, D.R., & NISWENDER, G.D., 1983. Characterization of hormonal patterns in the beef cow during postpartum anoestrus. *J. Anim. Sci.* 56, 445.

HUMPHREY, W.D., KORITNIK, D.R., KALTENBACH, C.C., DUNN, T.G. & NISWENDER, G.D., 1976. Progesterone and LH in postpartum suckled beef cows. *J. Anim. Sci.* 43, 290.

HUNTER, M.G., AYAD, V.J., GILBERT, C.L., SOUTHEE, J.A. & WATHES, D.C., 1989. Role of prostaglandin $F_2\alpha$ and oxytocin in the regression of GnRH-induced abnormal corpora lutea in anoestrous ewes. *J. Reprod. Fert.* 85, 551.

HUNTER. M.G. & SOUTHEE, J.A., 1987. Treatment with progesterone affects follicular steroidogenesis in anoestrous ewes. *Anim. Reprod. Sci.* 14, 273.

HUNTER, M.G., SOUTHEE, J.A. & LAMMING, G.E., 1988. Function of abnormal corpora lutea *in vitro* after GnRH-induced ovulation in the anoestrous ewe. *J. Reprod. Fert.* 84, 139.

HUNTER, M.G., SOUTHEE, J.A., McLEOD, B.J. & HARESIGN, W., 1986. Progesterone pretreatment has a direct effect on GnRH-induced preovulatory follicles to determine their ability to develop into normal corpora lutea in anoestrous ewes. *J. Reprod. Fert.* 76. 349.

INSKEEP, E.K. & MURDOCH, W.J., 1980. Relation of ovarian functions to uterine and ovarian secretion of prostaglandins during the estrous cycle and early pregnancy in the ewe and cow. In: *Reproductive Physiology III. Int. Rev. Physiol.* 22, 325. Ed. R.O. Greep. Baltimore, University Park Press.

INSKEEP, E.K., BRADEN, T.D., LEWIS, P.E., GARCIA-WINDER, M. & NISWENDER, G.D., 1988. Receptors for luteinizing hormone and follicle-stimulating hormone in largest follicles of postpartum beef cows. *Biol. Reprod.* 38, 587.

IRVIN, H.J., PFLANTZ, V.M., MORROW, R.E, DAY, B.N. & GARVERICK, H.A., 1981. GnRH induced release in suckled beef cows. II. The effects of exogenous corticoids and estradiol benzoate on luteinizing hormone release by GnRH. *Theriogenology* 16, 513.

IRVIN, H.J., ZAIED, A.A., DAY, B.N. & GARVERICK, H.A., 1981. GnRH induced LH release in suckled beef cows. I. The effects of days postpartum and estradiol-17β concentrations on the release of LH following administration of GnRH. *Theriogenology* 15, 443.

JENKIN, G., HEAP, R.B. & SYMONS, D.B.A., 1977. Pituitary responsiveness to synthetic LH-RH and pituitary LH content at various reproductive stages in sheep. *J. Reprod. Fert.* 49, 207.

JEWELEWICZ, R., DYRENFURTH, I., FERIN, M., BOGUMIL, J. & VANDE WIELE, R.L., 1977. Gonadotropin, estrogen and progesterone response to long term gonadotropin-releasing hormone infusion at various stages of the menstrual cycle. *J. Clin. Endocrin. Metab.* 45, 662.

JONES, L.S., OTTOBRE, J.S. & PATE, J.L., 1989. Positive regulation of luteinizing hormone (LH) receptors in bovine luteal cells. *J. Anim. Sci.* 67 (Suppl. 1), 412 (Abstr.).

KALTENBACH, C.C., GRABER, J.W., NISWENDER, G.D. & NALBANDOV, A.V., 1968. Luteotrophic properties of some pituitary hormones in nonpregnant or pregnant hypophysectomized ewes. *Endocrinology* 82, 818.

KARSCH, F.J., FOSTER, D.L., BITTMAN, E.L. & GOODMAN, R.R., 1983. A role for estradiol in enhancing luteinizing hormone pulse frequency during the follicular phase of the estrous cycle of the sheep. *Endocrinology* 113, 1333.

KARSCH, F.J., ROCHE, J.F., NOVEROSKE, J.W., FOSTER, D.L., NORTON, H.W. & NALBANDOV, A.V., 1971. Prolonged maintenance of the corpus luteum of the ewe by continuous infusion of luteinizing hormone. *Biol. Reprod.* 4, 129.

KEISLER, D.H., INSKEEP, E.K. & DAILEY, R.A., 1983. First luteal tissue in ewe lambs: Influence on subsequent ovarian activity and response to hysterectomy. *J. Anim. Sci.* 57, 150.

KEISLER, D.H., INSKEEP, E.K. & DAILEY, R.A., 1985. Roles of pattern of secretion of luteinizing hormone and the ovary in attainment of puberty in ewe lambs. *Domest. Anim. Endocrin.* 2, 123.

KESLER, D.J., GARVERICK, H.A., YOUNGQUIST, R.S., ELMORE, R.G. & BIERSCHWAL, C.J., 1977. Effect of days postpartum and endogenous reproductive hormones on GnRH-induced LH release in dairy cows. *J. Anim. Sci.* 46, 797.

KESLER, D.J., GARVERICK, H.A., YOUNGQUIST, R.S., ELMORE, R.G. & BIERSCHWAL, C.J., 1978. Ovarian and endocrine responses and reproductive performance following GnRH treatment in early postpartum dairy cows. *Theriogenology* 9, 363.

KESLER, D.J., WESTON, D.G., PIMENTAL, C.A., TROXEL, T.R., VINCENT, D.L. & HIXON, J.E., 1981. Diminution of the *in vitro* response to luteinizing hormone by corpora lutea induced by gonadotropin releasing hormone treatment of postpartum suckled beef cows. *J. Anim. Sci.* 53, 749.

KIRACOFE, G.H., SCHALLES, R.R. & MARION, G.B., 1969. Effect of wintering ration on reproductive phenomena in beef cows on range. *Kansas Agric. Exp. Sta. Bull.* 529.

KITTOK, R.J., STELLFLUG, J.N. & LOWRY, S.R., 1983. Enhanced progesterone and pregnancy rate after gonadotropin administration in lactating ewes. *J. Anim. Sci.* 56, 652.

KNIGHT, T.W., PETERSON, A.J. & PAYNE, E., 1978. The ovarian and hormonal response of the ewe to stimulation by the ram early in the breeding season. *Theriogenology* 10, 343.

KNIGHT, T.W., TERVIT, A.R. & FAIRCLOUGH, R.J., 1981. Corpus luteum function in ewes stimulated by rams. *Theriogenology* 15, 183.

KOOS, R.D. & HANSEL, W., 1981. The large and small cells of the bovine corpus luteum: Ultrastructural and functional differences. In: *Dynamics of ovarian function*. Eds. N.B. Schwartz and M. Hunzicker-Dunn. Raven Press, New York.

KOTWICA, J., 1988. Vascular component in the mechanism of luteolytic action of oxytocin given during first days of oestrous cycle in cattle. *11th Int. Congr. Reprod. A.I.* Dublin, Ireland, 37.

LAMMING, G.E., 1978. Reproduction during lactation. In: *Control of ovulation*. Eds. D.B. Crighton, G.R. Foxcroft, N.B. Haynes & G.E.

Lamming. Butterworths, London.

LAMMING, G.E., WATHES, D.C. & PETERS, A.R., 1981. Endocrine patterns of the post-partum cow. *J. Reprod. Fert. Suppl.* 30, 155.

LARSON, G.H., 1987. Effect of follicular fluid on development of follicles and function and life span of the corpus luteum. PhD. thesis. West Virginia University.

LARSON, G.H., LEWIS, P.E., DAILEY, R.A., INSKEEP, E.K. & TOWNSEND, E.C., 1987. Follicle stimulating hormone pattern and luteal function in ewes receiving bovine follicular fluid during three stages of the estrous cycle. *J. Anim. Sci.* 64, 1491.

LAUDERDALE, J.W., 1986. A review of patterns of change in luteal function. *J. Anim. Sci.* 62 (Suppl. 2), 79.

LAVOIE, V., HAN, D.K., FOSTER, D.B. & MOODY, E.L., 1981. Suckling effect on estrus and blood plasma progesterone in postpartum beef cows. *J. Anim. Sci.* 52, 802.

LEGAN, S.J. & KARSCH, F.J., 1979. Neuro-endocrine regulation of the estrous cycle and seasonal breeding in the ewe. *Biol. Reprod.* 20, 74.

LEGAN, S.J., I'ANSON, H., FITZGERALD, B.P. & AKAYDIN, M.S., 1985. Importance of short luteal phases in the endocrine mechanisms controlling initiation of oestrous cycles in anoestrous ewes. *Endocrinology* 117, 1530.

LESHIN, L.S., RUND, L.A., CRIM, J.W. & KISER, T.E., 1988. Morphological changes of luteinizing hormone-releasing hormone (LHRH) neurons during the post-partum period of beef cows. *J. Ani. Sci.* 66, (Suppl. 1), 414 (Abstr.).

LEWIS, G.S. & BOLT, D.J., 1987. Effects of suckling, progestogen-impregnated pessaries or hysterectomy on ovarian function in autumn-lambing postpartum ewes. *J. Anim. Sci.* 64, 216.

LEWIS, P.E., INSKEEP, E.K., DEAVER, D.R. & KEISLER, D.H., 1983. Effects of norgestomet on secretion of luteinizing hormone (LH) in postpartum beef cows. *J. Ani. Sci.*, 57 (Suppl. 1), 352 (Abstr.).

LEWIS, G.S., LISHMAN, A.W., BUTCHER, R.L., DAILEY, R.A. & INSKEEP, E.K., 1981. Factors affecting function of induced corpora lutea in postpartum anestrous ewes. *J. Anim. Sci.* 52, 1122.

LISHMAN, A.W., ALLISON, S.M.J., FOGWELL, R.L., BUTCHER, R.L. & INSKEEP, E.K., 1979. Follicular development and function of induced corpora lutea in underfed postpartum anestrous beef cows. *J. Anim. Sci.* 48, 867.

LITCH, S.J. & CONDON, W.A., 1988. Interaction of hCG and Lutalyse on steroidogenesis of bovine luteal cells. *Molec. Cell. Endocr.* 57, 81.

MALLORY, D.S., GUST, C.M. & DAILEY, R.A., Effects of pituitary stalk-transection and type of barrier on pituitary and luteal function during the estrous cycle of the ewe. *Dom. Anim. Endocr.* 3, 253.

MANNS, J.G., HUMPHREY, W.D., FLOOD, P.F., MAPLETOFT, R.J., RAWLINGS, N.C. & CHENG, K.W., 1983. Endocrine profiles and functional characteristics of corpora lutea following onset of postpartum ovarian activity in beef cows. *Can. J. Anim. Sci.* 63, 331.

MANNS, J.G., NISWENDER, G.D. & BRADEN, T., 1984. FSH receptors in the bovine corpus luteum. *Theriogenology* 22, 321.

MARTIN, G.B., OLDHAM, C.M. & LINSAY, D.R., 1980. Increased plasma LH levels in seasonally anovular Merino ewes following the introduction of rams. *Anim. Reprod. Sci.* 3, 125.

McCLELLAN, M.C., ABEL, J.H. & NISWENDER, G.D., 1977. Function of lysosomes during luteal regression in normally cycling and $PGF_{2}\alpha$-treated ewes. *Biol. Reprod.* 16, 499.

McCLELLAN, M.C., DIEKMON, M.A., ABEL, J.H. & NISWENDER, G.D., 1975. Luteinizing hormone, progesterone and the morphological development of normal and superovulated corpora lutea in sheep. *Cell. Tiss. Res.* 164, 291.

McCRACKEN, J.A., SCHRAMM, W. & OKULICZ, W.C., 1984. Hormone receptor control of pulsatile secretion of $PGF_{2}\alpha$ from the bovine uterus during luteolysis and its abrogation in early pregnancy. *Anim. Reprod. Sci.* 7, 31.

McLEOD, B.J. & HARESIGN, W., 1984a. Response of seasonally anoestrous ewes to six-hour periods of GnRH infusion administered on six consecutive days. *Theriogenology* 21, 791.

McLEOD, B.J. & HARESIGN, W., 1984b. Evidence that progesterone may influence subsequent luteal function in the ewe by modulating preovulatory follicle development. *J. Reprod. Fert.* 71, 381.

McLEOD, B.J., HARESIGN, W. & LAMMING, G.E., 1982a. The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injections of GnRH. *J. Reprod. Fert.* 65, 215.

McLEOD, B.J. HARESIGN, W. & LAMMING, G.E., 1982b. Response of seasonally anoestrous ewes to small-dose multiple injections of GnRH with and without progesterone pretreatment. *J. Reprod. Fert.* 65, 223.

McNATTY, K.P., 1979. Follicular determinants of corpus luteum function in the human ovary. *Adv. Exp. Med. Biol.* 112, 495.

McNATTY, J.P., BALL, K., GIBB, M., HUDSON, N. & THURLEY, D.C., 1982. Induction of cyclic ovarian activity in seasonally anoestrous ewes with exogenous GnRH. *J. Reprod. Fert.* 64, 93.

McNATTY, K.P., GIBB, M., DOBSON, C. & THURLEY, D.C., 1981. Evidence that changes in tonic luteinizing hormone secretion regulate the growth of the preovulatory follicle in the ewe. *J. Endocr.* 90, 375.

McNEILLY, A.S., HUNTER, M., LAND, R.B. & FRASER, H.M., 1981. Inadequate corpus luteum function after induction of ovulation in anoestrous ewes by LH-RH or an LH-RH agonist. *J. Reprod. Fert.* 63, 137.

MCNEILLY, A.S., O'CONNELL, M. & BAIRD, D.T., 1982. Induction of ovulation and normal luteal function by pulsed injections of luteinizing hormone in anoestrous ewes. *Endocrinology* 110, 1292.

MEISTERLING, E.M. & DAILEY, R.A., 1987. Use of concentrations of progesterone and estradiol-17β in milk in monitoring postpartum ovarian function in dairy cows. *J. Dairy Sci.* 70, 2154.

MILVAE, R.A. & HANSEL, W., 1980. Concurrent uterine venous and ovarian arterial prostaglandin F concentrations in heifers treated with oxytocin. *J. Reprod. Fert.* 60, 7.

MOOR, R.M., 1973. Oestrogen production by individual follicles explanted from ovaries of sheep. *J. Reprod. Fert.* 32, 545.

MOOR, R.M., 1974. The ovarian follicle of the sheep: Inhibition of oestrogen secretion by luteinizing hormone. *J. Endocr.* 61, 455.

MOOR, R.M., HAY, M.F., McINTOSH, J.E.A. & CALDWELL, B.V., 1973. Effect of gonadotrophins on the production of steroids by sheep ovarian follicles cultured *in vitro*. *J. Endocr.* 58, 599.

MORROW, D.A., ROBERTS, S.J., McENTEE, K. & GRAY, H.G., 1966. Postpartum ovarian activity and uterine involution in dairy cattle. *J. Am. Vet. Med. Assoc.* 149, 1596.

MOSS, G.E., PARFET, J.R., MARVIN, C.A., ALLRICH, R.D. & DIEKMAN, M.A., 1985. Pituitary concentrations of gonadotropins and receptors for GnRH in suckled beef cows at various intervals after calving. *J. Anim. Sci.* 60, 285.

MOSS, G.E., LEMENAGER, R.P., PARFET, J.R., ADAMS, B.M. & ADAMS, T.E., 1988. Concentrations of bioactive and immunoactive luteinizing hormone in bovine anterior pituitary tissue. *Dom. Anim. Endocr.* 5, 185.

MURDOCH, W.J., De SILVA, M. & DUNN, T.G., 1983. Luteal phase insufficiency in the ewe as a consequence of premature induction of ovulation by intrafollicular injection of gonadotropins. *J. Anim. Sci.* 57, 1507.

NANCARROW, C.D., RADFORD, H.M., SCARAMUZZI, R.J. & POST, T.B., 1977. Responses to injected oestrogen in suckled cows. *Theriogenology* 8, 192.

NEPHEW, K.P., FORD, S.P., DAY, M.L. & POPE, W.F., 1989. Extension of short cycles in postpartum beef cows by intrauterine treatment with catecholestradiol. *Dom. Anim. Endocr.* 6, 363.

NETT, T.M., 1987. Function of the hypothalamic-hypophysial axis during the postpartum period in ewes and cows. *J. Reprod. Fert. Suppl.* 34, 201.

NEWCOMB, R., BOOTH, W.D. & ROWSEN, L.E.A., 1977. The effect of oxytoxin treatment on the levels of prostaglandin F in the blood of heifers. *J. Reprod. Fert.* 49, 17.

NISWENDER, G.D., FARIN, C.E., GAMBONI, F., SAWYER, H.R. & NETT, T.M., 1986. Role of luteinizing hormone in regulating luteal function in ruminants. *J. Anim. Sci.* 62, (Suppl. 2), 1.

NISWENDER, D., SCHWALL, R.H., FITZ, T.A., FARIN, C.E. & SAWYER, H.R., 1985. Regulation of luteal function in domestic ruminants: new concepts. *Rec. Prog. Horm. Res.* 41, 101.

ODDE, K.G., WARD, H.S., KIRACOFE, G.H., McKEE, R.M. & KITTOK, R.J., 1980. Short estrous cycles and associated serum progesterone levels in beef cows. *Theriogenology* 14, 105.

OLDHAM, C.M. & MARTIN, G.B., 1979. Stimulation of seasonally anovular Merino ewes by rams. II. Premature regression of ram-induced corpora lutea. *Anim. Reprod. Sci.* 1, 291.

O'SHEA, J.D., 1987. Heterogeneous cell types in the corpus luteum of sheep, goats and cattle. *J. Reprod. Fert. Suppl.* 34, 71.

O'SHEA, J.D., CRAN, D.G. & HAY, M.F., 1979. The small luteal cell of the sheep. *J. Anat.* 128, 239.

O'SHEA, J.D., CRAN, D.G. & HAY, M.F., 1980. Fate of the theca interna following ovulation in the ewe. *Cell Tissue Res.* 210, 305.

O'SHEA, J.D., RODGERS, R.J. & WRIGHT, P.J., 1984. Morphometric analysis and function *in vivo* and *in vitro* of corpora lutea from ewes treated with LHRH during seasonal anoestrus. *J. Reprod. Fert.* 72, 75.

PARFET, J.R., MARVIN, C.A., ALLRICH, R.D., DIEKMAN, M.A. & MOSS, G.E., 1986. Anterior pituitary concentrations of gonadotropins, GnRH-receptors and ovarian characteristics following early weaning in beef cows. *J. Anim. Sci.* 62, 717.

PEARCE, D.T., MARTIN, G.B. & OLDHAM, C.M., 1985. Corpora lutea with a short life-span induced by rams in seasonally anovulatory ewes are prevented by progesterone delaying the preovulatory surge of LH. *J. Reprod. Fert.* 75, 79.

PEARCE, D.T., OLDHAM, C.M., HARESIGN, W. & GRAY, S.J., 1987. Effects of duration and timing of progesterone priming on the incidence of corpora lutea with a normal life-span in Merino ewes induced to ovulate by introduction of rams. *Anim. Reprod. Sci.* 13, 81.

PEARSON, H.G. & LISHMAN, A.W., 1989. Augmentation of GnRH-induced luteal function in lactating ewes by pre- or post-ovulatory administration of Naloxone or PMSG. *J. Anim. Sci.* 67 (Suppl. 1), 410 (Abstr.).

PEKALA, R.F., GNATEK, G.J., DUBY, R.T. & TRITSCHLER, J.P., 1983. Interaction of oxytocin and LH on progesterone synthesis by bovine luteal cells. *J. Anim. Sci.* 57, (Suppl. 1), 563 (Abstr.).

PETER, A.T., BOSU, W.T.U., LIPTRAP, R.M. & CUMMING, E., 1989. Temporal changes in serum prostaglandin $F_2\alpha$ and oxytocin in dairy cows with short luteal phases after the first postpartum ovulation. *Theriogenology* 32, 277–284.

PETERS, A.R., 1984. Effect of exogenous oestradiol-17-β on gonadotrophin secretion in postpartum beef cows. *J. Reprod. Fert.* 72, 473.

PETERS, A.R. & LAMMING, G.E., 1984. Reproductive activity of the cow in the postpartum period. II. Endocrine patterns and induction of ovulation. *Br. Vet. J.*, 140, 269.

PETERS, A.R., LAMMING, G.E. & FISHER, M.W., 1981. A comparison of plasma LH concentrations in milked and suckling post-partum cows. *J. Reprod. Fert.* 62, 567.

PETERS, A.R. & RILEY, G.M., 1982. Milk progesterone profiles and factors affecting postpartum ovarian activity in beef cows. *Anim. Prod.* 34, 145.

PEXTON, J.E., FORD, S.P., WILSON, L., BUTCHER, R.L. & INSKEEP, E.K., 1975. Prostaglandins F in uterine tissue and venous plasma of ewes with intrauterine devices. *J. Anim. Sci.* 4, 144.

PIRL, K.G. & ADAMS, T.E., 1987. Induction of precocious puberty in ewe lambs by pulsatile administration of GnRH. *J. Reprod. Fert.*, 80, 355.

PRATT, B.R., BERARDINELLI, J.G., STEVENS, L.P. & INSKEEP, E.K., 1982. Induced corpora lutea in the postpartum beef cows. I. Comparison of gonadotropin releasing hormone and human chorionic gonadotropin and effects of progestogen and estrogen. *J. Anim. Sci.* 54, 822.

PRIEDKALNS, J., WEBER, A.F. & ZEMJANIS, R., 1968. Qualitative and quantitative morphological studies of the cells of the membrane granulosa, theca interna and corpus luteum of the bovine ovary. *Z. Zellforsch. Mikrosk. Anat.* 85, 501.

PRYBIL, M.K. & BUTLER, W.R., 1978. The relationships between progesterone secretion and the initiation of ovulation in postpartum beef cows. *J. Anim. Sci.* 47 (Suppl. 1), 383.

RAHE, C.H., OWENS, R.E., FLEEGER, J.L., NEWTON, H.J. & HARMS, P.G., 1980. Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. *Endocrinology* 107, 498.

RAMIREZ-GODINEZ, J.A., KIRACOFE, G.H., CARNAHAN, D.L., SPIRE, M.F., BEEMAN, K.B., STEVENSON, J.S. & SCHALLES, R.R., 1982a. Evidence for ovulation and fertilization in beef cows with short estrous cycles. *Theriogenology* 17, 409.

RAMIREZ-GODINEZ, J.A., KIRACOFE, G.H. & McKEE, R.M., 1980. Norgestomet or GnRH effects on short-cycle incidence in anestrous cows after weaning. *J. Anim. Sci.* 51 (Suppl. 1), 318 (Abstr.).

RAMIREZ-GODINEZ, J.A., KIRACOFE, G.H., McKEE, R.M., SCHALLES, R.R. & KITTOK, R.J., 1981. Reducing the incidence of short cycles in beef cows with norgestomet. *Theriogenology* 15, 613.

RAMIREZ-GODINEZ, J.A., KIRACOFE, G.H., SCHALLES, R.R. & NISWENDER, G.D., 1982b. Endocrine patterns in the postpartum beef cow associated with weaning: A comparison of the short and subsequent normal cycles. *J. Anim. Sci.* 55, 153.

RAO, C.V., ESTERGREEN, V.L., CARMAN, F.R. & FRANDLE, R.A., 1979. Receptors for gonadotropin and prostaglandin $F_2\alpha$ receptors in bovine corpora lutea of early, mid and late luteal phase. *Acta. Endocrinol.* 91, 529.

RHIND, S.M., ROBINSON, J.J., CHESWORTH, J.M. & PHILLIPPO, M., 1980. Effects of season, lactation and plane of nutrition on the reproductive performance and associated plasma LH and progesterone profiles in hormonally treated ewes. *J. Reprod. Fert.*, 58, 127.

RICHARDS, J.S., 1980. Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiol. Rev.* 60, 51.

RICHARDS, J.S. & MIDGLEY, A.R., 1976. Protein hormone action: a key to understanding ovarian follicular and luteal cell development. *Biol. Reprod.* 14, 82.

RILEY, G.M., PETERS, A.R. & LAMMING, G.E., 1981. Induction of pulsatile LH release and ovulation in postpartum acyclic beef cows by repeated small doses of GnRH. *J. Reprod. Fert.* 63, 559.

ROBERTS, A.J., CHANG, C.F., SCHALLY, A.V. & REEVES, J.J., 1989. Luteinizing hormone concentrations in serum of postpartum beef cows injected with microencapsulated luteinizing hormone-releasing hormone. *J. Anim. Sci.* 67, 2730.

RODGERS, R.J. & O'SHEA, J.D., 1982. Purification, morphology, and progesterone production and content of three cell types isolated from the corpus luteum of the sheep. *Aust. J. Biol. Sci.* 35, 441.

RODGERS, R.J., O'SHEA, J.D. & FINDLAY, J.K., 1983. Progesterone production *in vitro* by small and large bovine luteal cells. *J. Reprod. Fert.* 69, 113.

RUTTER, L.M., CARRUTHERS, T.D. & MANNS, J.G., 1985. The postpartum induced corpus luteum: Functional differences from that of cycling cows and the effects of progesterone pretreatment. *Biol. Reprod.* 33, 560.

RUTTER, L.M. & RANDEL, R.D., 1984. Luteal competency during resumption of ovarian cyclicity in postpartum Brahman cows. *Theriogenology* 21, 713.

SASSER, R.G., NISWENDER, G.D. & NETT, T.M., 1977. Failure of LH and/or prolactin to prevent $PGF_2\alpha$-induced luteolysis of bovine corpora lutea. *Prostaglandins* 13, 1201.

SAVIO, J.D., BOLAND, M.P., HYNES, N., ROCHE, J.F., 1990. Resumption of follicular activity in the early post-partum period in dairy cows. *J. Reprod. Fert.* 88, 569.

SCHAMS, D., KRUIP, T.A.M. & KOLL, R., 1985. Oxytocin determination in steroid producing tissues and *in vitro* production in ovarian follicles. *Acta. Endocr. Copenh.* 109, 530.

SCHAMS, D., SCHALLENBERGER, E., HOFFMANN, B. & KARG, H., 1977. The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrus, ovulation and electrical resistance of the vaginal mucus. *Acta. Endocr. Copenh.* 86, 180.

SCHAMS, D., SCHALLENBERGER, E., MENZER, C., STANGL, J., ZOTTMEIER, K., HOFFMAN, B. & KARG, H., 1978. Profiles of LH, FSH and progesterone in postpartum dairy cows and their relationships to the commencements of cyclic functions. *Theriogenology* 10, 453.

SCHIRAR, A., MEUSNIER, C., PALY, J., LEVASSEUR, M.C. & MARTINET, J., 1989. Resumption of ovarian activity in post-partum ewes: Role of the uterus. *Anim. Reprod. Sci.* 19, 79.

SCHOMBERG, D.W., COUDERT, S.P. & SHORT, R.V., 1967. Effects of bovine luteinizing hormone and human chorionic gonadotrophin on the bovine corpus luteum *in vivo*. *J. Reprod. Fert.* 14, 277.

SEGUIN, B.E., OXENDER, W.D. & BRITT, J.H., 1977. Effect of human chorionic gonadotropin and gonadotropin releasing hormone on corpus luteum function and estrous cycle duration in dairy heifers. *Am. J. Vet. Res.* 38, 1153.

SHAREH, M., WARD, W.R, & BIRCHALL, K., 1976. Effects of continuous infusion of gonadotrophin releasing hormone in ewes at different times of the year. *J. Reprod. Fert.* 46, 331.

SHEFFEL, C.E., PRATT, B.R., FERRELL, W.L. & INSKEEP, E.K., 1982. Induced corpora lutea in the postpartum cow. II. Effects of treatment with progestogen and gonadotropins. *J. Anim. Sci.* 54, 830.

SHORT, R.E., BELLOWS, R.A., MOODY, E.L. & HOWLAND, B.E., 1972. Effects of suckling and masectomy on bovine postpartum reproduction. *J. Anim. Sci.* 34, 70.

SHORT, R.E., BELLOWS, R.A., STAIGMILLER, R.B., BERARDINELLI, J.G. & CUSTER, E.E., 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *J. Anim. Sci.* 68, 799.

SMITH, M.R., BURRELL, W.C., SHIPP, L.D., SHORT, L.R., SONGSTER, W.N. & WILTBANK, J.N., 1979. Hormone treatments and use of calf removal in postpartum beef cows. *J. Anim. Sci.* 48, 1285.

SMITH, M.F., GARVERICK, H.A., YOUNQUIST, R.S. & ZAHLER, W.L., 1986. Luteinizing hormone receptor concentrations, adenyl cyclase activity and phosphodiesterase activity of bovine corpora lutea: Comparison of short and normal estrous cycles. *Dom. Anim. Endocr.* 3, 127.

SMITH, M.F., LISHMAN, A.W., LEWIS, G.S., HARMS, P.G., ELLERSIECK, M.R., INSKEEP, E.K., WILTBANK, J.N. & AMOSS, M.S., 1983. Pituitary and ovarian responses to gonadotropin releasing hormone, calf removal and progestogen in anestrous beef cows. *J. Anim. Sci.* 57, 418.

SOUTHEE, J.A., HUNTER, M.G. & HARESIGN, W., 1988. Function of abnormal corpora lutea *in vivo* after GnRH-induced ovulation in the anoestrous ewe. *J. Reprod. Fert.* 84, 131.

SPICER, L.J., CONVEY, E.M., LEUNG, K., SHORT, R.E. & TUCKER, H.A., 1986. Anovulation in postpartum suckled beef cows. II. Associations among binding of ^{125}I-labeled gonadotropins to granulosa and thecal cells, and concentrations of steroids in serum and various sized ovarian follicles. *J. Anim. Sci.*62, 742.

SPICER, L.J., IRELAND, J.J. & ROCHE, J.F., 1981. Changes in serum LH, progesterone and specific binding of ^{125}I-hCG to luteal cells during regression and development of bovine corpora lutea. *Biol. Reprod.* 25, 832.

STAIGMILLER, R.B., ENGLAND, B.G., WEBB, R., SHORT, R.E. & BELLOWS, R.A., 1982. Estrogen secretion and gonadotropin binding by individual bovine follicles during estrus. *J. Anim. Sci.* 55, 1473.

STEVENSON, J.S. & BRITT, J.H., 1979. Relationships among luteinizing hormone, estradiol, progesterone, glucocorticoids, milk yield, body weight and postpartum ovarian activity in Holstein cows. *J. Anim. Sci.* 48, 570.

THATCHER, W.W., WILCOX, C.J., COLLIER, R.J., ELEY, D.S. & HEAD, H.H., 1980. Bovine conceptus-material interactions during the pre- and post-partum periods. *J. Dairy Sci.* 63, 1530.

TROXEL, T.R. & KESLER, D.J., 1984a. Ability of indomethacin to alter prostaglandin metabolite concentrations and to enhance function of induced corpora lutea in postpartum suckled beef cows. *J. Anim. Sci.* 59, 177.

TROXEL, T.R. & KESLER, D.J., 1984b. The effects of progestin and GnRH treatments on ovarian function and reproductive hormone secretions of anestrous postpartum suckled beef cows. *Theriogenology* 21, 699.

TROXEL, T.R., KESLER, D.J., NOBLE, R.C. & CARLIN, S.E., 1980. Ovulation and reproductive hormones following steroid pretreatment, calf removal and GnRH in postpartum suckled beef cows. *J. Anim. Sci.* 51, 652.

TROXEL, T.R., OPSOMER, M.J. & KESLER, D.J., 1984. The effect of days postpartum, indomethacin and oxytocin on prostaglandin metabolite concentrations in postpartum suckled beef cows. *Theriogenology* 22, 187.

UILENBROEK, J.T.J. & RICHARDS, J.S., 1979. Ovarian follicular development during the rat estrous cycle: gonadotropin receptors and follicular responsiveness. *Biol. Reprod.* 20, 1159.

URSELY, J. & LEYMARIE, P., 1979. Varying response to luteinizing hormone of two luteal cell types isolated from bovine corpus luteum. *Endocrinology* 83, 303.

VILLENEUVE, P., DAILEY, R.A., INSKEEP, E.K., GUILBAULT, L.A. & DUFOUR, J.J., 1989. Effect of multiple injections of PGF$_2\alpha$ on follicular development in early postpartum ewes. *J. Anim. Sci.* 67 (Suppl. 1), 406 (Abstr.).

VILLENEUVE, P., DUFOUR, J.J. & GUILBAULT, L.A., 1988. Influence of infusion of prostaglandin F$_2\alpha$ (PGF$_2\alpha$) and weaning on surface and histologic populations of ovarian follicles in early postpartum beef cows. *J. Anim. Sci.* 66, 3174.

WALTERS, D.L. & SCHALLENBERGER, E., 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the periovulatory phase of the oestrous cycle in the cow. *J. Reprod. Fert.* 71, 503.

WALTERS, D.L., SCHAMS, D. & SCHALLENBERGER, E., 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. *J. Reprod. Fert.* 71, 479.

WALTERS, D.L., SHORT, R.E., CONVEY, E.M., STAIGMILLER, R.B., DUNN, T.G. & KALTENBACH, C.C., 1982. Pituitary and ovarian function in postpartum beef cows. III. Induction of estrus, ovulation and luteal function with intermittent small-dose injections of GnRH. *Biol. Reprod.* 26, 655.

WALTON, J.S., McNEILLY, J.R., McNEILLY, A.S. & CUNNINGHAM, F.J., 1977. Changes in concentrations of follicle stimulating hormone, luteinizing hormone, prolactin and progesterone in the plasma of ewes during the transition from anoestrus to breeding activity. *J. Endocr.* 75, 127.

WANG, C.F., LASLEY, B.L., LEIN, A. & YEN, S.S.C., 1976. The functional changes of the pituitary gonadotrophins during the menstrual cycle. *J. Clin. Endocr. Metab.* 42, 718.

WARD, H.S., ODDE, K.G., KIRACOFE, G.H. & McKEE, R.M., 1979. Short estrous cycles after weaning in anestrous beef cows. *J. Anim. Sci.* 49 (Suppl. 1), 345 (Abstr.).

WEBB, R. & ENGLAND, B.G., 1982. Indentification of the ovulatory follicle in the ewe: Associated changes in follicular size, thecal and granulosa cell luteinizing hormone receptors, antral fluid steroids, and circulating hormones during the preovulatory period. *Endocrinology* 110, 873.

WEBB, R., LAMMING, G.E., HAYNES, N.B., HAFS, H.P. & MANNS, J.G., 1977. Response of cyclic and postpartum suckled cows to injection of synthetic LHRH. *J. Reprod. Fert.* 50, 203.

WEBB, R., LAMMING, G.E., HAYNES, N.B. & FOXCROFT, G.R., 1980. Plasma progesterone and gonadotrophin concentrations and ovarian activity in postpartum dairy cows. *J. Reprod. Fert.* 59, 133.

WEESNER, G.D., NORRIS, T.A., FORREST, D.W. & HARMS, P.G., 1987. Biological activity of luteinizing hormone in the peripartum cow: Least activity at parturition with an increase throughout the postpartum interval. *Biol. Reprod.* 37, 851.

WELLS, P.L., HOLNESS, D.H., FREYMARK, P.J., McCABE, C.T. & LISHMAN, A.W., 1985. Fertility in the Afrikaner cow. 2. Ovarian recovery and conception in suckled and non-suckled cows postpartum. *Anim. Reprod. Sci.* 8, 315.

WILLIAMS, L., 1989. Modulation of luteal activity in postpartum beef cows through changes in dietary lipids. *J. Anim. Sci.* 67, 785.

WILTBANK, J.N., ROTHLISBERGER, J.A. & ZIMMERMAN, D.R., 1961. Effect of human chorionic gonadotrophin on maintenance of the corpus luteum and embryonic survival in the cow. *Anim. Sci.* 20, 827.

WISE, M.E., GIRMUS, R.L. & RODRIQUEZ, R.E., 1989. Gonadotropin releasing hormone secretion during an estrus in the ewe. *Biol. Reprod.* 40 (Suppl. 1), 103 (Abstr.).

WISE, M.E., GLASS, J.D. & NETT, T.M., 1986. Changes in the concentration of hypothalamic and hypophyseal receptors for estradiol in pregnant and postpartum ewes. *J. Anim. Sci.* 62, 1021.

WODZICKA-TOMASZEWSKA, M., HECKER, J.F. & BRAY, A.R., 1974. Effect of day of insertion of intrauterine devices on luteal function in ewes. *Biol. Reprod.* 11, 79.

WRIGHT, P.J. & FINDLAY, J.K., 1977. LH release due to LH-RH or estradiol-17β (E_2)in postpartum ewes. *Theriogenology* 8, 191.

WRIGHT, P.J., GEYTENBEEK, P.E., CLARKE, I.J. & FINDLAY, J.K., 1981. Evidence for a change in oestradiol negative feedback and LH pulse frequency in postpartum ewes. *J. Reprod. Fert.* 61, 97.

WRIGHT, P.J., GEYTENBEEK, P.E., CLARKE, I.J. & FINDLAY, J.K., 1983. LH release and luteal function in postpartum acyclic ewes after the pulsatile administration of LH-RH. *Reprod. Fert.* 67, 257.

WRIGHT, P.J., GEYTENBEEK, P.E., CLARKE, I.J. & FINDLAY, J.K., 1984. Induction of plasma LH surges and normal luteal function in acyclic postpartum ewes by pulsatile administration of LH-RH. *J. Reprod. Fert.* 71, 1.

WRIGHT, J.M., KIRACOFE, G.H. & BEEMAN, K.B., 1988. Factors associated with shortened estrous cycles after abortion in beef heifers. *J. Anim. Sci.* 66, 3185.

YUTHASASTRAKOSOL, P., PALMER, W.M. & HOWLAND, B.E.,

with GnRH in experiment I. The ovaries of 17 cows were re-examined on day-56 postpartum to confirm the absence of CL and assess follicular activity. The cows were divided randomly and injected twice daily for 3 days with either 0 or 2 mg FSH (s.c.). Follicular development was estimated by palpating the ovaries again subsequent to FSH, the second examination being followed by an injection (i.m.) of 300 μg GnRH in all cows. Blood was drawn prior to FSH and again prior to GnRH for quantification of estradiol-17β and 7 and 14 days after GnRH for measurement of progesterone. Corpora lutea were detected by rectal palpation 7 and 14 days after GnRH.

Experiment III. Eighteen lactating, anovulatory 3-year-old Hereford cows were assigned at random to receive one of three treatments. These cows from the same herd used in experiments I and II, received 100% of NRC recommended energy and were studied 1 year later. On days 30, 34 and 37 postpartum the ovaries were palpated to assess ovarian activity. Twice daily (0600 and 1800 hr) hormonal treatments were made beginning on day 34 postpartum as follows: (1) corn oil (i.m.) on day -4 and saline (s.c.) on days -3, -2 and -1 (5 cows); (2) corn oil on day -4, and FSH on days -3 (.25 mg), -2 (.375 mg) and -1 (.5 mg) (6 cows); (3) estradiol benzoate (75 μg) on day -4, and FSH on days -3 (.25 mg), -2 (.375 mg) and -1 (.5 mg) (7 cows). The treatments given on day -1 also were given at 0600 hr on day 0, immediately prior to injection of 300 μg GnRH (i.m.).

Jugular venous blood collected by cannulae just prior to (0 hr) and every 30 min for 6 hr after GnRH were used to quantify plasma concentrations of LH. Samples taken by jugular venipuncture on day -4, -3, -2 and -1 and at 0, 8, 12 and 24 hr after GnRH on day 0 were assayed to quantify plasma concentrations of LH and estradiol-17β. Progesterone was measured in plasma from samples taken on days -8, -4 and 0 before GnRH and every 3 days from day 1 to 16 after GnRH. Cows were palpated *per rectum* on days 7 and 14 and again on day 30 to determine if new CL were formed spontaneously or cows had returned to anestrus subsequent to regression of induced CL.

Quantification of Hormones. Estradiol-17β, progesterone and LH were measured in plasma by radioimmunoassays (Butcher *et al.*, 1974; Butcher, 1977) validated for bovine plasma (Fogwell *et al.*, 1978). LH is reported as ng equivalants NIH-LH-B9 (.7×NIH-LH-S1)/ml.

Statistical Analyses. Data for experiments I and II were examined by least squares analysis of variance using models with terms for "nutritional level," "weight postpartum," "weight post-GnRH," "weight change postpartum to pre-GnRH," "weight change postpartum to post-GnRH," "FSH treatment," and interactions. Data on follicular development from experiment III were examined by analysis of variance and least significant difference. Chi-square was used to examine the data on occurrence of CL. Profiles of concentrations of hormones in all experiments were examined for differences among groups by analysis of variance using time as an independent continuous variable and the partitioned linear, quadratic and cubic regressions of hormonal concentration on time in the manner described by Fogwell *et al.* (1978).

RESULTS AND DISCUSSION

Experiment I. Animals fed the low energy ration initially weighed an average of 6.8 kg more than those on the high plane diet. Just prior to parturition this weight difference had decreased to a mean of 2.0 kg (379.1 *vs* 377.1). From parturition to 30 days postpartum, cows on the low plane lost 31.5 kg while those on the high plane lost 10.7 kg ($P<.1$). Thereafter they gained 1.1 (low) and 9.6 kg (high; $P<.05$) until 48 days postpartum.

The level of feeding had no effect on size of the largest follicle (high 11.6 ± .4 mm, low 10.9 ± .3) or the proportion of cows with a follicle ≥ 12 mm in diameter (12/20 high *vs* 11/23 low) prior to injection of FSH. The proportion of cows that had CL on day 7 after GnRH was not affected by treatment with FSH or plane of nutrition (table 1). Overall, neither body weight postpartum nor weight change after parturition appeared to determine whether or not a cow would produce a CL. Among the low-plane animals, nine of the 12 that lost the most weight had CL on day 7 as compared to four of the 11 that lost the least weight. Only seven of 21 cows in which the largest follicle in the ovaries, four days prior to GnRH, was less than 12 mm in diameter had CL on day 7 after GnRH in contrast to 16 of 22 of those with a follicle ≥ 12 mm.

Neither level of feeding nor FSH affected concentrations of estradiol-17β prior to (daily samples) or 8 hr after GnRH (overall mean 1.2 ± .1 pg/ml), so it was not possible to determine whether the level of feeding had any influence on ovarian response to FSH.

Pretreatment with FSH did not alter basal concentrations of LH (.2 ng/ml) or the pattern of release or the maximum concentration of LH (table 1) in response to GnRH. One cow (no. 38) on the high plane that did not receive FSH exhibited maximum LH of 252 ng/ml, whch was 111 ng/ml

1977. Release of LH in anoestrous and cyclic ewes. *J. Reprod. Fert.* 50, 319.

ZAIED, A.A., GARVERICK, H.A., BIERSCHWAL, C.J., ELMORE, R.G., YOUNGQUIST, R.S. & SHARP, A.J., 1980. Effects of ovarian activity and endogenous reproductive hormones on GnRH induced ovarian cycles in postpartum dairy cows. *J. Anim. Sci.* 50, 508.

ZOLLERS, W.G., GARVERICK, H.A., SILCOX, R.W., YOUNGQUIST, R.S., COPELIN, J.P. & SMITH, M.F., 1989. *In vivo* prostaglandin $F_{2}\alpha$ ($PGF_{2}\alpha$) secretion by endometrial tissue from postpartum beef cows expected to have a short or normal estrous cycle. *J. Anim. Sci.* 67 (Suppl. 1), 336 (Abstr.).

PAPER 36

BIOLOGY OF REPRODUCTION 48, 655–661 (1993)

Factors Affecting Fertility in the Postpartum Cow: Role of the Oocyte and Follicle in Conception Rate[1]

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ABSTRACT

Four experiments were designed to examine the contribution of the oocyte or the follicular, oviductal, or early uterine environments to low fertility associated with the first ovulation postpartum. At 17–25 days postpartum in experiments 1, 2, and 3, suckled beef cows were assigned at random to receive 6 mg norgestomet, via ear-implant, for 9 days (NOR) or to serve as controls (CON). Calves were weaned from all cows 7 days after assignment to treatment in order to induce estrus, an LH surge, ovulation, and subsequent formation of CL. As cows were detected to be in estrus, they were bred first by natural service and 12 h later by artificial insemination. In experiment 1, on Day 3 after estrus, the oviduct ipsilateral to the side of ovulation was removed and flushed for recovery of an embryo or oocyte. Rates of recovery (86%), fertilization (68%), and development of fertilized oocytes to the 4- to 8-cell stage (100%) did not differ between CON and NOR cows. In experiment 2, uteri were flushed nonsurgically on Day 6 after estrus. Rates of recovery of embryos from the uterus were similar between CON (86%) and NOR (71%) cows. In experiment 3, one half of the cows in each group (CON and NOR) were fed melengestrol acetate (MGA) beginning on Day 4 after estrus and continuing until Day 35. The remaining cows in each group served as controls. Treatment with NOR increased ($p < 0.05$) the proportion of cows that maintained pregnancy until Day 35 (9/22) as compared to controls (0/18). Supplementation with MGA failed to improve maintenance of pregnancy in cows that underwent early luteal regression. In experiment 4 progesterone (200 mg/day) was injected, beginning on Day 4 after cows were bred at first postpartum estrus, to produce luteal-phase concentrations in circulation. This treatment also failed to maintain pregnancy in CON cows with short-lived CL. In conclusion, low pregnancy rate in CON cows was due not only to premature regression of the CL; it may have resulted from ovulation of an oocyte with inherent or acquired defects preventing continued development, or from an oviductal or uterine environment hostile to the developing embryo.

INTRODUCTION

In the cow, the first ovulation postpartum usually is associated with development of a CL with a short life span [1–8]. Early weaning of calves from anestrous cows induces estrous behavior with subsequent formation of CL in about 4 days [9]. Unless luteal tissue has formed prior to this estrus, the resulting CL has a short life span [10–13], similar to that of first CL in suckled cows. Fertility associated with first ovulation, whether spontaneous [14] or induced by weaning [10, 11], is extremely low. At present, it is not known whether low fertility associated with the ovulation that precedes a short estrous cycle is entirely the result of premature regression of the CL and subsequent death of the embryo, or whether death of the embryo would occur independently of luteal regression.

For pregnancy to succeed, several processes are essential. First, an ovarian follicle must develop and ovulate an oocyte capable of being fertilized and of undergoing embryonic development. Second, the oviductal and uterine environments must be suitable for gamete transport, fertilization, and subsequent embryonic development. Finally, the CL must function for a sufficient period of time for maternal recognition of pregnancy and at a level that will maintain gestation.

The objectives of the present studies were as follows. In experiment 1 the objectives were to determine, in anestrous postpartum beef cows, a) whether ovulation occurs at first estrus after weaning at about 30 days postpartum, b) whether oocytes ovulated at the beginning of a short luteal phase are capable of being fertilized and of undergoing normal early development, c) whether pretreatment with progestogen affects early embryonic development, and d) whether preovulatory patterns of hormonal concentrations affect embryonic development. The objective in experiment 2 was to determine whether embryos conceived at the start of a short luteal phase are transported into the uterus. The objectives of experiment 3 were to determine, in cows provided exogenous progestogen following breeding, a) whether embryos conceived at the beginning of a short luteal phase are capable of establishing pregnancy, b) whether pretreatment with progestogen improves conception rate, and c) whether growth pattern of the ovulatory follicle or pattern of circulating estradiol-17β affects con-

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ception rate. In experiment 4, the objective was to ascertain whether provision of luteal-phase concentrations of exogenous progesterone allow establishment of pregnancy in cows with a short luteal phase.

MATERIALS AND METHODS

Multiparous beef cows that were in moderate body condition (scoring 4 to 7 on a scale of 1 to 9) [15] and that had calved during spring were used in these studies. Cows in experiments 1, 2, and 3 were assigned to receive, via an ear-implant, 6 mg of norgestomet (NOR; 17α-acetoxy-11β-methyl-19-nor-pregn-4-ene-3,20-dione; Sanofi Animal Health, Overland Park, KS) for 9 days beginning 17–25 days after parturition (NOR) or to serve as untreated controls (CON; Fig. 1). Palpation per rectum for CL at the start of the study and daily serum concentrations of progesterone (P_4) of < 1 ng/ml were used to verify that ovulation had not occurred before first estrus. Calves were weaned from all cows 7 days after assignment to treatment to induce estrus, an LH surge, ovulation, and subsequent formation of CL. Cows were observed twice daily for estrous behavior throughout each experiment. Intact bulls, fitted with chin-ball markers, were penned with the cows continuously from weaning to aid in estrous detection and for natural service. Cows were bred by natural service at onset of estrus (Day 0). They were bred again 12 h later by natural service, if still in standing estrus, or by artificial insemination if not standing. When cows exhibited estrous activity (attempted mounts by other animals and/or extensive paint markings from the chin-ball marker of the bull), but breeding by the bull was not observed, they were artificially inseminated at the time estrous activity was detected and again 12 h later. To confirm that artificial insemination was carried out near the time of the LH surge, measurements for an elevation of LH and/or a precipitous decline in concentrations of estradiol-17β (E_2) in serum from daily blood collections as well as ultrasonography for occurrence of follicular rupture were performed. Beginning with onset of estrus, ultrasonography of the ovary was performed twice daily (experiments 1 and 2) or daily (experiment 3) to determine the side and approximate time of ovulation via a real-time linear-array ultrasonic scanner (Pie Data 400, Pie Medical BV, Maastricht, The Netherlands) fitted with a 5-MHz transducer.

Experiment 1

Twenty-five CON and 26 NOR cows were started on this experiment. However, 11 cows were not bred due to failure to detect estrus by 10 days after weaning. Blood samples were collected via jugular venipuncture twice daily (0700 and 1800 h) from onset of treatment to the day after estrus and then every third day through Day 14. On Day 3 after estrus, the remaining 17 CON and 23 NOR cows were tranquilized with 20 mg xylazine. Under local anesthesia (lidocaine), a flank incision was made ipsilateral to the side of ovulation as determined previously by ultrasonography. The reproductive tract was exposed and the ovaries were examined for the presence of a CL with an ovulation papilla. The oviduct ipsilateral to the CL was removed and flushed (Dulbecco's PBS) as described by Ramirez-Godinez et al. [12]. The fluid was searched immediately via a stereoscopic microscope for an embryo or ovum, which, if recovered, was examined further for 1) fertilization, 2) stage of development, and 3) morphology.

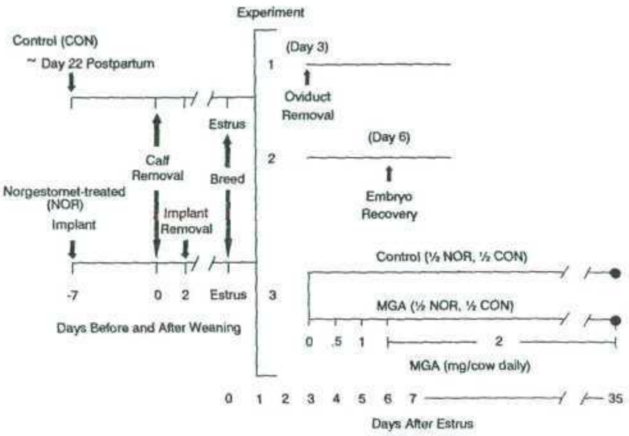

FIG. 1. Experimental procedures (experiments 1, 2, and 3). CON, control cows before first estrus; NOR, cows before first estrus receiving norgestomet for 9 days.

Experiment 2

Seven CON and 9 NOR cows were used in this experiment after removal of 3 CON and 1 NOR cow for failure to exhibit estrus by 10 days after weaning. Blood samples were collected twice daily from 2 days before onset of treatment through 1 day after estrus and then daily for 16 days after estrus. On Day 6 after estrus, each cow was given an epidural block (lidocaine) and the uterus was flushed by a nonsurgical technique for recovery of embryos [16]. Upon recovery of an ovum or embryo, it was classified as described for experiment 1.

Experiment 3

Thirty CON and 28 NOR cows were started on this experiment, but 7 CON and 4 NOR cows failed to exhibit estrus by 10 days after weaning and were removed from the study. Blood samples were collected from both groups at time of insertion of a NOR implant, twice daily from 2 days before weaning to the day after estrus, and then every third day to 35 days after breeding. One half of the cows on each treatment (CON and NOR) were individually fed melengestrol acetate (MGA) in their daily grain ration (0.9 kg) on Days 4 through 6 following breeding at first estrus and were group-fed thereafter. The dose (per cow daily) was 0.5 mg on Day 4, 1 mg on Day 5, and 2 mg from Day 6 until Day

35. The remaining cows from both groups received equal amounts of grain without MGA and served as controls for the MGA treatment (Fig. 1). Diameters of follicles ≥ 5 mm were measured every other day by ultrasonography, beginning 2 days before weaning and continuing until ovulation. Pregnancy status was determined by ultrasonography of the reproductive tract for the presence of a viable fetus on approximately Day 35.

Experiment 4

This experiment used 16 CON cows that had calved in late winter and early spring. Calves were weaned 25 to 30 days postpartum, 7 days after start of the study, and cows were bred at first estrus by natural service and 12 h later by artificial insemination. Blood samples were collected daily from 7 days before weaning of calves to onset of estrus, and then every other day to Day 35 after breeding. Beginning on Day 4 after estrus, each cow received exogenous P_4 (100 mg in 4 ml of corn oil containing 10% benzyl alcohol, s.c. twice daily) until Day 35. This treatment produced patterns of circulating P_4 similar to those of a normal luteal phase through Day 15 of the estrous cycle, followed by a plateau at 6–8 ng/ml beyond that time. This regimen of P_4 maintained pregnancy in early-weaned postpartum cows that had short-lived CL and that received good quality embryos on Day 7 after estrus [17]. Via ultrasonography (7.5-MHz transducer), structural presence of CL was determined at 4, 12, 15, 18, 21, and 35 days after breeding, and pregnancy status was determined at Day 35. Pregnancy rates were compared between cows classified as having either a short (< 15 days) or a normal (≥ 15 days) structural life span of the CL.

Blood Samples, Assays, and Statistical Analyses

Blood samples were allowed to clot at 4°C, and serum was collected after centrifugation and stored at −20°C. In experiments 1, 2, and 3, RIA were performed for P_4, E_2, LH, and FSH [18–21]. In experiment 4, assays for P_4 were performed to determine whether ovulation had occurred before weaning and to determine circulating concentrations of P_4 after breeding. Standards for LH and FSH were NIH-LH-B9 and NIAMDD-oFSH-RP-1, respectively. Intra- and interassay coefficients of variation, respectively, were 6% and 13% for P_4, 7% and 5% for E_2, 7% and 16% for LH, and 8% and 6% for FSH.

Data were examined by chi-square analysis for the effects of treatment and of other variables. For experiments 1 and 2, data for oocytes or embryos were analyzed only for cows without technical problems in the recovery procedures. To examine the effects of preovulatory concentrations of each hormone on recovery of oocytes or embryos, fertilization, and early development, cows in experiment 1 were divided into two equal groups according to high or low mean concentration of the hormone in question during the 3 days before the surge of LH. In experiment 3 a subgroup of 22 cows, which had data from ultrasonography before first postpartum estrus and which formed a CL of normal life span after the first estrus, were divided into two equal groups by size of the preovulatory follicle at 5 days before the LH surge. Data on these two groups were examined by chi-square analysis for effects of size of follicle on pregnancy at Day 35. Effects of follicular size on the fifth day before the LH surge on concentrations of E_2 during the 4 days before the LH surge were examined by analysis of variance [22].

RESULTS

Data combined from experiments 1, 2, and 3 showed that ovulation occurred by 10 days after weaning in 97% of cows, regardless of treatment, and in 100% of cows exhibiting estrus. Signs of estrous behavior preceded ovulation in 87% of cows that ovulated within 10 days after weaning, and the proportion did not differ between groups.

Experiment 1

Of the 40 cows that were inseminated, 1 CON and 3 NOR cows were deleted from analysis either because of a blockage of the oviduct or because of a technical problem in obtaining and flushing the oviduct. Measurement of P_4 in serum showed that 6 CON and 2 NOR cows had a luteal phase prior to first estrus. When only those cows without a prior luteal phase were considered, the rates for CON vs. NOR cows, respectively, of embryo or oocyte recovery (10/10 vs. 14/18) and of fertilization (7/9 vs. 8/13) did not differ ($p > 0.05$). The rates of recovery and fertilization were not different when the 8 cows with a luteal phase before first estrus were included (recovery, 15/16 vs. 16/20 and fertilization, 12/14 vs. 9/15 for CON vs. NOR, respectively; $p > 0.05$). Two cows (1 CON and 1 NOR) from which an empty zona pellucida had been recovered were excluded from calculations of fertilization rates.

All fertilized ova had developed to the 4- to 8-cell stage (12/12 in CON and 9/9 in NOR). A 7-cell embryo with the large nondividing cell exhibiting cytoplasmic degeneration (from a CON) and a 6-cell embryo with separation of cytoplasm from the cell membrane in 2 of the 4 smaller cells (from a NOR) were classified as abnormal. Dark areas occurred on some blastomeres of embryos (including the 7-cell embryo) from 6 CON and 5 NOR cows (Fig. 2). These areas, which varied in size from < 5% to > 50% of the plasma membranes, differed in size and shape among blastomeres and resembled dark areas in cells of dead embryos at Day 6 of gestation. As revealed by phase-contrast microscopy, these areas were at the internal surface of the plasma membrane and were composed of spherical particles or droplets of varying sizes (Fig. 3).

Data pooled from all cows showed that rates of ova recovery and fertilization were not affected by preovulatory

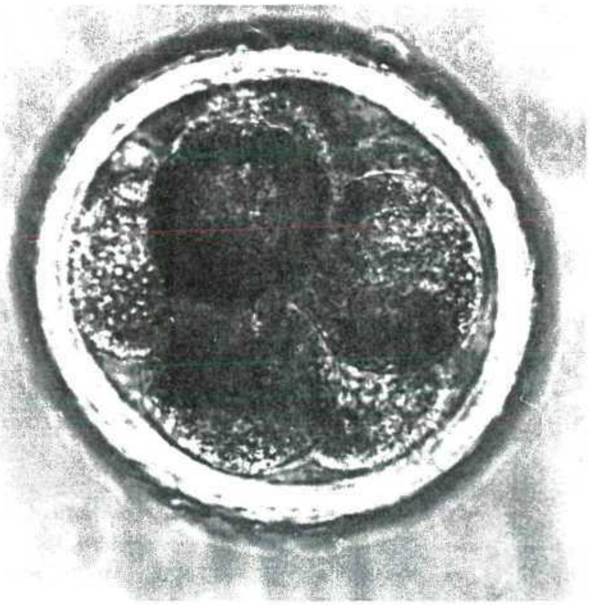

FIG. 2. Bright-field photomicrograph of an 8-cell embryo showing dark areas in some blastomeres (experiment 1). ×600.

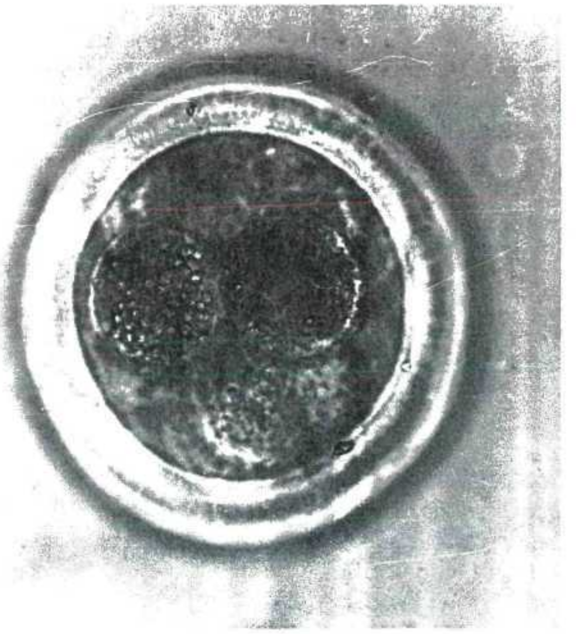

FIG. 3. Phase-contrast photomicrograph of the dark area of a blastomere in the 8-cell embryo shown in Figure 2. Spherical particles that form this dark area are against the internal surface of the cell membrane (experiment 1). ×600.

concentrations of LH, FSH, or E_2 during the 5 days before the LH surge, or by life span of CL. The proportion of embryos with dark areas against the internal surface of the plasma membrane of blastomeres did not differ ($p > 0.05$) between cows with low compared to high preovulatory concentrations of LH or FSH, or between cows having CL with short compared to normal life span. However, the proportion of recovered embryos with these dark areas or with abnormal development was higher ($p < 0.05$) for cows that had a prolonged elevation of E_2 (> 3 pg/ml for ≥ 3 days) before the LH surge (7/8) than for cows without a prolonged elevation of E_2 (5/13); the proportion of such embryos was also higher for cows with mean concentrations of $E_2 \geq 6.0$ pg/ml during the 3 days before the LH surge (8/10) compared with cows having lesser concentrations of E_2 (4/11).

Experiment 2

Among cows in estrus (n = 16), 2 NOR cows were removed from analysis due to technical problems during recovery. Between CON and NOR groups, respectively, rates of recovery of an embryo or ovum from the uterus (6/7 vs. 5/7), fertilization (4/6 vs. 5/5), and development to at least the 4-cell stage (3/4 vs. 5/5) did not differ. Three of 4 embryos recovered from CON cows were classified as being alive (normal morula) compared to only 1 of 5 in NOR cows. The other 5 embryos had died at the 4-cell to early morula stage of development.

Experiment 3

One NOR-treated cow stopped eating the feed containing MGA and was removed from the study. Measurement of P_4 in serum showed that 6 cows (5 CON and 1 NOR) had luteal function prior to first observed estrus. Pregnancy was maintained in all 6 of these cows (Table 1). The proportion of cows without a CL before first estrus that maintained pregnancy to Day 35 was higher ($p < 0.05$) in NOR-treated (9/22) than in CON (0/18) cows (Table 1). Supplementation with MGA did not affect the proportion of either CON or NOR cows maintaining pregnancy to Day 35 (Table 1). No pregnancies were maintained in either CON or NOR cows with a short-lived CL following breeding.

Data from ultrasonography of the ovulatory follicle were available for 22 cows (7 CON and 15 NOR) with a CL of normal life span after first estrus. A greater proportion ($p < 0.05$) of the cows with small size of the preovulatory follicle at 5 days before the LH surge (7.5 ± 1.8 mm) maintained pregnancy (10/11) than did cows with relatively larger follicles (12.9 ± 0.9 mm; 4/11; Fig. 4A). For the group of smaller follicles, growth rate to the LH surge was greater ($p < 0.05$) than for the group of larger follicles (1.5 ± 0.15 vs. 0.6 ± 0.13 mm/day); but size of the preovulatory follicle at time of the LH surge did not differ ($p > 0.05$) for follicles in the small (13.7 ± 0.8 mm) vs. large (15.7 ± 0.8 mm) classification. Concentrations of E_2 during the 4 days before the LH surge differed ($p < 0.05$) between cows with smaller vs. larger follicles at the fifth day before the LH surge (Fig. 4B). However, when cows were placed into two equal groups according to mean concentrations of E_2 during the 4 days before the LH surge, the proportion of cows

TABLE 1. Proportions of cows pregnant following pretreatment with norgestomet and supplemented with MGA after breeding (experiment 3).

		Cows pregnant at 35 days		
		First ovulation		Second ovulation[a]
Pretreatment	Supplement	Short-lived CL	Normal-lived CL	Normal-lived CL
Control	Control	0/8	0/0	3/3
	MGA	0/7	0/3	2/2
Norgestomet[b]	Control	0/3	5/9	0/0
	MGA	0/1	4/9	1/1

[a] Cows with a luteal phase before first postpartum estrus.

[b] Main effect of norgestomet on conception at first ovulation ($p < 0.05$).

that were pregnant was similar between cows classified as having low (5.0 ± 0.7 pg/ml: 8/11) compared to relatively high (8.6 ± 0.7 pg/ml; 6/11) E_2.

Experiment 4

Eight of 16 CON cows had short-lived CL (structure regressed < 15 days after breeding) as assessed by ultrasonography. Only 1 of these 8 cows had shown luteal function before breeding. Administration of exogenous P_4, which produced luteal-phase concentrations of P_4, failed to maintain pregnancy in those cows that underwent early structural luteal regression (0/8). Pregnancy was maintained to Day 18 after breeding in 6 of 8 cows that had a luteal structure, and the CL was present at Day 35 in each of the 6 pregnant cows. Seven of these 8 cows had shown a short luteal phase prior to first estrus. Treatment with P_4 produced similar circulating patterns and concentrations of P_4 in cows with short and normal luteal life span. Concentrations of P_4 to Day 15 were similar to those we previously reported for cycling cows [23], and plateaued at 6–8 ng/ml after Day 15.

DISCUSSION

Recovery of fertilized ova from the oviduct (experiment 1) of cows during a short estrous cycle verified the occurrence of ovulation, fertilization, and embryonic development to Day 3 in cows ovulating at an average of 32–34 days postpartum. Transport of embryos into the uterus during the first postpartum estrous cycle was confirmed by recovery of embryos or unfertilized oocytes on Day 6 from the uterus of 6 of 7 cows with short-lived CL (experiment 2). These data confirm those of Ramirez-Godinez et al. [12], who recovered 3 embryos from the oviducts of 4 cows and 1 ovum from the uterus in five attempts in cows with short estrous cycles.

In experiment 3, none of the CON cows (0/15) maintained pregnancy following insemination at estrus preceding a short-lived CL. Similarly, Odde et al. [10] and Ramirez-Godinez et al. [11] reported no pregnancies in a total of 25 postpartum cows mated at the weaning-induced estrus preceding a short-lived CL. In contrast, in our study pregnancy was maintained in 9 of 18 NOR-treated cows inseminated at the first postpartum ovulation and exhibiting a normal luteal phase (experiment 3); it was also maintained in 11 of 13 CON cows (experiments 3 and 4) that had a normal luteal phase following a short luteal phase before first post-

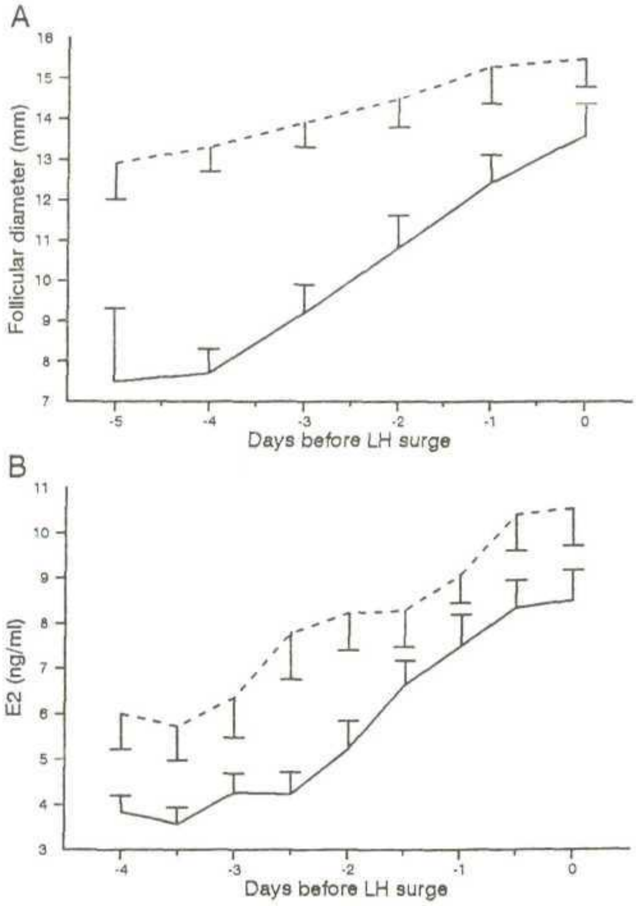

FIG. 4. Size of preovulatory follicles (panel A) and patterns of concentration of estradiol during the 4 days before the LH surge (panel B) for cows with preovulatory follicles of large (dashed lines) and small (solid lines) size (12.9 ± 0.9 and 7.5 ± 1.8 mm, respectively) at 5 days before the LH surge (experiment 3). These data represent only cows with a normal luteal phase after first postpartum estrus. The proportion of pregnancies was greater ($p < 0.05$) for cows with small compared to large preovulatory follicles at 5 days before the LH surge (small, 10/11 and large, 4/11). Concentrations of E_2 were greater ($p < 0.05$) in cows with large compared to small follicles.

partum estrus. Ramirez-Godinez et al. [11] reported 33% conception when cows were pretreated with NOR and mated at estrus beginning a normal luteal phase.

Wiltbank et al. [24] hypothesized that death of embryos after regression of the CL may be prevented if supplementation with a progestogen is begun before luteal regression. Supplementation of bilaterally ovariectomized heifers with 1 mg of MGA daily (beginning at time of surgery on Day 50 of pregnancy) or with 4 mg of MGA daily (beginning on Day 4 after estrus) maintained pregnancy in a high percentage of animals [25]. Inskeep and Baker [26], through supplementation with 4 mg/day of MGA, maintained pregnancy in 4 of 12 bilaterally ovariectomized postpartum cows following induced estrus and transfer of an embryo to the uterus. They concluded that once estrus has been induced, progestogen can replace the ovaries in preparing the uterus for pregnancy.

In the present studies, provision of 2 mg of MGA per day did not lead to maintenance of pregnancy in CON cows (0/10). Zimbelman and Smith [25] reported that feeding 1 mg/day of MGA starting at Day 4 after breeding maintained pregnancy in 6 of 6 bilaterally ovariectomized heifers, but that in unilaterally ovariectomized heifers with removal of the ovary containing the CL, all pregnancies were lost. To determine whether failure to conceive might be due to an inadequate dose of MGA in the presence of ovaries, CON cows (experiment 4) were injected with P_4 beginning on Day 4 after estrus (100 mg/head twice daily). Although luteal-phase concentrations of P_4 were achieved, pregnancy was not maintained in CON cows that formed short-lived CL (0/8). On the basis of these results, it is unlikely that premature regression of the CL is the only factor responsible for the low fertility associated with first ovulation in the postpartum cow.

Butcher et al. [17] reported that pregnancy was maintained in 28% of postpartum cows undergoing early luteal regression (CON) following injection of P_4 (100 mg/head twice daily from Day 4) and transfer of good quality frozen-thawed embryos on Day 7. In the same study, rate of pregnancy was 58% in NOR-treated cows receiving P_4 and embryos [17]. This is similar to the rate of pregnancy observed for NOR-treated cows (50%) that formed CL of normal life span in the present studies (experiment 3) and to the 55% overall calving rate from a single service in cycling cows [27]. One might speculate that increased fertility associated with NOR treatment could be due to synchronization of preovulatory endocrine and follicular events. It has been proposed that short-term increases in P_4 after the first ovulation serve as a priming or organizational mechanism that is required for establishment of normal estrous cyclicity and fertility [28–30].

It has been reported that treatment of anestrous cows with norgestomet before induced ovulation resulted in development of a single large follicle [31] and suppressed secretion of FSH [32]. In some cows, NOR prolonged development of the preovulatory follicle for an extended period of time. In the present study (experiment 3) a rapid rate of preovulatory growth of the ovulatory follicle, in cows with normal luteal function after first postpartum estrus, was associated with a greater conception rate than in cows with a slow and prolonged follicular growth. In cycling cows, providing small doses of progestogen after regression of CL resulted in large persistent follicles and a low conception rate [33–36]. When large doses of progestogen were provided, there was a turnover of developing follicles as well as improved conception rates. Norgestomet given by silastic implants following first postpartum ovulation, during and beyond a short luteal phase in cows with a good quality embryo placed into the uterus on Day 7, allowed large follicles to develop and failed to maintain pregnancy [17]. However, exogenous P_4 sufficient to produce luteal-phase concentrations of P_4, in postpartum cows receiving good quality embryos during a short luteal phase, suppressed development of large follicles and maintained pregnancy. This is in contrast to failure of identical treatment with P_4 (experiment 4) to maintain pregnancy in cows with their own embryos during a short luteal phase. These results implicate either the oviductal and/or uterine environment before Day 7 of gestation or inherent defects in the embryo as contributors to failure of conception during a short luteal phase.

In rats, prolongation of preovulatory concentrations of estrogen affected both the oocyte and the intrauterine environment so as to increase embryonic anomalies and embryonic death, respectively [37]. Duration of follicular development is inversely related to fertility in cattle [38, 39] and rats [37, 40]. In both CON and NOR-treated cows, prolonged follicular development could result in extended elevation of E_2 preceding ovulation. In those cows with normal CL function (experiment 3), persistence of the preovulatory follicle (larger size at 5 days before the LH surge and slower rate of growth) was associated with greater concentrations of E_2 and a lower rate of fertility. However, this alone fails to explain the differences in rates of pregnancy between CON (0%) and NOR-treated (50%) cows, since no difference in rate of growth of the preovulatory follicle was observed between treatments. A wide range in rate of growth of the preovulatory follicle was observed, from 0 to 2.5 mm/day. It was found that dark areas in blastomeres, possibly an early stage of embryonic death, were associated with prolonged high concentrations of E_2 in both CON and NOR-treated cows. Thus, the mechanisms by which persistent follicles and high concentrations of E_2 reduce fertility may include actions involving decreased luteal function, actions independent of luteal function, or both.

In conclusion, the oocyte released at the beginning of a short luteal phase was capable of being fertilized, undergoing early development, and being transported into the uterus. However, supplementation with progestogen (MGA or P_4), to replace the regressing CL, failed to maintain pregnancy.

Therefore, the low fertility associated with an ovulation that begins an estrous cycle with a short-lived CL is not due only to premature regression of the CL; it may result from ovulation of an oocyte with an inherent or acquired defect that prevents its development or an oviductal or uterine environment that is hostile to the developing embryo.

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REFERENCES

1. Menge AC, Mares SE, Tyler WJ, Casida LE. Variation and association among postpartum reproductive and production characteristics in Holstein-Friesian cows. J Dairy Sci 1962; 45:233–241.
2. Foote WD, Hunter JE. Postpartum intervals of beef cows treated with progesterone and estrogen. J Anim Sci 1964; 23:517–520.
3. Morrow DA, Roberts SJ, McEntee K, Gray HG. Postpartum ovarian activity and uterine involution in dairy cattle. J Am Vet Med Assoc 1966; 149:1596–1609.
4. Morrow DA, Roberts SJ, McEntee K. A review of postpartum ovarian activity and involution of the uterus and cervix in cattle. Cornell Vet 1969; 59:134–154.
5. Humphrey WD, Koritnik DR, Kaltenbach CC, Dunn TG, Niswender GD. Progesterone and LH in postpartum suckled beef cows. J Anim Sci 1976; 43(suppl 1):290 (abstract 307).
6. LaVoie V, Han DK, Foster DB, Moody EL. Suckling effect on estrus and blood plasma progesterone in postpartum beef cows. J Anim Sci 1981; 52:802–812.
7. Manns JG, Humphrey WD, Flood PF, Mapletoft RJ, Rawlings NC, Cheng KW. Endocrine profiles and functional characteristics of corpora lutea following onset of postpartum ovarian activity in beef cows. Can J Anim Sci 1983; 63:331–347.
8. Lishman AW, Allison SMJ, Fogwell RL, Butcher RL, Inskeep EK. Follicular development and function of induced corpora lutea in underfed postpartum anestrous beef cows. J Anim Sci 1979, 48:867–875.
9. Bellows RA, Short RE, Urick JJ, Pahnish OF. Effects of early weaning on postpartum reproduction of the dam and growth of calves born as multiples or singles. J Anim Sci 1974; 39:589–600.
10. Odde KG, Ward HS, Kiracofe GH, McKee RM, Kittok RJ. Short estrous cycles and associated serum progesterone levels in beef cows. Theriogenology 1980; 14:105–112.
11. Ramirez-Godinez JA, Kiracofe GH, McKee RM, Schalles RR, Kittok RJ. Reducing the incidence of short estrous cycles in beef cows with norgestomet. Theriogenology 1981; 15:613–623.
12. Ramirez-Godinez JA, Kiracofe GH, Carnahan DL, Spire MF, Beeman KB, Stevenson JS, Schalles RR. Evidence for ovulation and fertilization in beef cows with short estrous cycles. Theriogenology 1982; 17:409–414.
13. Ramirez-Godinez JA, Kiracofe GH, Schalles RR, Niswender GD. Endocrine patterns in the postpartum beef cow associated with weaning: a comparison of the short and subsequent normal cycles. J Anim Sci 1982; 55:153–158.
14. Graves WE, Lauderdale JW, Hauser ER, Casida LE. Relation of postpartum interval to pituitary gonadotropin, ovarian follicular development and fertility in beef cows. Univ Wis Madison Coll Agric Life Sci Res Div Res Bull 1968; 270:23–26.
15. Wiltbank JN, Rowden WW, Ingalls JE, Gregory KE, Koch RM. Effect of energy level on reproductive phenomena of mature Hereford cows. J Anim Sci 1962; 21:219–225.
16. Elsden RP, Hasler JF, Seidel GE Jr. Non-surgical recovery of bovine eggs. Theriogenology 1976; 6:523–532.
17. Butcher RL, Reber JE, Lishman AW, Breuel KF, Schrick FN, Spitzer JC, Inskeep EK. Maintenance of pregnancy in postpartum beef cows having short-lived corpora lutea. J Anim Sci 1992; 70:3831–3837.
18. Butcher RL. Changes in gonadotropins and steroids associated with unilateral ovariectomy in rats. Endocrinology 1977; 101:830–840.
19. Sheffel CE, Pratt BR, Ferrell WL, Inskeep EK. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. J Anim Sci 1982; 54:830–836.
20. Niswender GD, Midgley AR Jr, Monroe SE, Reichert LE Jr. Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-131I. Proc Soc Exp Biol Med 1968; 128:807–811.
21. Garcia-Winder M, Lewis PE, Deaver DR, Smith VG, Lewis GS, Inskeep EK. Endocrine profiles associated with life span of induced corpora lutea in postpartum beef cows. J Anim Sci 1986; 62:1353–1362.
22. Steel RGD, Torrie JH. Principles and Procedures of Statistics, 2nd ed. New York: McGraw-Hill, Inc.
23. Bryner RW, Garcia-Winder M, Lewis PE, Inskeep EK, Butcher RL. Changes in hormonal profiles during the estrous cycle in old lactating beef cows. Domest Anim Endocrinol 1990; 7:181–190.
24. Wiltbank JN, Hawk HW, Kidder HE, Black WG, Ulberg LC, Casida LE. Effect of progesterone therapy on embryo survival in cows of lowered fertility. J Dairy Sci 1956; 39:456–461.
25. Zimbelman RG, Smith LW. Maintenance of pregnancy in ovariectomized heifers with melengestrol acetate. J Anim Sci 1966; 25:207–211.
26. Inskeep EK, Baker RD. Successful transfer of bovine embryos into ovariectomized recipients. J Anim Sci 1985; 61(suppl 1):409 (abstract 491).
27. Sreenan JM, Diskin MG. The extent and timing of embryonic mortality in the cow. In: Sreenan JM, Diskin MG (eds.), Embryonic Mortality in Farm Animals. Dordrecht, Netherlands: Martinus Nijhoff Publishers; 1986: 1–11.
28. Henricks DM, Dickey JF, Hill JR, Johnston WE. Plasma estrogen and progesterone levels after mating, and during late pregnancy and postpartum in cows. Endocrinology 1972; 90:1336–1342.
29. Dickey JF, Henricks DM, Bierly S. Hormonal relationships in postpartum Holstein cows. J Anim Sci 1975; 41:348 (abstract 404).
30. Williams GL, Talavera F, Petersen BJ, Kirsch JD, Tilton JE. Coincident secretion of follicle-stimulating hormone and luteinizing hormone in early postpartum beef cows: effects of suckling and low-level increases of systemic progesterone. Biol Reprod 1983; 29:362–373.
31. Garcia-Winder M, Lewis PE, Townsend EC, Inskeep EK. Effects of norgestomet on follicular development in postpartum beef cows. J Anim Sci 1987; 64:1099–1109.
32. Breuel KF, Lewis PE, Inskeep EK, Butcher RL. Endocrine profiles and follicular development in the early-weaned post-partum beef cow. J Reprod Fertil 1992; (in press).
33. Wehrman ME, Roberson MS, Stumpf TT, Wolfe MW, Cupp AS, Kojima N, Werth LA, Kittok RJ, Kinder JE. Level of exogenous progesterone (P_4) influences the time to onset of estrus and conception rate after removal of the exogenous source of P_4 from beef cows. J Anim Sci 1991; 69(suppl 1):399 (abstract 504).
34. Sirois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 1990; 127:916–925.
35. Cupp A, Garcia-Winder M, Zamudio A, Mariscal V, Wehrman M, Kojima N, Peters K, Bergfeld E, Hernandez P, Sanchez T, Kittok R, Kinder J. Two concentrations of progesterone (P_4) in circulation have a differential effect on pattern of ovarian follicular development in the cow. Biol Reprod 1992; 46(suppl 1):106 (abstract 222).
36. Savio JD, Thatcher WW. Turnover of dominant ovarian follicles is regulated by progestins and dynamics of LH secretion in cattle. J Reprod Fertil 1990; Abstract Series No. 6:23 (abstract 37).
37. Butcher RL, Pope RS. Role of estrogen during prolonged estrous cycles of the rat on subsequent embryonic death or development. Biol Reprod 1979; 21:491–495.
38. Henricks DM, Hill JR, Dickey JF. Plasma ovarian hormone levels and fertility in beef heifers treated with melengestrol acetate (MGA). J Anim Sci 1973; 37:1169–1175.
39. Echternkamp SE, Hansel W. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum estrous cycle. J Anim Sci 1973; 37:1362–1370.
40. Butcher RL, Collins WE, Fugo NW. Altered secretion of gonadotropins and steroids resulting from delayed ovulation in the rat. Endocrinology 1975; 96:576–586.

PAPER 37

Maintenance of Pregnancy in Postpartum Beef Cows That Have Short-Lived Corpora Lutea[1,2]

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ABSTRACT: The first two experiments examined the role of the uterus in low pregnancy rates of beef cows induced to ovulate by early weaning. At 20 to 25 d postpartum, one-half of the cows in Exp. 1 and 2 received a s.c. implant containing 6 mg of norgestomet (NOR) for 9 d (NOR-pretreated) and the remaining cows were untreated controls (CON). Lengths of first postpartum luteal phase after weaning of calves at d 7 after implant insertion were expected to be normal in NOR-pretreated and short in CON cows. In Exp. 1, cows of both groups received an implant containing 3 mg of NOR at d 4 after first estrus and a silastic implant with 15 or 25 mg of NOR at d 7 after first estrus. At 7 d after first estrus, two embryos were transferred into the uterus of each cow and pregnancy was diagnosed by ultrasonography at d 35. Blood samples were collected daily from onset of treatment to d 8 after estrus and then every other day to d 24. Only 4 of 22 cows were pregnant at d 35, concentrations of estradiol (E_2) were elevated after luteolysis, and large follicles were present at d 35. In Exp. 2, all cows were injected with 100 mg of progesterone (P_4) twice daily from d 4 to 35 after first estrus. Embryos were transferred, pregnancy was diagnosed, and blood samples were collected as in Exp. 1, except blood sampling was continued to d 34. A subset of seven cows was examined by ultrasonography every other day from d 6 to 24 for change in size of each large follicle. Pregnancy rates in cows injected twice daily with 100 mg of P_4 were 5 of 18 in CON cows and 9 of 16 in NOR-pretreated cows ($P < .10$). In Exp. 2, waves of follicular growth occurred and concentrations of E_2 were low during d 6 to 24 after first estrus. A third experiment using nonlactating cows examined the ability of NOR implants, as used in Exp. 1, to suppress development of large follicles and to decrease secretion of E_2 after luteolysis induced at d 6 of the estrous cycle. Large (21.6 ± .8 mm), persistent follicles, elevated concentrations (5.9 ± .4 pg/mL) of E_2, and intermittent estrous activity without standing estrus or ovulation were observed after luteolysis. It is concluded that uteri of some postpartum cows with short-lived CL are capable of maintaining gestation, if provided good-quality embryos and twice-daily injections of P_4. However, NOR implants, as used in Exp. 1, were ineffective in maintaining pregnancy, suppressing secretion of E_2, and preventing persistence of large follicles.

Key Words: Postpartum Period, Beef Cows, Pregnancy, Uterus, Follicles, Embryos.

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Introduction

In beef cows, first spontaneous or induced postpartum ovulations are associated with corpora lutea (CL) of short life span (< 14 d; Ramirez-Godinez et al., 1981, 1982a,b; Pratt et al., 1982). However, treatment with progestogen before ovulation resulted in CL with normal life span (Ramirez-Godinez et al., 1981; Pratt et al., 1982; Sheffel et al., 1982). Ovulation and fertilization occur at the expected time after estrus preceding a short luteal phase (Graves et al., 1968; Ramirez-Godinez et al., 1982a). Breuel (1991) found that rates of recovery of embryos or ova (89%), fertilization rates (70%), and rates of development of fertilized ova to the two- to eight-cell stage (88%) at d 3 after estrus did not differ between norgestomet (NOR)-pretreated and control (CON) cows induced to ovulate by early weaning. Recovery of embryos on d 6 after estrus confirmed transport into the uterus in cows expected to have short (6 of 7) or normal (5 of 7) luteal phases. Feeding 2 mg of melengestrol acetate daily or injection of 100 mg of progesterone twice daily to d 35 after estrus, as replacement therapy for short-lived CL, did not maintain pregnancy in early-weaned postpartum cows (Breuel, 1991). It was concluded that short luteal phases are not the only limiting factor in low pregnancy rates. Other factors may include quality of oocytes (embryos) and(or) inadequate preparation of the uterus to support pregnancy.

The objective of the present studies was to test the null hypothesis that the ability of the early postpartum uterus to support pregnancy did not differ between beef cows with short or normal luteal phases when provided exogenous progestogen and normal embryos.

Materials and Methods

Experiment 1

Twenty-two multiparous cows that calved during fall were placed on treatment at 23 ± .5 d postpartum. Equal numbers of cows were assigned randomly to receive an ear implant containing 6 mg of norgestomet (17α-acetoxy-11β-methyl-19-norpreg-4-ene-3,20-dione) for 9 d (NOR-pretreated) or to serve as untreated controls (CON). Calves were weaned from all cows 7 d after assignment to treatment to induce first estrus and ovulation. Life span of induced CL was expected to be short in CON cows and normal in NOR-pretreated cows.

Cows were observed for estrous behavior twice daily for a minimum of 30 min at dawn and dusk, and detection of estrus was assisted by continuous exposure of cows to an androgenized cow fitted with a Chin-ball® marker. Initially, each cow in both treatment groups received one-half of a 6-mg NOR implant (3 mg) on d 4 postestrus and a silastic implant containing 25 mg of NOR on d 7. As the experiment progressed, overall pregnancy rates were low. Therefore, the last four cows in the study received a silastic implant containing 15 mg of NOR (n = 2) or 25 mg of NOR (n = 2) at d 4 instead of the 3 mg of NOR implant.

On d 7 after estrus, two frozen-thawed embryos were transferred nonsurgically into the uterine horn ipsilateral to the CL in each cow (Elsden and Seidel, 1982). Two embryos (excellent, good, or fair quality blastocysts and late morulae) were transferred to minimize variation in results due to individual embryos (Hart-Elcock et al., 1990). Each cow received either two good or one excellent and one fair embryo. As a control for transfer technique and to determine whether there were detrimental effects of NOR on pregnancy, embryos also were transferred into 11 nonlactating beef cows at d 7 after estrus. Four of these 11 cows received NOR implants at d 4 and 7, as described for postpartum cows. Ultrasonography (Pie Data 400 with a 7.5-MHz probe; Pie Medical, BV, Maastricht, The Netherlands) was performed transrectally at d 35 for diagnosis of pregnancy, which was confirmed by rectal palpation at d 60.

Blood samples were collected via jugular venipuncture daily (0700) from d −11 (d −9 = day of 6 mg of NOR implant) until d 8 after estrus and then every other day to d 24. A second sample was taken each day on d −9, −8, and −7 (1600) and a single sample was collected on d 35. Samples were allowed to clot at 4°C; serum was collected after centrifugation and stored at −20°C until assays were performed for estradiol-17β (E_2) and progesterone (P_4) by RIA (Butcher, 1977; Sheffel et al., 1982). Sensitivities of assays were 1 pg/tube for E_2 and 20 pg/tube for P_4. Intra- and interassay CV were, respectively, 5.2 and 6.2% for E_2 and 5.4 and 7.5% for P_4.

Pregnancy rates were compared by chi-square analysis and patterns of concentrations of E_2 and P_4 were examined by least squares analysis for a split-plot over time (Steel and Torrie, 1980). The GLM procedure of SAS (1988) was used for all analyses.

Experiment 2

In Exp. 1, progestational effects of NOR, delivered by implants that contained a total of up to 50 mg, seemed to have been inadequate. Secretion of E_2 was not suppressed and large follicles were retained, as observed with low doses of P_4 in nonpregnant cows (Sirois and Fortune, 1990). Therefore, in Exp. 2, P_4 was used as the supplemental progestogen after first estrus, at a dosage known to maintain pregnancy in cows after removal of the CL at d 58 to 68 of gestation (McDonald et al., 1952). Forty-one multiparous beef cows that calved in spring were assigned randomly within breed (Simmental, Angus, and crossbred), body condition score, and pretreatment (NOR-pretreated, n = 19 or CON, n = 22) as in Exp. 1, at 21.5 ± .2 d postpartum. Weaning, observation for estrus, and embryo transfer on d 7 were carried out as in Exp. 1. Progesterone at a dose of 100 mg in 4 mL of corn oil with 10% benzyl alcohol was injected s.c. behind the shoulders of each CON and NOR-pretreated cow, twice daily from d 4 to 35 after estrus. Ultrasonography was performed on d 15 for detection of presence or absence of a CL and on d 35 for detection of CL and pregnancy.

Blood samples were collected via jugular venipuncture daily (0700) from onset of treatment to d 8 and then every other day to d 34. Serum was collected and stored and assays were performed as in Exp. 1.

A subset of seven cows (four CON and three NOR-pretreated) was examined by ultrasonography every other day from d 6 to 24, to determine whether large follicles developed and persisted when 100 mg of P_4 was injected twice daily. Size and location of all follicles $\geq$ 5 mm were recorded. The pattern of change in size of each large follicle was used to assess whether large follicles persisted.

Measurements of P_4 in plasma showed that six cows (three CON and three NOR-pretreated) had luteal function (P_4 > 1 ng/mL) before first observed estrus and one CON cow had asynchrony of estrus and ovulation. These seven cows were removed from the study; nevertheless, four of the six cows with a prior luteal phase were pregnant by transfer of embryos. Intervals from weaning to estrus were examined by analysis of variance, and pregnancy rates were compared by a chi-square test (Steel and Torrie, 1980). Patterns of concentrations of E_2 and P_4 in four groups (nonpregnant NOR-pretreated, pregnant NOR-pretreated, nonpregnant CON, and pregnant CON) were examined using least squares analysis for a split-plot over time (Steel and Torrie, 1980). The model included pretreatment with NOR, pregnancy status, concentration of P_4, and interactions among these variables. The GLM procedure of SAS (1988) was used for all analyses.

Experiment 3

A high frequency of estrous activity during d 7 to 24 after estrus and presence of large follicles (20 to 25 mm) at diagnosis of pregnancy on d 35 were noted in Exp. 1. These observations are consistent with persistence of large follicles as opposed to the expected continuation of waves of follicular development during early pregnancy (Ginther et al., 1989). Therefore, a group of nine cycling, nonlactating cows was used to determine whether supplementation with NOR, as in Exp. 1, would result in persistence of large follicles in the absence of a CL. Each cow received an ear implant containing 15 (n = 3) or 25 (n = 6) mg of NOR at d 4 and a second implant with 25 mg of NOR at d 6 after spontaneous estrus. At the time of the second implant (d 6), each cow received an i.m. injection of 25 mg of prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) to induce luteolysis.

Size of all follicles $\geq$ 5 mm was measured by ultrasonography at d 4, 9, 18, and 24 after estrus to

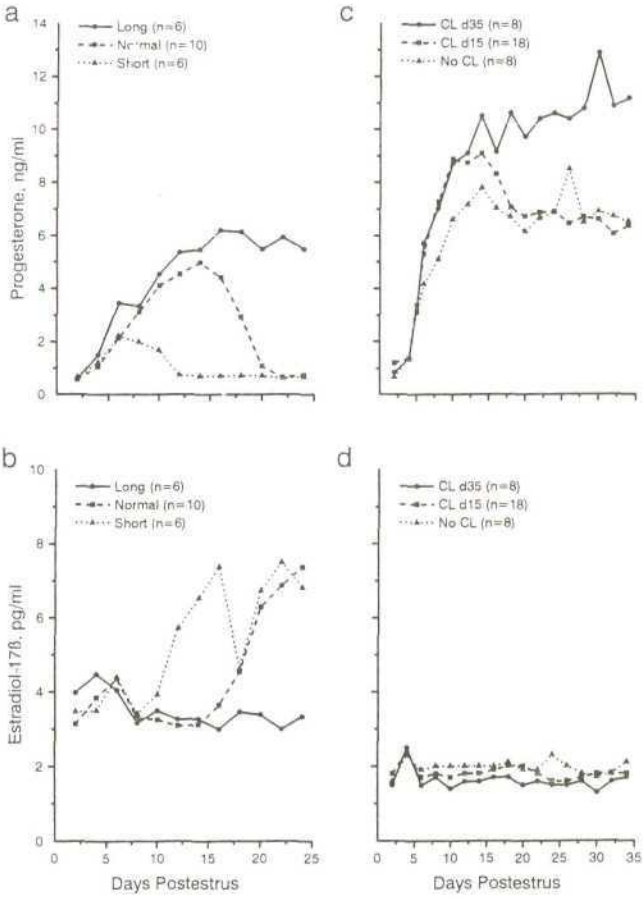

Figure 1. Concentrations of progesterone (P_4) and estradiol-17β (E_2) in peripheral serum. (a) Patterns of P_4 in cows grouped by length of luteal function during the presence of norgestomet implants in Exp. 1. (b) Patterns of E_2 in the same cows as in Panel a. (c) Patterns of P_4 in cows grouped by presence or absence of a corpus luteum at d 15 and 35 during treatment with 100 mg of P_4 twice daily in Exp. 2. (d) Patterns of E_2 in the same cows as in Panel c. Error mean squares were 1.13, 2.79, 2.44, and .17 for Panels a, b, c, and d, respectively.

Table 1. Pregnancy rate at day 35 in relation to prior corpus luteum (CL) and length of luteal phase (Exp. 1)

Length of luteal phase	Pregnancy rate: Control, Prior CL	Control, No prior CL	NOR-pretreated, Prior CL	NOR-pretreated, No prior CL
< 10 d	0/1	0/2	0/1	0/2
16 to 20 d	1/4	—	0/3	0/3
≥ 24 d	1/2	1/2	—	1/2

determine whether large follicles, similar in size to those observed at d 35 in Exp. 1, were present. Blood samples were collected daily from d 1 to 9 and every 3rd d from d 12 to 24. Serum was processed and assays were performed for P_4 and E_2 as in Exp. 1.

Results

Experiment 1

There was a high incidence (50%) of formation of CL ($P_4 > 1$ ng/mL) before first observed estrus (4 of 11 NOR-pretreated, all 4 before implant insertion; 7 of 11 CON cows, 4 before and 3 after weaning). Eight of 11 luteal phases before first estrus were of short duration (< 14 d). Luteal phases after first estrus were short (< 10 d), normal (16 to 20 d), or prolonged (≥ 24 d) in 3, 4, and 4 of 11 CON cows and 3, 6, and 2 of 11 NOR-pretreated cows, respectively (Table 1). None of the six cows with short luteal phases after estrus was pregnant, whereas one of 10 cows with normal luteal phases was pregnant at d 35, but not pregnant at d 60. Of the six cows (four CON and two NOR-pretreated) with luteal function ≥ 24 d, three were pregnant at d 35, but only two (both CON cows) maintained pregnancy to d 60. The four cows (included in the above results) that received either a 15- or 25-mg implant instead of a 3-mg implant at d 4, in addition to the 25 mg at d 7 (three with short and one with normal luteal phases), were not pregnant. However, transfer of embryos to cycling cows, as a control for technique, resulted in pregnancy in two of four cows given NOR implants and four of seven cows without implants, for a total of 54% pregnancy rate.

Patterns of concentrations of E_2 were related to length of luteal phase. Concentration of E_2 was affected by concentration of P_4 (regression of −.53; $P < .001$; Figure 1a,b), but not by any other treatment or condition. The peak in concentrations of E_2 at d 12 to 16 in cows with a short-lived CL was due to very high levels in one cow. A second ovulation had not occurred ($P_4 < 1$ ng/mL) by d 24 in these cows with NOR implants. Large follicles (20 to 25 mm) were present in 10 of 19 cows without CL at time of diagnosis for pregnancy (d 35). Estrous activity (including mounting of other cows, attempted mounting by the androgenized cow, vulval swelling, and mucous discharge), but no standing estrus, was noted in 7 of 11 CON and 7 of 11 NOR-pretreated cows after regression of the CL during the first 24 d after first estrus, even though NOR implants were present. These periods of estrous activity varied in duration from 1 to 7 d and occurred twice in seven cows. In 12 of 14 cows, one period of activity began 1 to 2 d after P_4 declined to < 1 ng/mL, when concentrations of E_2 were elevated (5.5 ± .5 pg/mL) in peripheral serum.

Experiment 2

Interval from weaning to estrus was 5.8 ± .8 and 5.6 ± .6 d for CON and NOR-pretreated cows, respectively. There was a trend ($P < .10$) toward a decreased pregnancy rate in CON cows (28%) compared with NOR-pretreated cows (56%). Data regarding presence or absence of CL at d 15 and 35, relative to pregnancy status, are summarized in Table 2. Eight of 18 CON cows did not have echogenic CL by ultrasonography at d 15. Three of these eight cows maintained pregnancy. Two of the remaining 10 CON cows (CL present at d 15, absent at d 35) maintained pregnancy. Corpora lutea were present in all NOR-pretreated cows at d 15. Two NOR-pretreated cows were pregnant at d 35 without echogenic CL. Seven of eight NOR-pretreated cows with a CL at d 35 were pregnant.

Concentrations of P_4 (effect of endogenous and exogenous) are plotted in Figure 1c by length of luteal life span that was determined by ultrasonography. A decline in concentrations of P_4 of 2 to 3 ng/mL occurred between d 14 and 20 in pregnant CON cows and nonpregnant NOR-pretreated cows, whereas in nonpregnant CON cows, P_4 reached a plateau after d 10 (data not shown). Cows with a CL at d 35 showed a small increase in P_4 from d 10 to 35 (Figure 1c). Concentrations of P_4

Table 2. Relationship between pregnancy and presence of a corpus luteum (CL) at days 15 and 35 after estrus (Exp. 2)

		No CL d 15		CL d 15 No CL d 35		CL d 15 and 35		
Treatment	n	Preg[a]	Not preg	Preg	Not preg	Preg	Not preg	Overall % pregnant
Norgestomet	16	0	0	2	6	7	1	56%
Controls	18	3	5	2	8	0	0	28%[b]

[a] Preg = pregnant.
[b] Differs ($P < .10$) from norgestomet-treated, by chi-square test.

(ng/mL) during d 2 to 34 were greater in pregnant cows (7.8 ± .5), especially NOR-pretreated cows (8.3 ± .3), than (6.3 ± .4) in nonpregnant cows (pregnancy status, $P < .05$; pregnancy × time and pretreatment × pregnancy × time, $P < .01$). Concentrations of E_2 during the period of treatment with P_4 were decreased in association with increased concentrations of P_4, pretreatment with NOR, and prolonged structural life span of CL, and there was a $P_4 \times$ pretreatment interaction ($P <$ .05; Figure 1d).

In the subset of seven postpartum cows examined by ultrasonography every other day (d 6 to 24), waves of follicular growth and regression were observed. The number of dominant follicles > 10 mm per cow that underwent waves of growth and regression by d 24 varied from two to three follicles (mean 2.1 ± .1), and another one or two (mean 1.4 ± .2) follicles > 10 mm were in a growth phase at d 24. The diameter (Figure 2a) of the largest follicle in each cow during d 6 to 24 ranged from 12 to 15 mm (mean 13.9 ± .4 mm). Concentrations of E_2 and P_4 are depicted in Figure 2a for these seven cows. Concentrations of E_2 were low (range of means per cow 1.4 ± .1 to 2.4 ± .1, overall mean 1.8 ± .1 pg/mL), and P_4 ranged from 5.6 ± .5 to 11.9 ± 1.0 (mean 7.9 ± .8 ng/mL).

Experiment 3

In nine cycling cows with NOR implants (15 or 25 mg on d 4 and 25 mg on d 6) and $PGF_{2\alpha}$-induced luteolysis at d 6, seven cows had one, and two cows had two large follicles that persisted to d 24. The average largest diameter of these follicles was 21.6 ± .8 mm and occurred at either d 18 or 24 (Figure 2b). Size of the largest nonpersistent follicle ranged from 6 to 15 mm (mean 10.4 ± 1.1 mm). Concentrations of E_2 remained relatively high (mean per cow ranged from 4.7 ± .7 to 8.1 ± 1.2 pg/mL), whereas P_4 remained at basal levels (< 1 ng/mL) during d 7 to 24 (Figure 2b). Estrous activity, as described for Exp. 1, was observed for periods of 1 to 5 d in seven of nine cows, but none of these cows was in standing estrus.

Discussion

Providing 25 mg of NOR in a silastic implant was not sufficient to maintain pregnancy from transferred embryos after the first postpartum ovulation in cows with short or normal duration of luteal function. Although NOR prevented ovulation, it did not suppress follicular growth or secretion of E_2 after the CL had regressed. An inadequate level of progestogen also was indicated by estrous activity in 14 of 22 cows after luteal regression, although none of the cows exhibited standing estrus. Pregnancy was maintained by two implants, each containing 15 mg of NOR, in cows ovariectomized on d 10 of gestation (R. J. Favero, E. A. Henderson, and D. J. Kesler, 1988–1989 Beef Cattle Research Report, Univ. of Illinois). Zimbelman and Smith (1966) reported that 1 mg of melengestrol acetate daily was adequate to maintain pregnancy in cows when both ovaries were removed at d 56 of gestation, whereas 4 mg (but not 1 mg) maintained gestation when one ovary without a CL remained. Likewise, McDonald et al. (1952) found that a higher dose of P_4 was required to maintain pregnancy in cows after removal of the CL than in ovariectomized cows.

Injection of 100 mg of P_4 twice daily resulted in a pregnancy rate of 28% in CON cows with transferred embryos, which establishes that the uterus in some cows during the first postpartum estrous cycle is capable of maintaining gestation. None of these cows had a previous luteal phase and they were expected to have a high incidence of short-lived CL. Treatment with exogenous P_4 prevented confirmation of functional life span of CL by measurement of serum P_4, but eight cows (three pregnant) did not have echogenic CL by ultrasonography at d 15. In the remaining 10 CON cows (two pregnant), the CL was echogenic at d 15, but not at d 35; some of the cows with an echogenic CL at d 15 could have had a shorter than normal duration of luteal function.

There was a trend for an increased pregnancy rate (56%) in NOR-pretreated compared with CON cows (28%; $P < .10$). The greater concentration of

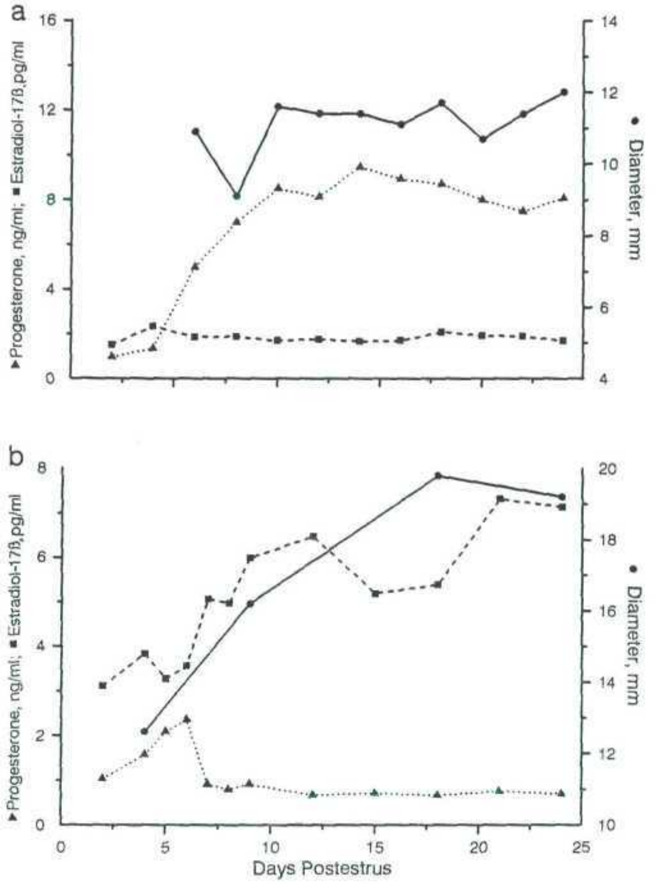

Figure 2. (a) Patterns of concentrations of progesterone (P_4), concentrations of estradiol-17β (E_2), and diameters of the largest follicle during treatment of cows with 100 mg of P_4 twice daily from d 2 through 24 postestrus (Exp. 2). (b) Patterns of concentrations of P_4, concentrations of E_2, and diameters of the largest follicle on d 4, 9, 18, and 24 after estrus in cows with two implants, each containing 25 mg of norgestomet, and in which the corpus luteum was regressed with $PGF_{2\alpha}$ given at d 6 (Exp. 3). Error mean squares were 3.84 and .11 for P_4, .19 and 3.98 for E_2, and 4.42 and 13.96 for follicular diameter in Panels a and b, respectively.

P_4 in eight NOR-pretreated cows with prolonged presence of a CL (seven of eight pregnant) cannot be ruled out as contributing to this increased pregnancy rate. However, neither feeding of melengestrol acetate (2 mg/d) nor injection of 100 mg of P_4 twice daily maintained any pregnancies after breeding at first postpartum estrus in intact cows with a short-lived CL (Breuel, 1991). Thus, although pregnancy was maintained after transfer of good-quality embryos in 28% of CON cows with a short luteal phase and provided with exogenous P_4, pregnancy was not maintained by exogenous P_4 in cows with their own embryo. We interpret this to mean that either 1) the oocyte contributes to failure of pregnancy, or 2) the oviduct and(or) uterus has a lethal effect before d 7 of gestation, or 3) both the oocyte and reproductive tract contribute to early embryonic death.

Injections of 100 mg of P_4 twice daily maintained concentrations of P_4 at approximately 5 to 7 ng/mL after regression of CL, kept E_2 concentrations < 2 pg/mL, prevented formation of large follicles, and allowed waves of follicular growth and atresia in Exp. 2. In contrast, NOR implants, as used in Exp. 1 and 3, allowed development of large, persistent follicles (20 to 25 mm) that secreted greater amounts of E_2 (mean = 5 pg/mL in Exp. 3). These findings are consistent with those of Sirois and Fortune (1990), who found that treatment with high concentrations of P_4 produced waves of growth and atresia of follicles, whereas low concentrations of P_4 resulted in persistence of follicles. Similarly, Custer et al. (1991) reported that feeding melengestrol acetate (.5 mg/d) resulted in persistence of the dominant follicle, whereas use of P_4-releasing intravaginal devices allowed follicular turnover after luteolysis. The persistence of large follicles during periods of low progestogen is associated with increased frequency of pulsatile secretion of LH (Roberson et al., 1989; Savio et al., 1990). Elevated concentrations of E_2 could be due to an increase in pulsatile secretion of LH or to reduced suppression of E_2 by an action of P_4 directly on the follicle (Fogwell et al., 1978). High levels of E_2 or other factors associated with persistent follicles could have been responsible for loss of pregnancy in Exp. 1, because larger doses of P_4 were required to maintain gestation in the presence of ovaries without CL than in ovariectomized cows (Mcdonald et al., 1952). Use of embryo transfer in conjunction with steroid treatment of ovariectomized ewes (Moore, 1985; Wilmut et al., 1985) shows that there is a need for critical timing and concentrations of steroids before, during, and after estrus for survival and development of embryos.

In summary, ovulation occurred at the beginning of a short luteal phase in the postpartum cow, the embryo was transported into the uterus, and some embryos developed to the morula stage (Graves et al., 1968; Ramirez-Godinez et al., 1982a; Breuel, 1991). However, cows bred at the first postpartum ovulation failed to maintain pregnancy, even when provided exogenous progestogen (Breuel, 1991). The uterus of some early postpartum cows can maintain pregnancy, as shown in the present study, by providing exogenous P_4 and transferring good-quality embryos into the uterus.

Implications

The uterus of the beef cow during the short luteal phase after first postpartum ovulation is capable of maintaining pregnancy, when good-quality embryos and exogenous progesterone are provided. Thus, the short-lived corpus luteum is not the only cause of low pregnancy rate at first postpartum estrus. The lower pregnancy rate in control cows than in cows treated with norgestomet before first ovulation is interpreted to mean that a lower-quality oocyte or a hostile environment in the oviduct and(or) uterus before d 7 also is involved. Additional experiments are needed before information from this study can be applied fully to improving conception rate at first postpartum estrus.

Literature Cited

Breuel, K. F. 1991. Factors affecting fertility in the postpartum beef cow. Ph.D. Dissertation. West Virginia University, Morgantown.

Butcher, R. L. 1977. Changes in gonadotropins and steroids associated with unilateral ovariectomy of the rat. Endocrinology 101:830.

Custer, E. E., W. E. Beal, S. J. Hall, and A. W. Meadows. 1991. Effect of melengestrol acetate (MGA) and progesterone-releasing intravaginal devices (PRID) on follicular development in cattle. J. Anim. Sci. 69(Suppl. 1):137 (Abstr.).

Elsden, R. P., and G. E. Seidel, Jr. 1982. Procedures for recovery, bisection, freezing and transfer of bovine embryos. Bull. 2, Anim. Reprod. Lab., Colorado State University, Fort Collins.

Fogwell, R. L., C. W. Weems, G. S. Lewis, R. L. Butcher, and E. K. Inskeep. 1978. Secretion of steroids after induced luteal regression in beef heifers: Effects of $PGF_{2\alpha}$ and removal of corpora lutea. J. Anim. Sci. 46:1718.

Ginther, O. J., L. Knopf, and J. P. Kastelic. 1989. Ovarian dynamics in heifers during early pregnancy. Biol. Reprod. 41:247.

Graves, W. E., J. W. Lauderdale, E. R. Hauser, and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotropins, ovarian follicular development and fertility in beef cows. In: Studies on the Postpartum Cow. p 23. Univ. of Wisconsin College of Agric. and Life Sci. Bull. No. 270. Madison.

Hart-Elcock, L., R. D. Baker, and H. W. Leipold. 1990. Growth of the early bovine fetus. J. Vet. Med. Ser. A 37:294.

McDonald, L. M., R. E. Nichols, and S. H. McNutt. 1952. Studies on corpus luteum ablation and progesterone replacement therapy during pregnancy in the cow. Am. J. Vet. Res. 13:446.

Moore, N. W. 1985. The use of embryo transfer and steroid hormone replacement therapy in the study of prenatal mortality. Theriogenology 23:121.

Pratt, B. R., J. G. Berardinelli, L. P. Stevens, and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cows. I. Comparison of gonadotropin releasing hormone and human chorionic gonadotropin and effects of progestogen and estrogen. J. Anim. Sci. 54:822.

Ramirez-Godinez, J. A., G. H. Kiracofe, D. L. Carnahan, M. F. Spire, K. B. Beeman, J. S. Stevenson, and R. R. Schalles. 1982a. Evidence for ovulation and fertilization in beef cows with short estrous cycles. Theriogenology 17:409.

Ramirez-Godinez, J. A., G. H. Kiracofe, R. M. McKee, R. R. Schalles, and R. J. Kittock. 1981. Reducing the incidence of short estrous cycles in beef cows with norgestomet. Theriogenology 15:613.

Ramirez-Godinez, J. A., G. H. Kiracofe, R. R. Schalles, and G. D. Niswender. 1982b. Endocrine patterns in the postpartum beef cow associated with weaning: A comparison of the short and subsequent normal cycles. J. Anim. Sci. 55:153.

Roberson, M. S., M. W. Wolfe, T. T. Stumpf, R. J. Kittock, and J. E. Kinder. 1989. Luteinizing hormone secretion and corpus luteum function in cows receiving two levels of progesterone. Biol. Reprod. 41:997.

SAS. 1988. SAS User's Guide: Statistics. (Version 6 Ed.). SAS Inst. Inc., Cary, NC.

Savio, J. D., W. W. Thatcher, L. Badinga, and R. L. de la Sota. 1990. Turnover of dominant ovarian follicles is regulated by progestins and dynamics of LH secretion in cattle. J. Reprod. Fertil. 6:23 (Abstr.).

Sheffel, C. E., B. R. Pratt, W. L. Ferrell, and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. J. Anim. Sci. 54:830.

Sirois, J., and J. E. Fortune. 1990. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: A model for studying ovarian follicular dominance. Endocrinology 127:916.

Steel, R.G.D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Book Co., New York.

Wilmut, I., D. I. Sales, and C. J. Ashworth. 1985. The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. Theriogenology 23:107.

Zimbelman, R. G., and L. W. Smith. 1966. Maintenance of pregnancy in ovariectomized heifers with melengestrol acetate. J. Anim. Sci. 25:207.

PAPER 38

The effect of restricted suckling on LH and ovarian steroids after induced ovulation in *Bos taurus* and *Bos indicus* cows

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A total of 37 first-calf cows and their calves, comprising 19 Drakensbergers and 18 Hereford × Simmentalers, were subjected to either normal or once-daily suckling for 15 days, commencing at either day 35 or 60 post-partum in a 2 × 2 × 2 factorial experiment. All cows received common hormone therapy during the normal or restricted suckling periods. A Norgestomet ear implant was inserted in conjunction with an oestrogen injection on the first day, and removed on the tenth day of the variable suckling periods. The cows were injected with gonadotropin releasing hormone (GnRh) 30 h after the removal of the ear implants, and inseminated 18 and 42 h after the GnRh injection. There was a tendency for a greater ($P \geqslant 0,05$) proportion of the cows suckled once daily to ovulate than those suckled normally, and this effect was most marked in the Drakensberger cows treated between days 60 and 75 post-partum. In the Drakensbergers, restricted suckling tended to increase the proportion of cows which conceived to the fixed-time inseminations, and to decrease the incidence of 'short' oestrous cycles following GnRh. Tonic luteinizing hormone (LH) levels, the release of LH in response to GnRh and oestrogen secretion were not affected by any of the variables studied.

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'n Totaal van 37 eerstekalf-koeie en hulle kalwers, wat uit 19 Drakensbergers en 18 Hereford × Simmentalers bestaan het, is onderwerp aan óf normale soging óf soging een keer per dag in 'n 2 × 2 × 2-faktoriale proefontwerp. Die twee sogingsbehandelings het óf op dag 35 óf dag 60 postpartum 'n aanvang geneem en het 15 dae geduur. Alle koeie het standaard-hormoonterapie ontvang gedurende die periodes van normale of beperkte soging. 'n Norgestomet-oorinplanting is tesame met 'n estrogeeninspuiting op die eerste dag toegedien en op die tiende dag van die normale of beperkte sogingsperiodes verwyder. Die koeie is 30 uur na die verwydering van die oorinplanting met gonadotropien-vrystellingshormoon (GnVh) ingespuit, en 18 en 42 uur daarna geïnsemineer. Meer koeie het geneig om tydens beperkte soging te ovuleer as tydens normale soging. Hierdie effek was veral merkbaar in die Drakensbergerkoeie wat behandel is tussen dae 60 en 75 postpartum. Beperkte soging het in die Drakensbergers gelei tot 'n vermeerdering in die persentasie besette koeie asook 'n vermindering in die voorkoms van 'kort' siklusse na GnVh-toediening. Basale luteïniserende hormoon-(LH)-konsentrasies, die afskeiding van LH na die GnVh-inspuiting en estrogeensekresie is nie deur behandeling beïnvloed nie.

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Introduction

Reducing the interval between calving and reconception is a priority for efficient reproductive performance in beef herds. The duration of the post-partum anoestrous period is influenced by a number of factors, including lactation (Laster, Glimp & Gregory, 1973). The stimulus of suckling by the calf, and not the process of lactation *per se*, is important in beef cows (Wiltbank & Cook, 1958; Wetteman, Turman, Wyatt & Totusek, 1978). Measures such as early weaning (Smith & Vintcent, 1972), temporary weaning (Hollness, Hopley & Hale, 1978) and restricted suckling (Randel, 1981) have been found to hasten the re-initiation of oestrous cycles after calving. The physiological mechanisms whereby suckling depresses ovarian function are not clear, and results relating to the influence of suckling on hormone secretion are somewhat inconsistent. Echternkamp (1978), Radford, Nancarrow & Mattner (1978) and Forrest, Rhodes & Randel (1980) showed that suckling depresses tonic LH secretion, although Chang, Gimenez & Henricks (1981) and Williams, Kotwica, Slanger, Olsen, Tilton & Johnson (1982) were unable to demonstrate such an effect. Suckling also depressed LH release in response to gonadotropin releasing hormone (GnRh) in certain studies (Carter, Dierschke, Rutledge & Hauser, 1980; Troxel, Kesler, Noble & Carlin, 1980) but not in others (Echternkamp, 1978). The object of this study was therefore to determine the effect of suckling on LH, oestrogen and progesterone secretion and on ovarian function at different stages of the post-partum period. *Bos taurus* and *Bos indicus* cows were used in this experiment, since evidence exists to indicate that the problem of lactation anoestrus is greater, and overall reproductive ability lower in *Bos indicus* than in European breeds of cattle (Coetzer, Mentz, Vermeulen & Coetzee, 1975; Adeyemo & Heath, 1980).

Procedure

Animals

A total of 37 first-calf cows and their calves, comprising 19 Drakensbergers *(Bos indicus)* and 18 Hereford × Simmentaler (British crosses) were used in the experiment. When calving commenced on 11 August the cows were approximately three years old. Calving continued for 45 days. Once the cows had calved they were placed in feeding pens and fed a ration consisting of *ad lib* maize silage, approximately 3 kg *Eragrostis curvula* hay and 0,3 kg high protein concentrate per cow per day.

Treatments

The experiment incorporated a 2 × 2 × 2 factorial design and involved a comparison of the reproductive performance of Drakensberger and British-cross cows subjected to either normal or once-daily suckling during two stages of the post-partum period, *viz* days 35 – 50 and 60 – 75. Following calving each breed type was divided into four groups, each balanced for cow and calf masses and date of calving. The four groups were randomly allocated to four treatments (Table 1).

Table 1 Outlay of experimental treatments employed

Breed	Days post-partum during which suckling intensity was varied	Suckling intensity	*n*	Treatment
British-cross	35 – 50	Normal	5	1
		Restricted	5	2
	60 – 75	Normal	4	3
		Restricted	4	4
Drakensberger	35 – 50	Normal	5	5
		Restricted	5	6
	60 – 75	Normal	4	7
		Restricted	5	8

In view of the well established need for a preovulatory increase of blood progesterone levels in anoestrous cows (Donaldson, Basset & Thorburn, 1970; Yuthasastrakasol, Palmer & Howland, 1975), all cows in the experiment were subjected to progesterone and oestrogen therapy, followed by an injection of GnRh, as standard treatment during the 15-day restricted or normal suckling periods. A Norgestomet ear implant (Intervet S.A.) was inserted in conjunction with an I.M. injection of 3 mg Norgestomet and 6 mg oestradial valerate on the first day, and removed on the tenth day of the normal or restricted suckling periods. On day 11 of these periods (30 h after the removal of the ear implants) the cows' ability to release LH and to ovulate was challenged further by injecting them (I.M.) with 500 μg GnRh (Abbott). At 18 and 42 h after the GnRh injection (48 and 72 h after implant removal) fixed-time inseminations were performed on the cows.

Cows subjected to restricted suckling were allowed access to their calves between 08h00 and 08h30 each morning during the 15-day period of restricted suckling. After suckling, the cows were kept in feeding pens approximately 400 m from the calves. The latter were housed in partly enclosed pens with free access to water, a concentrate mixture (80 % maize meal, 20 % lucerne meal) and *Eragrostis curvula* hay.

Measurements obtained

All cows were joined with masculinized teaser cows between 06h00 and 07h00, and again between 17h00 and 18h00 each day from day 20 post-partum until five days after the second fixed-time insemination was performed. The cows were rectally palpated for the presence of *corpora lutea* immediately prior to the insertion of the ear implants, and again approximately one week after the injection of GnRh. Conception rates were established from subsequent calving records.

Cows and calves were weighed at fortnightly intervals throughout the experiment. The body condition of each cow was assessed (thin = 1, fat = 5) immediately prior to the insertion of the ear implants by the scoring system described by Van Niekerk & Louw (1982).

The influence of treatment on tonic LH secretion was investigated by bleeding the cows at intervals of 15 min for a period of 2 h immediately prior to the insertion of the ear implants. This schedule was repeated five days after implant insertion, and 2 h after implant removal. LH was also measured in samples obtained at intervals of 30 min for a total period of 9 h after the GnRh injection. Indwelling polyethylene catheters (Clay Adams) were inserted into the jugular vein immediately prior to the sequential collection of samples described above. The cows were restrained in feeding stanchions during the periods of frequent blood collection.

Blood samples were obtained *via* venipuncture from all cows once daily for five days prior to, and at six-hourly intervals for 18 h prior to the GnRh injection for subsequent analysis of total oestrogens. Progesterone concentrations were determined in samples obtained at intervals of three days for a period of 20 days after the GnRh injection.

Blood samples were collected into heparinized syringes, centrifuged within 30 min of collection and stored at – 15°C pending analysis.

Hormone determinations

LH was measured in plasma according to the radioimmunoassay described by Niswender, Reichert, Midgley & Nalbandov (1969) and validated by Lishman (1972) in this laboratory. When samples collected to measure tonic LH secretion were analysed, the assay was modified slightly to obtain greater sensitivity. The initial dilution of anti-LH serum was changed from 1:100 000 to 1:160 000, and the incubation of antiserum with standards and unknown plasma samples prior to the addition of labelled tracer was increased from 24 to 48 h. The inter- and intra- assay coefficients of variation for LH were 12,3 and 4,3 %, respectively. Progesterone and oestrogen were measured according to the methods described by Butcher, Collins & Fugo (1974). Recovery of labelled progesterone added to plasma varied from 92,8 to 96,3 %, and the within- and between- assay coefficients of variation were 8,8 and 12,7 %, respectively. Total oestrogens were determined, since oestradiol-17β obtained after column chromotography was undetectable. Levels were corrected for recovery of tritiated oestrogen, which varied from 72,9 to 80,0 %. The within- and between- assay coefficients of variation were 14,2 and 18,6 %, respectively.

Statistical analysis

Analyses of variance were used to test the effect of treatment on the different parameters of hormone secretion. An analysis of covariance was applied to test whether tonic LH levels before, differed from those measured during the normal or restricted suckling periods, and the Chi-squared test to determine whether treatment affected ovulatory responses and conception rates. Regression analyses were used to study the relationship between the different parameters measured.

Results

Ovulatory response of treated cows

None of the cows had exhibited oestrus or had palpable *corpora lutea* prior to the insertion of the progesterone ear implants at the commencement of the normal or restricted suckling periods. The ovulatory response of the cows (Table 2) was gauged by rectal palpation of the ovaries approximately one week after the GnRh injection. In addition, the pattern of progesterone secretion during the 21 days which followed the administration of GnRh was used to indicate ovulations. It is evident from Table 2 that there was a tendency for a greater ($P \geqslant 0,05$) proportion of the cows suckled once daily to

Table 2 Number of cows which ovulated, conceived and underwent 'short' cycles in response to GnRh

Breed	Days post-partum treated	Suckling intensity	n	No. of cows which ovulated in response to GnRh	No. of cows which conceived to fixed-time inseminations	No. of cows which underwent 'short cycles'*
British-cross	35 – 50	Normal	5	2 (40 %)	1 (20%)	0
		Restricted	5	3 (60 %)	0	0
	60 – 75	Normal	4	3 (75 %)	1 (25%)	1 (25%)
		Restricted	4	3 (75 %)	2 (50%)	1 (25%)
Drakensberger	35 – 50	Normal	5	3 (60 %)	0	2 (40%)
		Restricted	5	4 (80 %)	2 (40%)	1 (20%)
	60 – 75	Normal	4	2 (50 %)	1 (25%)	1 (25 %)
		Restricted	5	5 (100%)	3 (60%)	0

* Progesterone levels elevated above 0,5 ng/ml for only six days after GnRh administration in these cows

ovulate in response to GnRh than those suckled normally, and this effect was most marked in the Drakensberger cows treated between days 60 and 75 post-partum. A greater proportion of the Drakensbergers conceived when suckled once daily than when suckled normally (Table 2). This effect was more marked in the cows treated between days 60 and 75 post-partum than for cows treated earlier. None of the cows which ovulated displayed overt oestrus within three days of removing the ear implants. A varying proportion (0 – 40 %) of the cows allocated to each treatment underwent 'short cycles' (cows which ovulated, but where progesterone levels were subsequently higher than 0,5 ng/ml for only six days). Progesterone levels exceded 0,5ng/ml for at least 12 days in cows which displayed 'normal cycles' (Figure 1). The incidence of 'short cycles' was not affected by treatment in the British-cross cows, but 50 % less 'short cycles' occurred in the Drakensberger cows suckled once daily than in those suckled normally (Table 2).

Hormone levels

Concentrations of oestrogen in samples obtained on a daily basis for five days prior to the removal of the ear implants and then on a six-hourly basis between the removal of the implants and the GnRh injection fluctuated considerably from day to day in all the cows. Changes in the levels of oestrogen over the aforementioned periods did not follow any particular pattern, and consequently the area under the curve obtained by plotting oestrogen levels against time in each individual cow was calculated to obtain an estimate of the total quantity of oestrogen secreted. The mean area under the oestrogen curve and the mean peak oestrogen level were not affected by treatment. However, maximum oestrogen levels tended to be higher in cows suckled once daily than in those suckled normally (Table 3).

Tonic LH levels fluctuated at relatively low levels during each of the two-hour periods during which samples were obtained at intervals of 15 min. Mean tonic LH levels prior to the commencement of the normal and restricted suckling periods were not affected by breed of cow and stage post-partum. Furthermore, the once-daily suckling regime did not

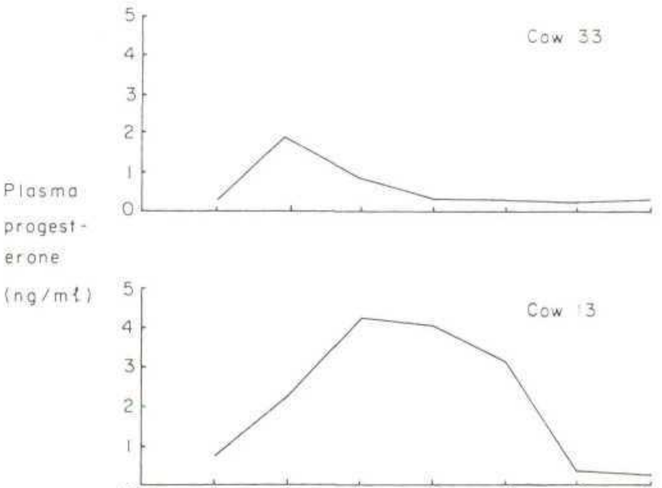

Figure 1 The pattern of progesterone secretion following GnRh in Cow 33 which displayed a 'short' cycle and cow 13 which displayed a 'normal' cycle.

Table 3 Mean area under oestrogen curve and maximum oestrogen level measured

Breed	Days post-partum treated	Suckling intensity	n	Mean area under oestrogen curve (mm^2)	Mean maximum oestrogen level measured over sampling period (pg/ml)
British-cross	35 – 50	Normal	5	1227,9 ± 133,4	18,8 ± 1,42
		Restricted	5	1345,2 ± 509,2	25,4 ± 10,8
	60 – 75	Normal	4	1205,9 ± 37,1	8,8 ± 3,4
		Restricted	4	1402,5 ± 441,9	45,7 ± 33,3
Drakensberger	35 – 50	Normal	5	1053,5 ± 275,3	17,0 ± 1,9
		Restricted	5	805,5 ± 113,5	23,6 ± 11,1
	60 – 75	Normal	4	740,3 ± 90,7	11,9 ± 1,7
		Restricted	5	2323,7 ± 1242,3	64,4 ± 37,3

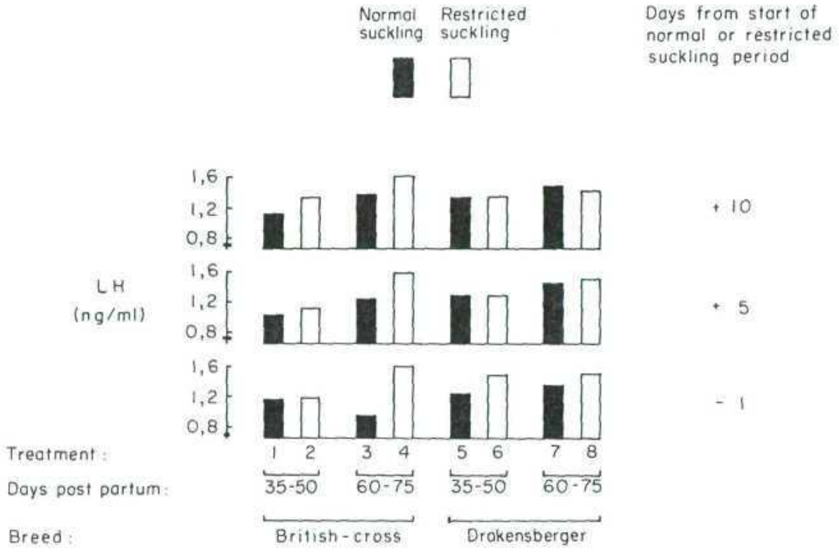

Figure 2 Mean tonic LH levels prior to and during the periods of normal or restricted suckling.

Table 4 Mean area under curve, maximum level and duration of LH surge

Breed	Days post-partum treated	Suckling intensity	*n*	Mean area under LH curve (mm^2)	Mean maximum LH level reached (ng/ml)	Mean duration of LH surge (h)*
British-cross	35 – 50	Normal	5	225,9 ± 40,7	101,0 ± 17,6	6,9 ± 0,6
		Restricted	5	202,4 ± 27,5	88,7 ± 12,0	6,2 ± 0,3
	60 – 75	Normal	4	124,6 ± 20,3	60,7 ± 6,7	6,5 ± 0,0
		Restricted	4	185,1 ± 47,0	76,9 ± 19,2	6,6 ± 0,4
Drakensberger	35 – 50	Normal	5	197,5 ± 19,7	88,8 ± 12,9	6,9 ± 0,4
		Restricted	5	173,7 ± 77,7	78,0 ± 11,0	6,1 ± 0,5
	60 – 75	Normal	4	193,6 ± 41,5	88,9 ± 17,5	7,3 ± 0,4
		Restricted	5	163,8 ± 37,1	72,2 ± 16,7	5,7 ± 1,0

* Levels > 10ng/ml.

influence tonic LH levels, since there were no significant differences between mean levels measured before and during the restricted suckling periods (Figure 2).

An LH surge was measured in all cows following the injection of GnRh. Treatment did not influence the area under the LH curve, the maximum LH level and the duration of the LH surge (Table 4). Data relating to LH release in response to GnRh in the Drakensberger and British-cross breeds were subsequently pooled in order to increase the number of cows at each of the two post-partum stages and suckling intensities employed. Mean pooled LH levels are diagrammatically illustrated in Figure 3, from which it is evident that LH surges tended to be smaller in cows suckled once daily than those suckled normally between days 35 and 50 post-partum. Between days 60 and 75 the cows suckled normally and once daily, secreted similar quantities of LH.

There were no significant correlations between the secretory patterns of the different hormones studied, the body-mass changes in the cows and their ovulatory response.

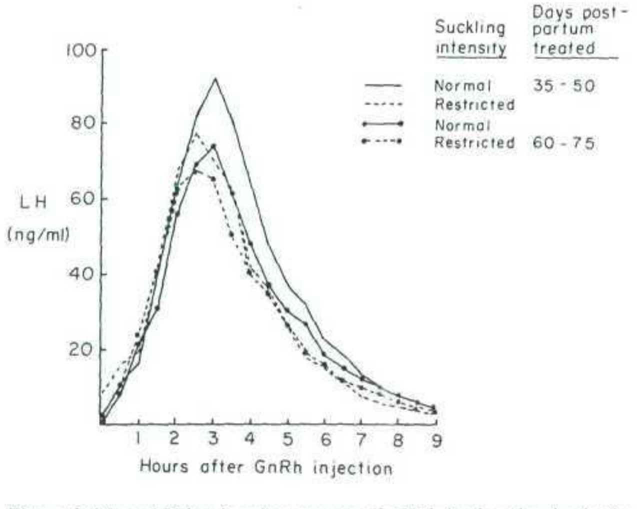

Figure 3 Mean LH levels subsequent to GnRh injection (levels obtained in two breeds pooled)

Body-mass changes

Mean masses of the Drakensberger cows within five days of calving (461,5 ± 10,2 kg) did not differ from those of the British-cross cows (456,2 ± 9,9 kg). All cows used in this study underwent mass losses ranging from 3,2 to 30,9 kg between parturition and the onset of the normal or restricted suckling periods, but these were not affected by treatment or breed. Mean condition scores in cows treated between days 60 and 75 post-partum (2,89 ± 0,08) tended to be lower than those in cows treated between days 35 and 50 (3,13 ± 0,1). Mean growth rates in the calves between birth and 90 days were not affected by the suckling intensity employed.

Discussion

In the present study the ovulatory response of the cows to the injection of GnRh was not significantly influenced by the breed of cow, the stage post-partum or the suckling intensity employed. However, there was a tendency for a greater proportion of the cows suckled once daily to ovulate in response to GnRh than those suckled normally, and this effect was more marked in the Drakensberger than in the British-cross cows (Table 2). It could be postulated that this improved ovulatory response may have been due to relatively higher pituitary LH levels, which in turn would be expected to result in the release of relatively larger quantities of LH into the bloodstream following GnRh injection in the cows suckled once daily than in those suckled normally. However, the suckling intensity employed did not influence the quantity of LH released following GnRh injection, in fact cows suckled normally tended to release more LH than those suckled once daily at the earlier post-partum stage (Figure 3). This finding is at variance with that of Carter *et al.* (1980) and Troxel *et al.* (1980), who showed that suckling depresses LH release in response to GnRh in beef cows. Our results are however in agreement with those of Echternkamp (1978), who showed that the GnRh- induced LH release was not influenced by suckling intensity. The discrepancies described above may be due to differences in the stages post-partum during which the suckling intensities were varied, the degree of suckling stimuli imposed, the genetic constitution of the cows and the numbers of cattle used in the different studies.

The magnitude of the increase in ovulatory response owing to restricted suckling tended to be greater in the Drakensberger than in the British-cross cows (Table 2). This finding lends support to the contention that reproductive ability in *Bos indicus* cows differs inherently from that in *Bos taurus* females. For example, *Bos indicus* cows display a greater proportion of ovulations without oestrus than European breeds (Van der Westhuysen, 1972), and Randel & Moseley (1977) found that peak LH levels at the time of oestrus are significantly lower in Brahman than in Hereford heifers. Griffin & Randel (1977) found that *Bos indicus* cows release less LH in response to GnRh than *Bos taurus* cows, a finding which was not substantiated in the present study (Table 4).

The once-daily suckling regime used in the present study tended to reduce the incidence of 'short' luteal phase cycles in the Drakensberger, but not in the British-cross cows (Table 2). Carter *et al.* (1980) and Reeves & Gaskins (1981) found a greater incidence of 'short' cycles in non-suckled cows or cows nursed once daily than in normally suckled *Bos taurus* cows injected with GnRh. However, in these investigations GnRh was administered at an earlier stage post-partum than in the present study, and this could account for the different results obtained.

Tonic LH levels were not affected by breed of cow, stage post-partum and suckling intensity employed (Figure 2). In a number of other studies suckling has been shown to cause varying levels of depression on tonic LH concentrations (Carruthers, Convey, Kesner, Hafs & Cheng, 1980; Carruthers & Hafs, 1980; Forrest *et al.*, 1980). In the present study the mean tonic LH level measured over all the treatments was 1,32 ± 0,04 ng/ml which is similar to levels reported during the post-partum period by Ingalls, Convey & Hafs (1973), Edgerton & Hafs (1973) and Forrest *et al.* (1980). It should be noted though that in the present study LH concentrations in the majority of samples were measurable at, or just above, the lower limit of sensitivity for the LH assay. It is thus possible that the assay was not sensitive enough, or the number of animals used was too small to detect differences in tonic LH secretion owing to treatment. The finding that LH levels prior to and after the insertion of the progesterone ear implants did not differ supports this conclusion, since Chang *et al.* (1981) and Ireland & Roche (1982) have shown that tonic LH levels are suppressed by progesterone therapy.

It was anticipated that oestrogen levels measured in the present study would provide an indication of follicular development following the withdrawal of the progesterone ear implants, and the effect thereon of treatment. However, oestrogen concentrations varied considerably within and between individual cows (Table 3), and it is evident that a larger number of animals than was used in this experiment are necessary to measure the effect of treatment on oestrogen secretion. Such studies appear warranted, since Bellin, Hinshelwood, Robinson, Ax & Hauser (1982) found that fewer and smaller follicles, which contained smaller quantities of oestrogen, occurred in suckled than in non-suckled beef cows.

The number of cows which conceived to the fixed-time inseminations, relative to the number which ovulated over all treatments was generally dissapointing (Table 2). The stress of frequent handling imposed on the cows may have contributed to this phenomenon. However, the beneficial effect of restricted suckling on the ovulatory response obtained in this and other trials (Randel, 1981) indicate that it may hold potential as a means of increasing reproductive rates in beef herds. The use of restricted suckling techniques as a means of reducing post-partum anoestrous periods in first calvers, late season calvers and *Bos indicus* females clearly warrants attention under practical farming conditions.

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References

ADEYEMO, O. & HEATH, E., 1980. Plasma progesterone concentration in *Bos taurus* and *Bos indicus* heifers. *Theriogenology* 14, 411.

BELLIN, M.E., HINSHELWOOD, M.M., ROBINSON, G.M., AX, R.L. & HAUSER, E.R., 1982. Distribution of follicles and intrafollicular estrogen in suckled and non-suckled post-partum beef cows. *J. Anim. Sci.* 55, Suppl. 1, 338.

BUTCHER, R.L., COLLINS, W.E. & FUGO, N.W., 1974. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol 17-β throughout the four day estrus cycle of the rat. *Endocrinology* 94, 1704.

CARRUTHERS, T.D., CONVEY, E.M., KESNER, J.S., HAFS, H.D. & CHENG, K.W., 1980. The hypothalamo-pituitary gonadotrophic axis of suckled and non-suckled dairy cows post-partum. *J. Anim. Sci.* 51, 949.

CARRUTHERS, T.D. & HAFS, H.D., 1980. Suckling and four-times daily milking: Influence on ovulation, estrus and serum luteinizing hormone, glucocorticoids and prolactin in post-partum Holsteins. *J. Anim. Sci.* 50, 919.

CARTER, M.L., DIERSCHKE, D.J., RUTLEDGE, J.J. & HAUSER, E.R., 1980. Effect of gonadotropin-releasing hormone and calf removal on pituitary-ovarian function and reproductive performance in post-partum beef cows. *J. Anim. Sci.* 51, 903.

CHANG, C.H., GIMENEZ, T. & HENRICKS, D.M., 1981. Modulation of reproductive hormones by suckling and exogenous gonadal hormones in young beef cows post-partum. *J. Reprod. Fert.* 63, 31.

COETZER, W.A., MENTZ, A.H., VERMEULEN, J.A. & COETZEE, J.E., 1975. Resultate verkry met K.I. by Afrikaner- en Jerseykoeie onder ekstensiewe toestande in Noord-Kaapland. *S. Afr. J. Anim. Sci.* 5, 11.

DONALDSON, L.E., BASSET, J.M. & THORBURN, G.D., 1970. Peripheral plasma progesterone concentration of cows during puberty, oestrous cycles, pregnancy and lactation, and the effects of undernutrition or exogenous oxytocin on progesterone concentration. *J. Endocr.* 48, 599.

ECHTERNKAMP, S.E., 1978. Stimulation of estrogen and luteinizing hormone secretion in post-partum beef cows. *J. Anim. Sci.* 47, 521.

EDGERTON, L.A. & HAFS, H.D., 1973. Serum luteinizing hormone, prolactin, glucocorticoid, and progestin in dairy cows from calving to gestation. *J. Dairy Sci.* 56, 541.

FORREST, P.K., RHODES, T.C. & RANDEL, R.D., 1980. Effect of bleeding stress and variable suckling intensity upon serum luteinizing hormone in Brangus heifers. *Theriogenology* 13, 321.

GRIFFIN, J.L. & RANDEL, R.D., 1977. Luteinizing hormone response of ovariectomized Brahman and Hereford cows to 500 μg GnRh. *Abstracts 69th Ann. Mtg Amer. Soc. Anim. Sci.* University of Wisconsin, 164.

HOLLNESS, D.H., HOPLEY, J.D.H. & HALE, D.H., 1978. The effects of plane of nutrition, live weight, temporary weaning and breed on the occurrence of oestrus in beef cows during the post-partum period. *Anim. Prod.* 26, 47.

INGALLS, W.G., CONVEY, E.M. & HAFS. H.D., 1973. Bovine serum LH, GH and prolactin during late pregnancy, parturition and early lactation. *Proc. Soc. Exp. Biol. Med.* 143, 161.

IRELAND, J.J. & ROCHE, J.F., 1982. Effect of progesterone on basal LH and episodic LH and FSH secretion in heifers. *J. Reprod. Fert.* 64, 295.

LASTER, D.B., GLIMP, H.A. & GREGORY, K.E., 1973. Effects of early weaning on post-partum reproduction of cows. *J. Anim. Sci.* 36, 734.

LISHMAN, A.W., 1972. *The influence of variations in the level of nutrition on reproduction in the ewe.* Ph.D. Thesis. University of Natal.

NISWENDER, G.D., REICHERT, L.E., MIDGLEY, A.R. & NALBANDOV, A.V., 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology* 84, 1166.

RADFORD, H.M., NANCARROW, C.D. & MATTNER, P.E., 1978. Ovarian function in suckling and non-suckling beef cows post-partum. *J. Reprod. Fert.* 54, 49.

RANDEL, R.D., 1981. Effects of once-daily suckling on post-partum interval and cow-calf performance of first-calf Brahman × Hereford heifers. *J. Anim. Sci.* 53, 755.

RANDEL, R.D. & MOSELY, W.M., 1977. Serum luteinizing hormone surge and progesterone near estrus in Brahman compared to Brahman × Hereford and Hereford heifers. *Abstracts 69th Ann. Mtg. Amer. Soc. Anim. Sci.* University of Wisconsin, 189.

REEVES, J.J. & GASKINS, C.T., 1981. Effect of once-a-day nursing on rebreeding efficiency of beef cows. *J. Anim. Sci.* 53, 889.

SMITH, L.E. & VINTCENT, C.K., 1972. Effects of early weaning and exogenous hormone treatment on bovine post-partum reproduction. *J. Anim. Sci.* 35, 1228.

TROXEL, T.R., KESLER, D.J., NOBLE, R.C. & CARLIN, S.E., 1980. Ovulation and reproductive hormones following steroid pretreatment, calf removal and GnRh in post-partum suckled beef cows. *J. Anim. Sci.* 51, 652.

VAN DER WESTHUYSEN, J.M., 1972. Differences in progesterone synchronized oestrus and ovulation in three cattle breeds. *S. Afr. J. Anim. Sci.* 2, 91.

VAN NIEKERK, A. & LOUW, B.P., 1982. *Condition scoring of beef cattle.* Bull. N14/82. Dept. Agric. Natal Region.

WETTEMAN, R.P., TURMAN, E.J., WYATT, R.D. & TOTUSEK, R., 1978. Influence of suckling intensity on reproductive performance of range cows. *J. Anim. Sci.* 47, 342.

WILLIAMS, G.L., KOTWICA, J., SLANGER, W.D., OLSON, D.K., TILTON, J.E. & JOHNSON, L.J., 1982. Effect of suckling on pituitary responsiveness to gonodotropin-releasing hormone throughout the early post-partum period of beef cows. *J. Anim. Sci.* 54, 594.

WILTBANK, J.N. & COOK, A.C., 1958. The comparitive reproductive performance of nursed cows and milked cows. *J. Anim. Sci.* 17, 640.

YUTHASASTRAKOSOL, P., PALMER, W.M. & HOWLAND, B.E., 1975. Luteinizing hormone, oestrogen and progesterone levels in peripheral serum of anoestrous and cyclic ewes as determined by radioimmunoassay. *J. Reprod. Fert.* 43, 57.

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mbryo mortality and early post-oestrous cycle embryonic death estimated from oestrous cycle lengths and milk progesterone analysis

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An estimate of embryo mortality (cycles longer than 28 days) was obtained from milk progesterone analysis and delayed return rate in two dairy herds. Oestrus cycle lengths were measured, and cycles grouped according to whether artificial insemination (AI) had, or had not, been performed. Early post-oestrous cycle embryonic death (cycles extended beyond the average of 21 days, yet less than 28 days in length) was estimated by examining the changes in distribution of the two groups of oestrous cycle lengths. Embryo mortality occurring between 28 and 75 days after AI was measured at 15,2% using progesterone analysis and 21,6% using delayed returns. A 23,3% increase ($P < 0{,}001$) in the number of extended cycles (22 – 28 days) in the mated group of cows was found. This change in distribution of the length of mated and unmated groups of cycles was significantly different, and was assumed to represent early post-oestrous cycle embryonic death. Some implications of these findings are discussed.

Embriomortaliteit (siklusse langer as 28 dae) is geskat vanuit melkprogesteroonbepalings en vertraagde terugkeertempo in twee melkkuddes. Siklusse is ingedeel in twee groepe volgens die voorkoms al dan nie van kunsmatige inseminasie (KI) en die sikluslengtes is gemeet. Vroeë embrio-afsterwing (siklusse langer as die gemiddeld van 21 dae, maar korter as 28 dae) is geskat deur die voorkoms van 'n verspreidingsverandering in estruslengte tussen die twee groepe. In die geïnsemineerde groep is 'n toename van 23,3% ($P < 0{,}001$) in die aantal verlengde siklusse (22 – 28 dae) gevind, terwyl embriomortaliteit tussen 28 en 75 dae na inseminasie bepaal is as 15,2% en 21,6% met behulp van melkprogesteroonbepalings en terugkeertempo onderskeidelik. Die verandering in die verspreiding van sikluslengte tussen geïnsemineerde en nie-geïnsemineerde groepe is betekenisvol verskillend, en word beskou as verteenwoordigend van vroeë embriomortaliteit. Enkele implikasies hiervan word kortliks bespreek.

Keywords: Embryo mortality, dairy cows, oestrous cycle lengths, milk progesterone analysis

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roduction

s widely considered that, in dairy cows, an increase in interval between service and return to oestrus ond the usual range of 17 – 25 days reflects embryo rtality (Erb & Holtz, 1958). Estimates of such losses e been based on the decline in non-return rate, ughter experiments, and recently, analysis of milk gesterone content. In artificially inseminated herds se estimates have ranged from 7,2% (Kummerfeld, enacu & Foote, 1978) to 24,7% (Bulman & mming, 1979).

he major portion of embryonic losses occurs well ore day 15 after service (Ayalon, Weis & Lewis, 8; Boyd, Bacsich, Young & McCracken, 1969). proximately 40% of inseminations are followed by roductive failure during this time (Pope & inburne, 1980). Neither the delayed returns nor the k progesterone method is capable of detecting these ly events. Cows experiencing such early death of the bryo will return to oestrus after the same interval as nated animals. However, the length of the oestrous le may be extended slightly. Some early death of the bryo may therefore be represented by extension of cycle to more than the average cycle length of unmated cows during the same post-partum period (Kummerfeld, *et al.*, 1978).

The aim of this analysis was to determine the extent of embryo mortality and early post-oestrous cycle embryonic death in two dairy herds where samples were being obtained for milk progesterone analysis. From the results it was possible to evaluate the importance of embryo mortality in commercial dairying.

Procedure

Whole milk samples (afternoon milking) were collected daily from 44 cows in two herds located on two research farms in the Natal Midlands. Cows were initially chosen for study on the basis of a poor reproductive history (long inter-calving periods and more than two inseminations) although all cows showed clinically normal reproductive tracts at the start of the study. However, when it appeared that previous reproductive history had little influence on the following reproductive period (Butterfield, 1986), cows were chosen for the study at random. Sampling was started between 10 and 20 days after calving and continued until pregnancy was confirmed by rectal palpation of the reproductive organs 60 days after service. Sampling was resumed if a cow returned to service after pregnancy had been confirmed. The routine for detection of oestrus in both herds consisted of twice daily observation prior to milking supplemented by casual observation of the herds during daylight hours.

The milk samples were analysed for progesterone content using the radioimmunoassay method as described by Butterfield (1986). A progesterone profile was plotted for each of the 44 cows under observation. The data obtained from these profiles were compared with observations for oestrus and insemination dates. Oestrous cycles which occurred before 30 days post-partum were excluded from the analysis. The lengths of 150 oestrous cycles were determined from progesterone profiles and oestrous observations and categorized into either mated (AI performed at oestrus) or unmated (no AI performed at oestrus) cycle groups.

Where expected heat was not observed, the end of the previous cycle was taken to have occurred at the mid-point of the trough where progesterone levels were less than 1 ng/ml milk. In fact, 33 out of a possible 150 heats were missed. The estimate of embryo mortality in the cow cycles was based on a sustained production of progesterone during the first 28 days after breeding, followed by a sudden decline in progesterone levels, with a return to cyclic progesterone patterns during the 28 to 75-day interval. This pattern was assumed to indicate that pregnancy had been initiated and terminated.

Embryo mortality was also estimated using the expected date of return to oestrus following insemination as basis, i.e. the delayed returns method. The decline in non-return rate during the interval between 28 and 75 days then estimated the proportion of cows in which the embryo died (Kummerfeld, *et al.*, 1978). This analysis was performed to determine the inaccuracy of the delayed returns method with respect to unobserved oestrous periods.

The incidence of early embryonic death was calculated after determination of the average length of an oestrous cycle in an unmated cow. Any increase in frequency of long cycles among inseminated cows could thus be assumed to represent early embryonic death. The difference in distribution of cycle lengths for mated and unmated oestrous cycles was thus analysed.

Results

An estimate of embryo mortality (cycles longer than 28 days) of 15,2% was calculated from the milk progesterone profiles. Of this, 58% occurred during the period between 30 and 38 days after insemination. This indicated that embryos enter a critical survival stage during this time.

Using the delayed returns method of estimating embryo mortality it was found that 21,6% of the cow cycles represented delayed returns to oestrus, which was indicative of embryo loss. The discrepancy between this result and that calculated from milk progesterone profiles was due to unobserved oestrous periods.

The estimate of early embryonic death was based on the calculation of the mean length of oestrous cycles of cows which had not been inseminated at oestrus. The value obtained from this calculation was 21,02 days. Using this value the mated and unmated oestrous cycles were distributed according to length of cycle and the difference in distribution calculated (Table 1).

Table 1 Length and distribution of oestrous cycles in mated and unmated dairy cows

Days after previous oestrus	Mated *n*	Mated %	Unmated *n*	Unmated %	Differences %
0 – 21	23	29,1	48	67,6	–38,5
22 – 28	44	55,7	23	32,0	+23,2
0 – 28	67	84,8	71	100,0	–15,2
> 28	12	15,2	0	0	+15,2
Total	79	100,0	71	100,0	

A marked change in the distribution of cycle lengths before and after insemination was evident (Table 1). The inseminated group had 38,5% fewer cycles of 21 days of less, 23,3% more cycles 22 – 28 days long and 15,2% more cycles longer than 28 days. According to the X^2 test, these differences were significant ($P < 0,001$). It was expected that, as a result of embryo mortality, the length of some oestrous cycles would be extended after AI. In fact, after insemination 70,9% of oestrous cycles were longer than 21 days compared to 32,4% such cycles in uninseminated cows ($P < 0,01$). Insemination of dairy cows thus causes a change in the distribution of oestrous cycle lengths.

Further evidence of this change is shown by the shift in distribution of cycle lengths of inseminated cows (Figure 1). This explains why the average cycle length of the mated group of cows was 25,54 days compared to 21,01 days for the unmated oestrous cycles. However, cycles longer than 28 days added considerable bias to the calculations. Exclusion of these long cycles from the analysis resulted in a mean cycle length of 22,35 days for the mated group. The 1,34 days difference between the means did not seem to warrant further analysis.

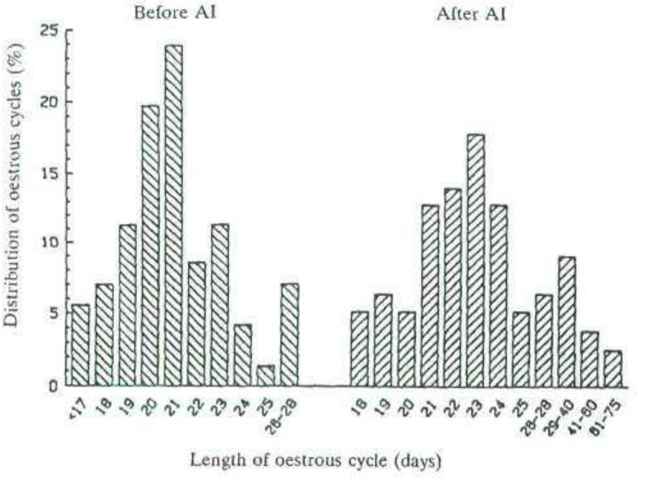

Figure 1 Distribution of oestrous cycle lengths before and after AI

lowever, as the standard deviation of the mean of both roups was similar (2,30 vs 2,29). the difference in ariance about the means was compared. The difference as significant at the 1% level (t = 3,22, 136 *df*).

These results demonstrated that when dairy cows had ie chance to become pregnant, but appeared not to onceive, the length of oestrous cycles was increased. he implication of this finding is important to dairy herd eproductive management.

iscussion

he analysis of milk progesterone levels is probably the iost accurate non-slaughter method of estimating mbryo mortality. Bias may be introduced to the stimate in cases of prolonged luteal activity in the non-regnant cow, but in the absence of any apparent clinical bnormality the incidence of this is very low, pproximately 1,5% (Bulman & Lamming, 1977). The 5,2% incidence of embryo mortality occurring after 28 ays compares with the 12,5% estimate of Claus, Karg, wiauer, von Butler, Pirchner & Rattenberger (1983) nd the 17% estimate calculated by MacFarlane, Booth, eas & Lowman (1977) using similar methods.

Using the delayed return rate, the estimate of embryo iortality was 21,6%. A similar level of 22,7% was found y Kummerfeld, *et al.* (1978) when using the same echnique. The difference between the 15,2% estimate nd the 21,6% estimate demonstrated that a portion of ie estimate obtained using delayed returns was biased ue to unobserved heats. In this study, the bias itroduced was fairly low (6,4%) as a result of efficient eat detection programmes in the herds involved. On irms where the efficiency of oestrous observation is oor, estimates of embryo mortality could be onsiderably influenced by unobserved heats.

The most important finding of this research was that ie oestrous cycles of inseminated cows were longer than iose of uninseminated cows. Kummerfeld, *et al.* (1978) uggested that cycles extended beyond 21 days in iseminated cows were associated with early post-estrous cycle embryonic death, but could show no tatistical significance. However, Boyd (1973) found a ignificant difference between inter-oestrous intervals efore and after first AI, with 90,5% of pre-insemination ycles of normal length (18 – 24 days) compared with 3,5% of post-insemination cycles. Although Boyd's tudy was based on the delayed returns method, and bias ue to unobserved heats is possible, the results are still ighly significant for the present investigation.

The results of this analysis demonstrate that there is a ignificant difference between the lengths of inseminated nd non-inseminated cycles. As all the cycles occurred uring the same post-partum period, the difference in ength must be the result of AI. Early post-oestrous cycle mbryonic death is thus incriminated.

In the field of reproductive management of dairy erds, emphasis has been placed on improving onception rates (Watson, 1982; Watson & McDonald, 984). The factors influencing the conception rate of iseminated dairy cows have been investigated (Shanks, Freeman & Berger, 1979; Stevenson, Schmidt & Call, 1983), but it seems that the emphasis should be placed on improving embryo survival.

When death of the conceptus occurs sufficiently early, the length of the oestrus cycle may be extended slightly or not affected at all. As there is no subsequent visible infertility, the economic consequences are the same as failure of fertilization. In fact, a large proportion of the estimated fertilization failure could probably be ascribed to early embryonic death. Failure to conceive may be replaced by failure to maintain the conceptus.

Bishop (1964) contended that a considerable part of embryo mortality was unavoidable, should be regarded as normal, and was a means of eliminating unfit genotypes at low biological cost. However, the extent of the embryo mortality phenomenon measured in this study is reproductively inefficient, and as such would be unacceptable to the commercial dairy farmer. Factors affecting embryo survival should thus be investigated further.

The increase in the length of oestrous cycles of inseminated cows was also relevant to the milk progesterone pregnancy test. Conventionally, the sampling day for the test is 21 – 22 days after AI (Pennington, Spahr & Lodge, 1976; Pope, Majzlik, Ball & Leaver, 1976). The results of this paper indicated that sampling at a slightly later date, say 24 – 25 days after AI, might prove to be more accurate. Heap, Holdsworth, Gadsby, Laing & Walters (1976) and Butterfield (1986) found that maximum accuracy for pregnancy diagnosis was obtained from samples taken 24 days after AI. These results endorse the findings of this trial.

Conclusion

If the increase in frequency of long cycles (longer than 21 days) among inseminated cows is assumed to represent embryonic death, the incidence of this phenomenon totalled nearly 40% (23,2% and 15,2%) in this study. Poor dairy herd management could compound the problem considerably, resulting in drastic economic consequences. Further research is thus warranted.

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References

AYALON, N., WEIS, Y. & LEWIS, I., 1968. Fertility losses in normal cows and repeat breeders. *Proc. 6th Int. Cong. Anim. Reprod. A.I.*, Paris, vol. 1, 393.

BISHOP, M.W., 1964. Paternal contribution to embryonic death. *J. Reprod. Fert.* 7, 383.

BOYD, H., BACSICH, P., YOUNG, A. & McCRACKEN, J.A., 1969. Fertilization and embryonic survival in dairy cattle. *Br. Vet. J.* 125, 87.

BOYD, H. 1973. Oestrous cycles in Ayrshire Cows before and after insemination. *Vet. Rec.* 92, 427.

BULMAN, D.C. & LAMMING, G.E., 1977. Cases of prolonged luteal activity in the non-pregnant dairy cow. *Vet. Rec.* 100, 550.

BULMAN, D.C. & LAMMING, G.E., 1979. The use of milk progesterone analysis in the study of oestrus detection, herd fertility and embryonic mortality in dairy cows. *Br. Vet. J.* 135., 559.

BUTTERFIELD, W.A., 1986. The progesterone assay of milk as an indicator of the reproductive status of the dairy herd. M.Sc. thesis. University of Natal.

CLAUS, R., KARG, H., ZWIAUER, D., VON BUTLER, PRICHNER, F. & RATTENBERGER, 1983. Analysis of factors influencing reproductive performance of the dairy cow by progesterone assay in milk fat. *Br. Vet. J.* 139, 29.

ERB, R.E. & HOLTZ, E.W., 1958. Factors associated with estimated fertilization and service efficiency of cows. *J. Dairy Sci.* 41, 1541.

HEAP, R.B., HOLDSWORTH, R.J., GADSBY, J.E., LAING, J.A. & WALTERS, D.E., 1976. Pregnancy diagnosis in the cow from milk progesterone concentration. *Br. Vet. J.* 132, 445.

KUMMERFELD, H.L., OLTENACU, E.A.B. & FOOTE, R.H., 1978. Embryonic mortality in dairy cows estimated by non-returns to service, oestrus and cyclic milk progesterone patterns. *J. Dairy Sci.* 61, 1773.

MACFARLANE, J.S., BOOTH, J.M., DEAS, D.W. & LOWMAN, B.W., 1977. Pregnancy test and evaluation of embryonic and foetal mortality based on progesterone concentrations in fore-milk. *Vet. Rec.* 100, 565.

PENNINGTON, J.A., SPAHR, S.L. & LODGE, J.R., 1976. Factors affecting progesterone in milk for pregnancy diagnosis in dairy cattle. *Br. Vet. J.* 132, 487.

POPE, G.S., MAJZLIK, BALL, P.J.H. & LEAVER, J.D., 1976. Use of progesterone concentration in plasma and milk in the diagnosis of pregnancy in domestic cattle. *Br. Vet. J.* 132, 497.

POPE, G.S. & SWINBURNE, J.K., 1980. Hormones in milk : their physiological significance and value as diagnostic aids. *J. Dairy Res.* 47, 427.

SHANKS, R.D., FREEMAN, A.E. & BERGER, P.J., 1979. Relationship of reproductive factors with interval and rate of conception. *J. Dairy Sci.* 62, 74.

STEVENSON, J.S., SCHMIDT, M.K. & CALL, E.P., 1983. Estrous intensity and conception rates in Holsteins. *J. Dairy Sci.* 66, 275.

WATSON, E.D., 1982. Ovarian findings in relation to the onset of oestrus and conception rates in dairy cattle. *Vet. Rec.* 110, 277.

WATSON, E.D. & McDONALD, B.J., 1984. Failure of conception in dairy cattle: progesterone and estradiol-17 concentrations and the presence of ovarian follicles in relation to the timing of AI. *Br. Vet. J.* 140, 398.

TABLE 1. EFFECTS OF PLANE OF NUTRITION AND FSH ON RESPONSES OF POSTPARTUM ANESTROUS COWS TO GnRH, EXPERIMENT I

Level of feeding	Dose of FSH (mg/day)	No. of cows	Cows with CL on day 7 after GnRH	Maximum concentration of LH±SE (ng/ml)
High	0	7	4	82.2±32.6[a]
	.25	7	3	55.0± 9.9
	.5	6	3	59.4±18.6
Low	0	8	4	49.0±14.8
	.25	8	3	49.6± 8.0
	.5	7	6	60.6±16.9

[a] Cow no. 38 had an extremely high value of 252. When that value is deleted, the mean for the remaining six cows is 53.9 ng/ml.

higher than the next highest animal in this treatment group. If values for this cow are omitted, then the high dosage of FSH may have increased the maximum level of LH.

The pattern of release of LH (figure 1) was altered ($P<.05$) by plane of nutrition. The high plane of feeding increased the slope of the rise in LH so that the maximum occurred 30 min earlier than in underfed animals. The mechanism by which level of feeding and possibly FSH influenced release of LH may be through their effects on follicular development, even though effects of level of feeding on size of follicles were not detected here. (The number of degrees of freedom available to test for this effect was considerably fewer than for the pattern of release of LH.) This suggestion is based upon the finding that cows with a largest follicle measuring 10, 12 or 14 mm in diameter prior to GnRH released considerably more LH than those with follicles < 10 mm (figure 2). The effects of size of follicle were reflected both in the pattern of release ($P<.01$) and in the area under the profile of LH ($P<.05$). The maximum value for LH was not correlated significantly with the concentration of estradiol, either 24 hr before or at the time of, injection of GnRH (pooled within $r=.1$ and $-.1$, respectively).

The profiles of plasma progesterone in cows with CL for each plane of nutrition and dose of FSH are shown in figure 3. There was a significant FSH×time interaction ($P<.05$) and a trend towards an FSH×plane of nutrition interaction ($P<.08$). Apparently FSH led to an increase in progesterone 7 days after GnRH, except in the high plane animals that received the higher dose of FSH. Cows that lost more weight from parturition

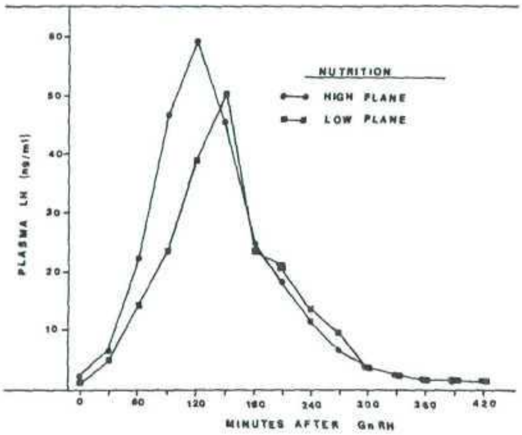

Figure 1. Effects of plane of nutrition on profiles of LH induced by GnRH in postpartum anestrous beef cows.

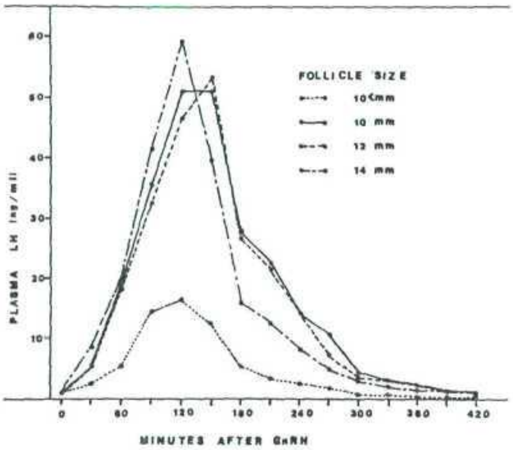

Figure 2. Effects of sizes of follicles on profiles of LH induced by GnRH in postpartum anestrous beef cows.

TABLE 2. CHANGES IN OVARIAN FOLLICLES AND SECRETION OF STEROIDS IN COWS TREATED WITH FSH PRIOR TO RE-INJECTION WITH GnRH, EXPERIMENT II

FSH pre-treatment	n	Days post-partum	Diameter (mm) of largest follicle		Plasma estradiol-17β (pg/ml)		Cows with CL	Plasma progesterone (ng/ml)	
			Pre-FSH	Post-FSH	Pre-FSH	Post-FSH		Day 7	Day 14
4.0 mg/day	8	55.1	9.7±.6	13.5±.5*	1.5±.2	3.7±.4	7	1.6±.2	.1±.1
Control	9	56.7	10.0±.7	11.8±.6	1.8±.2	2.2±.3	5	1.0±.2	.2±.1

*P<.05.

to the time GnRH was given had more progesterone on day 7 ($P<.05$), but this was not accompanied by an increased life span of CL.

When the ovaries were palpated on day 14 post-GnRH, cow no. 38 (high plane, control) was the only animal to have re-ovulated since day 7. Her plasma progesterone level fell from .7 ng/ml on day 7 to .2 ng/ml on day 10, but then rose again to 1.4 and 2.9 ng/ml on days 14 and 18, respectively. In all other cows the CL palpated on day 7 had regressed (21) or were greatly reduced in size (1) by day 14 without a new ovulation having occured. The mean concentrations of progesterone on days 10 and 14 were less than one-half as great as on day 7 (figure 3), reflecting a shortened life span of the induced corpora lutea.

Experiment II. A positive correlation ($r=.70$; $P<.01$) was observed between body weights of these 17 cows at 48 days postpartum and concentration of estradiol-17β (table 2) prior to commencement of treatment with FSH at 56 days postpartum. Concentration of estradiol-17β and size of follicle were not associated at that time, but were associated immediately prior to injection of GnRH on day 59 ($r=.53$; $P<.05$). Mean estradiol (pg/ml) ranged from .7 in one animal with an 8 mm follicle to 1.8, 2.4, 2.2 and 15.0 in cows with 10, 12, 14 and 16 mm follicles, respectively.

Level of feeding did not influence the diameter of the largest follicle immediately prior to GnRH. Treatment with FSH (4 mg/day) for 3 days resulted in a greater ($P<.05$) mean diameter of the largest follicle than in the control animals (table 2). Despite this effect and the association of estradiol with size of follicle, plasma estradiol-17β in cows treated with FSH was not greater than in untreated controls (table 2).

Although a greater proportion of cows that were injected with FSH (88%) than control cows (56%) had CL after GnRH, the difference was not significant (table 2) and concentrations of progesterone were not affected by treatment. According to palpation and progesterone measured on day 14 (table 2), the induced CL were short-lived.

Experiment III. Size of ovaries, number of follicles ⩾ 10 mm, sizes of follicles per ovary and the size of the largest follicle on day 37 did not vary with hormonal treatments or over time (table 3). Similarly the profiles of estradiol-17β were parallel over time, averaging 4 pg/ml, except for an increase to nearly 14 pg/ml on day -3 in cows receiving exogenous estradiol on day -4 (day 34 postpartum). Clearly, follicular activity as measured by several end points was not affected by either treatment.

Profiles of concentrations of LH after GnRH were parallel among all three groups of cows. In addition, the peak concentrations and areas under the curves did not differ among groups.

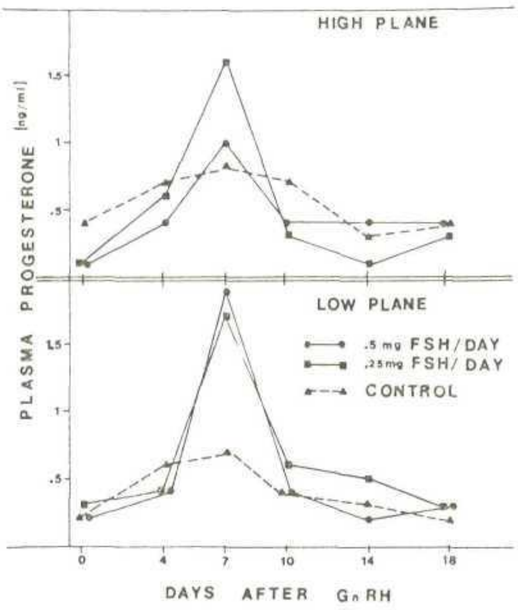

Figure 3. Effects of plane of nutrition and FSH treatment on profiles of progesterone after GnRH in postpartum anestrous beef cows.

TABLE 3. EFFECTS OF HORMONAL TREATMENTS ON FOLLICULAR DEVELOPMENT IN POSTPARTUM ANESTROUS BEEF COWS. EXPERIMENT III[a]

Treatment	No. of cows	Combined ovarian size (mm)			No. of follicles ≥10 mm			Size of follicles (mm)			Largest follicles per ovary (mm)
					Day postpartum						
		30	34	37	30	34	37	30	34	37	37
Control	5	4344±499	3937±385	4178±486	.6±.2	.7±.2	.8±.2	7.4±2.0	6.9±1.9	8.1±1.8	12.4± .7
FSH	6	3921±386	3529±288	3852±399	.7±.2	.8±.2	.8±.1	6.8±1.7	7.7±1.7	9.3±1.7	13.3±1.0
Estradiol-17-β plus FSH	7	3740±432	3420±398	3777±431	.7±.2	.8±.2	.6±.1	6.6±1.6	6.6±1.6	7.8±1.7	13.0± .9

[a] Values presented are mean±standard error. See text for details of treatments with FSH and estradiol-17β.

Sixty-one percent of all cows treated with GnRH had palpable CL in the ovaries on day 7. There was a tendency for fewer cows treated with estradiol plus FSH to have CL on day 7 than in the other two groups (2/7 *vs* 9/11; P<.1). At least one cow in each group and five in total had a CL with a very short life span, as judged by its absence from the ovaries on day 13. Nine of the 11 (82%) cows with CL on day 7 had ovulated spontaneously by day 30 (68 days postpartum). Fewer cows treated with estradiol plus FSH had CL on day 30 (1/7) than in the other groups (8/14; P<.05). The location of CL on day 30 (same or opposite ovary as day 7), did not vary with treatment.

Progesterone (ng/ml) in cows with induced CL averaged only 1.4±.3 on day 7 and .9±.4 on day 13 compared to usually reported values of 4 to 5 at those stages of a normal cycle. Profiles of progesterone did not vary among the three groups of hormonally-treated cows.

Discussion

The question arises as to why there was an apparent response in occurrence of CL to FSH in the energy-restricted animals but not among cows fed the high energy ration in experiment I. Examination of the results does not support the idea that the high-plane cows tended to be above a threshold and therefore could not respond to small doses of FSH. The most likely explanation appears to be that although the cows were allocated randomly to treatments the high-plane cows assigned to the control group possessed an advantage since the population of follicles within the ovaries reflected a more active condition than cows in the remaining groups. This contention is supported by the tendency for a CL occurring in response to GnRH to be greater when the diameter of the largest ovarian follicle was 12 mm or greater. Because of this finding and the increase in diameter of the largest follicle under the influence of exogenous FSH in experiment II, it is suggested that attention should be given to the ovarian situation when selecting the dose of FSH and the duration of treatment. In view of the lack of association between the peak concentration of LH and the formation of CL, it is doubtful whether the reduction in release of LH associated with inadequate follicular development warrants adjustment of the dose of GnRH according to the follicular population.

When estrous cycles resume spontaneously after parturition in cows the first overt estrus is preceded usually by a 4- to 5-day period during which plasma levels of progesterone are elevated (Donaldson *et al.*, 1970; Henricks *et al.*, 1972;

Dickey *et al.*, 1975; Castenson *et al.*, 1976; Humphrey *et al.*, 1976). Treatment of lactating, anestrous beef cows with GnRH can induce luteal function for a number of days (Britt *et al.*, 1975; Webb *et al.*, 1975; present experiments). Questions arise as to why (1) the luteal function is usually of short duration and (2) the elevation in progesterone levels is not followed by further spontaneous ovulations. Since it has been demonstrated that the major luteotropin in the bovine is LH (Hansel and Siefart, 1967), it is tempting to consider the possibility of quantitative deficiencies (concentration and/or duration) in the LH needed for sustained luteal function. Since there was great variation in the peak concentration, but induced CL were always short-lived, the deficiency probably does not rest in this area. The only common factor regarding release of LH was the short duration of some 6 hr compared to 10 hr or more in the cycling cow (Lemon *et al.*, 1975). This appears a more fertile ground for exploration; indeed when the duration of the peak has been prolonged in ewes by employing divided doses of GnRH (Restall *et al.*, 1977), the abnormal CL were reported to be eliminated.

A further possibility for the premature regression of the CL is that the follicle which ovulated was incompetent or deficient in some receptor for gonadotropin. This contention is supported by the finding that treatment with FSH prior to GnRH tended to increase progesterone one week after formation of the CL (figure 3, table 2). Although FSH affects both size of follicle and secretion of estrogen, the latter, via its effect on the pituitary and hypothalamus, may be the more important aspect regarding luteal function. Consequently, ovarian follicles may reflect the hypothalamic-pituitary situation rather than ovarian readiness as such.

When attention is focused on the failure to initiate regular ovulations, the case of cow no. 38 is of considerable interest. During the 22-day period from prior to GnRH until the end of experiment I this animal gained 34.5 kg, whereas her calf weighed 13.7 kg (19.3%) less than the mean for the group. This cow was the only animal that re-ovulated after regression of the GnRH-induced CL and her release of LH after GnRH reached an exceptionally high peak value. It is tempting to attribute the atypical gain in weight to some change in the nutritional status of the cow, but the possibility of a reduced frequency of suckling by the calf, perhaps due to illness, cannot be eliminated in this particular case.

In sheep, pituitary reserves of LH are low during early lactation (Restall and Starr, 1977) and in beef heifers the feeding of a low energy diet reduced the LH reserves (Beal *et al.*, 1975). However, differences in the level of nutrition during pregnancy did not influence the peak level of LH following GnRH in heifers (Cummins *et al.*, 1975). It remains to be demonstrated therefore, whether the delayed and reduced response of LH to GnRH in the underfed, lactating cows (figure 1) was simply the result of deficient pituitary reserves or whether inadequate sensitization by ovarian steroids was also involved. Estradiol did not vary with plane of nutrition and, in contrast to the report of Fernandes *et al.* (1978) in dairy cattle, maximum LH was not correlated with concentrations of estradiol prior to GnRH.

Ovarian follicular populations and secretion of estradiol-17β were not affected by exogenous FSH or estradiol and FSH in experiment III. Also, the formation and life-span of induced CL were not affected by these treatments. Dose of FSH may have been too low since follicular development was increased in postpartum cows with 4 mg of FSH per day (experiment II). Similarly, the dose the estradiol used may not have accomplished physiological concentrations of estradiol within the follicle; microgram concentrations are known to occur in follicular-fluid (England *et al.*, 1973); thus potential effects of estradiol on receptors for FSH may not have been obtained. Possibly, the anestrous ovaries require low level stimulation by FSH over longer periods rather than higher or graded doses administered acutely (4 days). Because of these questions, these data do not clarify whether ovarian insensitivity is a possible explanation for low occurrence or short-life of CL induced by GnRH. It may be important to note that in the 3-year-old cows (experiment III) six of 11 induced CL were palpable on day 13 in contrast to one of 35 in the 2-year-old cows in the previous experiments. Even so, progesterone had decreased by day 13.

The presence of CL on day 30 after GnRH, particularly in those cows which had induced CL of apparently nearer normal life span indicates possible resumption of estrous cycles. This hypothesis deserves more critical investigation because of the possibility of a short intervening period of anestrus.

From these data, it appears that investigations of postpartum anestrous in beef cows should consider the mechanism by which increases in progesterone prior to first estrus affect follicular development.

LITERATURE CITED

Apgar, J., D. Aspros, J. E. Hixon, R. R. Saatman and W. Hansel. 1975. Effect of restricted feed intake on the sensitivity of the bovine corpus luteum to LH *in vitro*. J. Anim. Sci. 41:1120.

Baker, R. D., B. R. Downey and H. J. Brinkley. 1973. Induction of ovulation in pigs with gonadotrophin releasing hormone. J. Anim. Sci. 37:1376.

Beal, W. E. R. E. Short, R. B. Staigmiller, R. A. Bellows, C. C. Kaltenbach and T. G. Dunn. 1978. Influence of dietary energy intake on bovine pituitary and luteal function. J. Anim. Sci. 46:181.

Britt, J. H., T. E. Kiser, B. E. Seguin, H. D. Hafs, W. D. Oxender and H. D. Ritchie. 1975. Fertility after GnRh and $PGF_{2\alpha}$ in suckling cows. J. Anim. Sci. 41:345 (Abstr.).

Butcher, R. L., W. E. Collins and N. W. Fugo. 1974. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrus cycle of the rat. Endocrinology 94:1704.

Butcher, R. L. 1977. Changes in gonadotropins and steroids associated with unilateral ovariectomy of the rat. Endocrinology 101:830.

Casida, L. E., W. E. Graves, J. W. Lauderdale, J. W. Riesen, S. Saiduddin, E. R. Hauser and W. J. Tyler. 1968. Studies on the postpartum cow. Univ. of Wisconsin Res. Bull. 270.

Castenson, P. E., A. M. Sorenson, Jr., C. R. Cobos and J. L. Fleeger. 1976. Source of postpartum P and 20β-OHP preceding estrus in heifers. J. Anim. Sci. 43:277 (Abstr.).

Cummins, L. J., I. A. Cumming, M. A. Knight and R. A. S. Lawson. 1975. LH release by cows in response to GnRH during the post-partum period. J. Reprod. Fertil. 43:397.

Dickey, J. F., D. M. Henricks and S. Bierly. 1975. Hormonal relationship in post-partum Holstein cows. J. Anim. Sci. 41:348 (Abstr.).

Donaldson, L. E., J. M. Bassett and G. D. Thorburn. 1970. Peripheral plasma progesterone concentration of cows during puberty, oestrous cycles, pregnancy and lactation, and the effects of under-nutrition or exogenous oxytocin on progesterone concentration. J. Endocrinol. 48:599.

Dunn, T. G., J. E. Ingalls, D. R. Zimmerman and J. N. Wiltbank. 1969. Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. J. Anim. Sci.29:719.

Dunn, T. G., J. Rone, C. C. Kaltenbach, L. A. van der Walt, M. L. Riley and A. M. Akbar. 1974. Hormone changes during underfeeding of beef cows. J. Anim. Sci. 39:206 (Abstr.).

Edgerton, L. A. and H. D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451.

England, B. G., H. J. Karavolas, E. R. Hauser and L. E. Casida. 1973. Ovarian follicular estrogens in Angus heifers. J. Anim. Sci. 37:1176.

Fernandes, L. C., W. W. Thatcher, C. J. Wilcox and E. P. Call. 1978. LH release in response to GnRH during the postpartum period of dairy cows. J. Anim. Sci. 46:443.

Fogwell, R. L., C. W. Weems, G. S. Lewis, R. L. Butcher and E. K. Inskeep. 1978. Secretion of steroids after induced luteal regression in beef heifers: effects of $PGF_{2\alpha}$ and removal of corpora lutea. J. Anim. Sci. 46:1718.

Fortune, J. E. and D. T. Armstrong. 1978. Hormonal control of 17β-estradiol biosynthesis in proestrous rat follicles: estradiol production by isolated theca versus granulosa. Endocrinology 102:227.

Goldenberg, R. L., J. L. Vaitukaitis and G. T. Ross. 1972. Estrogen and follicle stimulating hormone interactions on follicle growth in rats. Endocrinology 90:1492.

Gombe, S. and W. Hansel. 1973. Plasma luteinizing hormone (LH) and progesterone levels in heifers on restricted energy intakes. J. Anim. Sci. 37:728.

Hansel, W. and K. H. Siefart. 1967. Maintenance of luteal function in the cow. J. Dairy Sci. 50:1948.

Henricks, D. M., J. F. Dickey, J. R. Hill and W. E. Johnston. 1972. Plasma estrogen and progesterone levels after mating, and during late pregnancy and post-partum in cows. Endocrinology 90:1336.

Humphrey, W. D., D. R. Koritnik, C. C. Kaltenbach, T. G. Dunn and G. D. Niswender. 1976. Progesterone and LH in postpartum suckled beef cows. J. Anim. Sci. 43:290.

Lemon, M., J. Pelletier, J. Saumande and J. P. Signoret. 1975. Peripheral plasma concentrations of progesterone, oestradiol-17β and luteinizing hormone around oestrus in the cow. J. Reprod. Fertil. 42:137.

Louvet, J. P. and J. L. Vaitukaitis. 1976. Induction of follicle-stimulating hormone (FSH) receptors in rat ovaries by estrogen priming. Endocrinology 99:758.

NRC. 1970. Nutrient requirements of beef cattle. National Research Council, National Academy of Sciences, Washington, DC.

Perkins, J. L. and H. E. Kidder. 1963. Relation of uterine involution and postpartum interval to reproductive efficiency in beef cattle. Anim. Sci. 22:313.

Restall, B. J. and B. G. Starr. 1977. The influence of season of lambing and lactation on reproductive activity and plasma LH concentrations in Merino ewes. J. Reprod. Fertil. 49:297.

Restall, B. J., R. D. Kearins and B. G. Starr. 1977. Studies of pituitary function in lactating ewes. J. Reprod. Fertil. 49:291.

Richards, J. S., J. J. Ireland, M. C. Rao, G. A. Bernath, A. R. Midgley, Jr., and L. E. Reichert, Jr. 1976. Ovarian follicular development in the rat: Hormone receptor regulation by estradiol, follicle-stimulating hormone and luteinizing hormone. Endocrinology 99:1562.

Short, R. E., R. A. Bellows, E. L. Moody and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. J. Anim. Sci. 34:70.

Spitzer, J. C., G. D. Niswender, G. E. Seidel, Jr. and J. N. Wiltbank. 1975. Fertilization and endocrinology in underfed heifers. J. Anim. Sci. 41:380 (Abstr.).

Steger, R. W., J. J. Peluso, J. A. Mitchell and E. S. E. Hafez. 1975. Photoperiod effect on gonadotropin releasing hormone induced ovulation in the immature rat. Biol. Reprod. 13:597.

Webb, R., N. B. Haynes, H. D. Hafs and G. E. Lamming. 1975. The response of postpartum suckler cows to injection of gonadotropic releasing hormone. Soc. for the Study of Fertility Annu. Conf., Birmingham, England.

Whitman, R. W., E. E. Remmenga and J. N. Wiltbank. 1975. Weight change, condition and beef cow reproduction. J. Anim. Sci 41:387 (Abstr.).

Whitmore, H. L., W. J. Tyler and L. E. Casida. 1974. Effects of early postpartum breeding in dairy cattle. J. Anim. Sci. 38:339.

Wiltbank, J. N. 1974. Management programs to increase reproduction efficiency of beef herds. J. Anim. Sci. 38:(Suppl. I):58.

PITUITARY AND OVARIAN RESPONSES TO GONADOTROPIN RELEASING HORMONE, CALF REMOVAL AND PROGESTOGEN IN ANESTROUS BEEF COWS[1,2]

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Summary

Effects of Syncro-Mate B (SMB) and(or) short-term calf removal (CR) on pituitary and ovarian responses after gonadotropin releasing hormone (GnRH) were studied in two experiments. In Exp. 1, 24 thin, 2-yr-old, anestrous, Brahman crossbred cows were allotted randomly to a 2 × 2 factorial design involving 27-h CR and SMB. All cows were injected (iv) with 150 μg GnRH at 24 h after removal of implants and(or) calves. Blood samples were collected at −30, 0, 15, 30, 45, 60, 90, 120 and 180 min post-GnRH. The GnRH-induced release of luteinizing hormone (LH) differed among treatments ($P<.05$). The release of LH was higher in cows with CR than in control cows ($P<.05$). Although pretreatment with SMB increased the quantity of LH release compared with the CR and control cows, SMB + CR did not show an additive effect over SMB alone. In Exp. 2, 49 mature Charolais × Hereford cows were allotted randomly to one of the following groups: control, 24-h CR or 32-h CR. Follicular size was measured by palpation per rectum at the time of CR. All cows received 300 μg GnRH (im) at 24 h after CR, at which time calves were returned to cows in the 24-h CR group. The pattern of release of LH over time was similar to the control and 24-h CR groups; however, LH release was greater ($P<.05$) in the 32-h CR group. A linear effect of follicular size on the pattern of release of LH after GnRH injection was observed regardless of treatment. Plasma concentration of progesterone at 7 and 14 d after GnRH, indicated that function and lifespan of the induced corpora lutea were not altered by CR or follicular size. These results indicate that pretreatment with SMB increased the release of LH to exogenous GnRH. Furthermore, CR also increased the responsiveness of the pituitary to GnRH, provided that the calves were not returned during the period of induced LH release.

(Key Words: Anestrous Cows, Gonadotropin Releasing Hormone, Progestogen, Calf Removal.)

Introduction

Numerous investigators have attempted to induce an ovulatory estrus in anestrous cows suckling calves (see reviews by Inskeep and Lishman, 1978; Wettemann, 1980). In addition to various hormonal treatments, methods of reducing suckling frequency such as once daily suckling (Reeves and Gaskins, 1981; Randel, 1981), short-term calf removal (Smith et al., 1979), and early weaning (Bellows et al., 1974) have decreased the postpartum interval. Although the inhibitory effect of the suckling stimulus on bovine reproductive performance is well known (Edgerton, 1980), the mechanism

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by which suckling prolongs anestrus in cows is unclear.

The suckling stimulus reportedly decreases the systemic concentration of luteinizing hormone (LH; Short et al., 1972; Randel et al., 1976; Carruthers et al., 1980); however, the hypothalamic content of gonadotropin releasing hormone (GnRH; Carruthers et al., 1980) and the pituitary content of LH (Saiduddin et al., 1968; Walters et al., 1982a) are similar for suckled and nonsuckled cows. Consequently, suckling may decrease the responsiveness of the adenohypophysis to GnRH. The purpose of the present study was to determine whether administration of GnRH after short-term calf removal (CR) and(or) progestogen treatment would result in a greater release of LH, a higher incidence of corpus luteum formation and(or) improved luteal function in anestrous beef cows.

Materials and Methods

Exp. 1. Twenty-four, thin, 2-yr-old Brahman crossbred cows were allotted (six cows/group) by body condition and days postpartum (average 160 and range 138 to 189 d postpartum) to a 2 × 2 factorial design involving CR and Syncro-Mate B (SMB). The experiment, performed at Beeville, Texas, was conducted in two replicates. All cows were injected (iv) with 150 μg of GnRH (courtesy of Dr. M. Monahan, Salk Institute) diluted in sterile physiological saline. Cows allotted to the SMB, CR or SMB + CR groups received GnRH 24 h after removal of the implant and(or) calf. The calves, which were removed from the cows for 27 h (i.e., until completion of sampling), were kept in a dry pen and received a palatable diet containing 12 to 15% crude protein (ad libitum).

The SMB[5] treatment consisted of a 9-d ear implant containing 6 mg of norgestomet (17α acetoxy-11β methyl-19 nor preg-4-ene 3, 20 dione) and an im injection containing 3 mg of norgestomet and 6 mg of estradiol valerate given at the time the implant was placed into the ear.

The cows were observed for estrus twice daily from parturition until the end of the experiment and only cows that stood to be ridden by other cows were considered to be in estrus. The ovaries of all cows were examined per rectum for corpora lutea biweekly before the experiment. Only anestrous cows, as determined by estrous detection and palpation per rectum, were used in the study. Seven cows were detected in estrus after the first replicate and were replaced by seven anestrous cows at the same stage postpartum and having a similar body condition for the second replicate. During the experiment, the cows were maintained in a drylot and received a diet of approximately 7.5 kg Sudan grass hay that provided 16 Mcal of metabolizable energy$\cdot$head$^{-1}\cdot$d^{-1}.

Cow body condition was measured subjectively at the start of the experiment by one technician. The fat cover over the back and ribs was manually palpated and the cows were assigned a body condition score ranging from one to nine indicating the lowest to highest body condition, respectively. The means ($\bar{x}$ ± SD) for cow body condition and body weight at the start of the experiment were 3.6 ± .7 and 290.5 ± 33.7 kg, respectively.

Blood samples were collected via puncture of a tail vessel at −30, 0, 15, 30, 45, 60, 90, 120 and 180 min postinjection of GnRH and allowed to clot at 4 C. After centrifugation, the serum was collected and frozen at −20 C until assayed for LH by radioimmunoassay (Forrest et al., 1980).

Exp. 2. Mature Charolais × Hereford cows (average 24 d and range 19 to 33 d postpartum) were allotted at random within age to one of the following groups: control, 24-h CR or 32-h CR. The experiment was conducted at Wardensville, West Virginia, with three replicates of cows (total n = 49). Cows were fed mixed grass hay and a daily supplement of 45 g magnesium oxide (Mg0) in 1 kg of a mixture of ground ear corn, oats and soybean oil mean (4:4:1), which was begun 4 mo before calving. The supplement was increased to 2 kg 4 wk before calving, with the level of Mg0 remaining at 45 g. All cows were in moderate body condition when the experiment was conducted. Observations for estrus were made twice daily from the day before GnRH until 14 d after GnRH. The ovaries of all cows were examined per rectum for diameter of the largest follicle and absence of corpora lutea 1 d before treatment with GnRH and for induced corpora lutea 7 and 14 d after GnRH.

All cows were injected (im) with 300 μg GnRH (generously donated by R. H. Rippel, Abbott Laboratories). For 24-h CR, calves were separated from their dams for 24 h and returned

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[5] G. D. Searle and Co., Chicago, IL.

as GnRH was injected. For 32-h CR, calves were not returned to their dams until 8 h after injection of GnRH. While calves were removed, they were housed as in Exp. 1, in a barn 1.6 km from the barn where the cows were maintained. In the first and third replicates (34 cows), the cows were placed into stanchions and fitted with iv catheters 2 h before injection of GnRH. Jugular blood samples were collected just before GnRH and thereafter at 30-min intervals for 7 h. Progesterone was measured in blood samples collected from all 49 cows at 7 and 14 d after injection of GnRH. After separation by centrifugation, the plasma samples were stored at −20 C until assayed for LH (Fogwell et al., 1978) or progesterone (Berardinelli et al., 1979).

Statistical Analyses. Profiles of concentrations of LH in both experiments were examined for differences among groups by analysis of variance using time as an independent continuous variable as described by Fogwell et al. (1978). In Exp. 1, data were transformed to logarithms and the linear, quadratic and cubic regression of hormonal concentrations on time were partitioned. In Exp. 2, the linear effect of follicular size was included in the final regression model and both linear and quadratic terms were used to describe the rise and fall of LH. Differences between replicates were not significant in either experiment, so replicate was eliminated from the final models.

Effects of treatment and follicular size on proportions of cows forming corpora lutea in Exp. 2 were examined by chi-square. Concentrations of progesterone on d 7 and 14 after GnRH in Exp. 2 were compared by analysis of variance for effects of treatment and follicular size.

Results

Exp. 1. Regardless of treatment, LH began to increase by 15 min after GnRH administration and the maximum concentration of LH occurred at 120 min postinjection (figure 1).

The pattern of release of LH was modified by pretreatment with SMB (P<.01). The GnRH-induced release of LH was greater in the cows receiving SMB or SMB + CR than the release of LH for cows in the control and CR groups. While there was not a significant effect of CR over time in the groups receiving SMB, the interaction of SMB × CR over time was significant (P<.05). Consequently, the pattern of LH release differed among treatments except for the SMB and SMB + CR groups. The release of LH was higher in cows with CR compared with control cows.

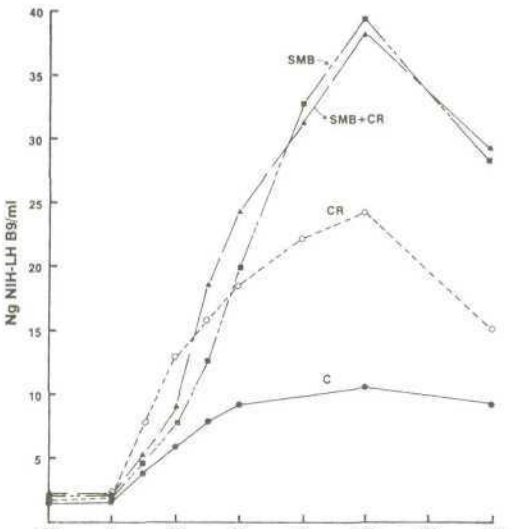

Figure 1. The GnRH-induced release of LH for control (C), 27-h calf removal (CR), Syncro-Mate B (SMB) and Syncro-Mate B + 27-h calf removal (SMB + CR) groups (six cows/group). Each cow was injected with 150 μg of GnRH at 24 h after implant and(or) calf removal.

Exp. 2. Patterns of release of LH, whether examined as the arithmetic or logarithmic values over time, were altered by treatment (P<.05). The effect was due primarily to calves being absent during the GnRH-induced release of LH. Patterns in control cows and those with 24-h CR did not differ and are illustrated together in figure 2 (part B). The amount of LH released was greater in cows with 32-h CR (figure 2, part A). Regardless of treatment, there was a linear effect of follicular size on the pattern of LH release (P<.05), which also is illustrated in the computed arithmetic patterns presented in figure 2. The regression equation from which these curves were generated accounted for 69% (R^2) of the variation in LH over time in this study.

The percentage of cows forming corpora lutea was related to the size of the largest follicle as was seen with release of LH. Corpora lutea and increases in plasma progesterone were detected in 50% of two cows in which the largest follicles were less than 10 mm, 67% of six cows with 10-mm follicles, 73% of 30 cows

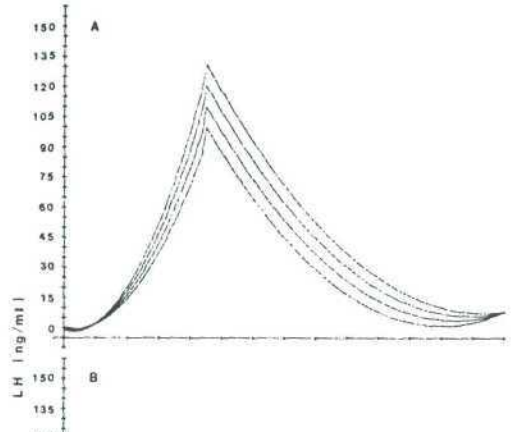

Figure 2. Patterns of LH in response to GnRH (300 μg im) in (A) cows with calves removed 24 h before GnRH and returned 8 h after GnRH, and (B) cows with calves suckling or calves removed 24 h before GnRH and returned as GnRH was injected. Curves are presented as a regression model considering the time of peak LH and linear and quadratic terms in time to and from the peak of LH. Separate curves in ascending order reflect the linear effects of follicular size 24 h before GnRH in 2-mm increments, from 10 to 16 mm in A and from 8 to 16 mm in B. There were no cows with 8-mm follicles in the group where calves were removed for 32 h (A).

with 12- or 14-mm follicles, 88% of eight cows with 16-mm follicles and 100% of three cows with follicles greater than 16 mm in diameter. However, neither the overall nor the linear effect of follicular size was significant by chi-square.

The apparent lifespan and level of function of the induced corpora lutea, as judged by systemic concentration of progesterone on d 7 and 14, did not differ among treatments (table 1) or follicular size (not shown). Concentrations of plasma progesterone on d 7 in most cows having induced corpora lutea were lower than would be expected in normal, cycling cows. All but eight of the 37 cows with corpora lutea had apparently undergone complete luteal regression by d 14. None of the cows showed estrus during the experimental period.

Discussion

The increased pituitary responsiveness to GnRH after pretreatment with SMB is in agreement with a report by Troxel et al. (1980). Presently, it is unclear whether the increased responsiveness of the adenohypophysis after SMB is due to an increase in synthesis of LH and(or) a prior inhibition by progestogen of release of LH, resulting in hormone storage.

The inhibitory effect of progesterone on LH release has been reported in ewes (Karsch et al., 1977) and cattle (Hansel and Echternkamp, 1972). Furthermore, the GnRH-induced release of LH during the luteal phase of the cycle was diminished in ewes (Reeves et al., 1971) and cows (Kittock et al., 1973); consequently progesterone may inhibit the release of LH in response to the neurohormone. Alternatively, Zolman et al. (1974) reported that the progesterone level on d 15 and 20 of the bovine cycle was not related to GnRH-induced release of LH. They suggested that changes in pituitary responsiveness to GnRH after a decrease in systemic concentrations of progesterone may be time dependent.

Walters et al. (1982b) reported that SMB suppressed basal concentrations of LH in anestrous cows. This effect of SMB might decrease endogenous GnRH secretion from the hypothalamus. Removal of the progestogen implant may allow a greater release of endogenous GnRH, which might have a self-priming effect (Staigmiller et al., 1981) on the adenohypophysis before the injection of exogenous GnRH.

Another method by which SMB pretreatment might increase the pattern of release of LH after GnRH injection involves increased estrogen secretion. Progestogen pretreatment has been associated with increased concentrations of estrogen in anestrous cows (Troxel et al., 1980; Sheffel et al., 1982). Estrogens reportedly increase the responsiveness of the adenohypophysis to GnRH (Beck and Convey, 1974; Kesler et al., 1977).

In cows not receiving SMB pretreatment, CR (27 or 32 h) increased pituitary responsiveness to GnRH, as reported by Troxel et al. (1980). Additional evidence for the increased release of LH after withdrawal of the suckling stimulus has been shown in vitro. Pituitaries collected

TABLE 1. RESPONSE OF LACTATING CROSSBRED BEEF COWS TO GnRH: OCCURRENCE OF LUTEAL STRUCTURES AND SYSTEMIC CONCENTRATIONS OF PROGESTERONE[a]

	Mean					
					Progesterone in plasma (ng/ml)[b]	
Treatment	No. of cows	Days postpartum[b]	Size of follicle, mm[b]	Cows forming corpora lutea, %	d 7	d 14
Control	16	23.7 (3.2)	13.6 (3.1)	69	1.5 (.8)	.8[c] (1.7)
Calf removed 24 h	17	23.8 (4.4)	12.7 (1.9)	82	1.4 (.9)	.4[c] (.7)
Calf removed 32 h	16	24.4 (4.0)	13.4 (3.0)	75	1.4 (.8)	.8[c] (1.1)

[a] Neither variable differed with treatment.

[b] Values in parentheses are standard deviations.

[c] Two cows in the control group, two cows with calves removed for 24 h and four cows with calves removed for 32 h exhibited increasing levels of progesterone from d 7 to d 14; one such animal had a luteinized follicle and two may have formed corpora lutea in response to an endogenous surge of LH unrelated to the exogenous GnRH (definite luteal structures were not palpable in these two cows on d 7).

from anestrous cows at 24, 48, 72 or 96 h after CR and incubated with GnRH, accumulated more LH in the media than pituitaries from control (suckled) cows (Walters et al., 1982a).

It is not clear whether the critical factor was the duration of CR or the fact that suckling did not occur while LH was being released. The suppressive effect of suckling, specifically during action of exogenous GnRH, was demonstrated by the similarity of the pattern of GnRH-induced release of LH in the control cows and those in the 24-h CR group, to which calves were returned when GnRH was injected. In a previous study (Walters et al., 1982b), the circulating basal concentration of LH in cows was decreased at the time of calf return.

The increased release of LH over time in cows not suckled during the period of action of GnRH may have been due to a greater sensitivity of the pituitary releasing mechanism. Saiduddin et al. (1968) and Walters et al. (1982a) reported no difference in pituitary concentrations of LH between suckled and nonsuckled cows; consequently, an increase in synthesis of LH after CR seems less likely.

As previously mentioned, the animals in Exp. 1 were in poor body condition, which partially explains why the cows were still anestrus at 138 to 189 d postpartum. Several reports indicate that cows in poor body condition have prolonged postpartum intervals (Whitman et al., 1975; Dunn and Kaltenbach, 1980). It is important to note that short term CR increased the release of LH in response to GnRH in both Exp. 1 and 2 even though the cows were in poor and moderate body condition, respectively.

Greater release of LH with increasing follicular size as seen in Exp. 2 has been reported in anestrous 2-yr-old Hereford cows treated with GnRH (Lishman et al., 1979). A similar relationship to increasing concentrations of estrogen was observed in dairy cows (Fernandes et al., 1978). It would appear that in the early postpartum cow, ovarian follicular status reflects the readiness of the pituitary to respond to GnRH. In turn, cows with larger follicles tended to form corpora lutea, although the corpora lutea did not necessarily form from the largest palpable follicle and were sometimes in the opposite ovary.

Luteal function or lifespan as measured by concentrations of progesterone on d 7 and 14 post-GnRH was usually limited and was not related to the pattern of release of LH. In other studies, induced corpora lutea had a short lifespan (Lishman et al., 1979), except when pretreated with progestogen (Pratt et al., 1982; Sheffel et al., 1982). Additionally, corpora lutea formed spontaneously at the first estrus after weaning had a short lifespan and could not maintain pregnancy unless estrus was preceded by progestogen treatment (Ramirez-Godinez et al., 1981). Induced corpora lutea collected from anestrous cows and ewes did not produce as much progesterone in response to LH in vitro as corpora lutea collected from cycling animals (Kesler et al., 1981; McNeilly et al., 1981).

That estrus was not observed during induction of, or after early regression of, corpora lutea in Exp. 2 is not surprising because these cows averaged only 24 d postpartum at treatment. Lishman et al. (1979) observed that only one of 23 young cows with corpora lutea induced on d 34 postpartum ovulated again after the induced corpora lutea regressed. Alternatively, Pratt et al. (1982) observed estrus on d 8 to 12 after treatment in 11 of 37 mature cows that were over 30 d postpartum when they received GnRH or human chorionic gonadotropin.

It is concluded that pretreatment with SMB before injection of GnRH increased release of LH in response to GnRH. Calf removal for 24 h had a similar effect, provided that the calves were not returned during the period of induced release of LH. Patterns of release of LH were directly proportional to follicular size. Neither CR nor follicular size affected the lifespan of corpora lutea induced by GnRH.

Literature Cited

Beck, T. W. and E. M. Convey. 1974. LH after estradiol and GnRH in spayed heifers. J. Anim. Sci. 39:198 (Abstr.).

Bellows, R. A., R. E. Short, J. J. Urick and O. F. Pahnish. 1974. Effects of early weaning on postpartum reproduction of the dam and growth of calves born as multiples or singles. J. Anim. Sci. 39:589.

Berardinelli, J. G., R. A. Dailey, R. L. Butcher and E. K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49:1276.

Carruthers, T. D., E. M. Convey, J. S. Kesner, H. D. Hafs and K. W. Cheng. 1980. The hypothalamo-pituitary gonadotrophic axis of suckled and nonsuckled dairy cows postpartum. J. Anim. Sci. 51:949.

Dunn, T. G. and C. C. Kaltenbach. 1980. Nutrition and the postpartum interval of the ewe, sow and cow. J. Anim. Sci. 51(Suppl. II):29.

Edgerton, L. A. 1980. Effect of lactation upon the postpartum interval. J. Anim. Sci. 51(Suppl. II):40.

Fernandes, L. C., W. W. Thatcher, C. J. Wilcox and E. P. Call. 1978. LH release in response to GnRH during the postpartum period of dairy cows. J. Anim. Sci. 46:443.

Fogwell, R. L., C. W. Weems, G. S. Lewis, R. L. Butcher and E. K. Inskeep. 1978. Secretion of steroids after induced luteal regression in beef heifers: Effects of PGF$_2\alpha$ and removal of corpora lutea. J. Anim. Sci. 46:1718.

Forrest, D. W., J. L. Fleeger, C. R. Long, A. M. Sorensen, Jr. and P. G. Harms. 1980. Effect of exogenous prolactin on peripheral luteinzing hormone levels in ovariectomized cows. Biol. Reprod. 22:197.

Hansel, W. and S. E. Echternkamp. 1972. Control of ovarian function in domestic animals. Amer. Zool. 12:225.

Inskeep, E. K. and A. W. Lishman. 1978. Factors affecting postpartum anestrus in beef cattle. Beltsville Symp. on Agr. Res. (BARC) III Anim. Reprod. p 277.

Karsch, F. J., S. J. Legan, R. L. Hauger and D. L. Foster. 1977. Negative feedback action of progesterone on tonic luteinizing hormone secretion in the ewe: Dependence on the ovaries. Endocrinology 101:800.

Kesler, D. J., H. A. Garverick, R. S. Youngquist, R. G. Elmore and C. J. Bierschwal. 1977. Effect of days postpartum and endogenous reproductive hormones on GnRH-induced LH release in dairy cows. J. Anim. Sci. 45:797.

Kesler, D. J., P. G. Weston, C. A. Pimentel, T. R. Troxel, D. L. Vincent and J. E. Hixon. 1981. Diminution of the in vitro response to luteinizing hormone by corpora lutea induced by gonadotropin releasing hormone treatment of postpartum suckled beef cows. J. Anim. Sci. 53:749.

Kittock, R. J., J. H. Britt and E. M. Convey. 1973. Endocrine response after GnRH in luteal phase cows and cows with ovarian follicular cysts. J. Anim. Sci. 37:985.

Lishman, A. W., S.M.J. Allison, R. L. Fogwell, R. L. Butcher and E. K. Inskeep. 1979. Follicular development and function of induced corpora lutea in underfed postpartum anestrous beef cows. J. Anim. Sci. 48:867.

McNeilly, A. S., M. Hunter, R. G. Land and H. S. Fraser. 1981. Inadequate corpus luteum function after induction of ovulation in anestrous ewes by LH-RH or an LH-RH agonists. J. Reprod. Fertil. 61:137.

Pratt, B. R., J. G. Berardinelli, L. P. Stevens and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cow. I. Comparison of gonadotropin releasing hormone and human chorionic gonadotropin and effects of progestogen and estrogen. J. Anim. Sci. 54:822.

Ramirez-Godinez, J. A., G. H. Kiracofe, R. M. McKee, R. R. Schalles and R. J. Kittock. 1981. Reducing the incidence of short estrous cycles in beef cows with norgestomet. Theriogenology 15:613.

Randel, R. D. 1981. Effect of once daily suckling on postpartum interval and cow-calf performance of first-calf Brahman X Hereford heifers. J. Anim. Sci. 53:755.

Randel, R. D., R. E. Short and R. A. Bellows. 1976. Suckling effect on LH and progesterone in beef cows. J. Anim. Sci. 42:267 (Abstr.).

Reeves, J. J., A. Arimura and A. V. Schally. 1971. Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH-RH) at various stages of the estrous cycle in sheep. J. Anim. Sci. 32:123.

Reeves, J. J. and C. T. Gaskins. 1981. Effect of once-a-day nursing on rebreeding efficiency of beef cows. J. Anim. Sci. 53:889.

Saiduddin, S., J. W. Risen, W. J. Tyler and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotropins, ovarian follicular development and fertility of dairy cows. Univ. Wisconsin Res. Bull. 270:15.

Sheffel, C. E., B. R. Pratt, W. L. Ferrell and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. J. Anim. Sci. 54:830.

Short, R. E., R. A. Bellows, E. L. Moody and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. J. Anim. Sci. 34:70.

Smith, M. F., W. C. Burrell, L. D. Shipp, L. R. Sprott, W. N. Songster and J. N. Wiltbank. 1979. Hormone treatments and use of calf removal in postpartum beef cows. J. Anim. Sci. 48:1285.

Staigmiller, R. B., R. E. Short and R. A. Bellows. 1981. Response to multiple GnRH injections in prepuberal beef heifers. Biol. Reprod. 24(Suppl. 1):72A.

Troxel, T. R., D. J. Kesler, R. C. Noble and S. E. Carlin. 1980. Ovulation and reproductive hormones following steroid pretreatment, calf removal and GnRH in postpartum suckled beef cows. J. Anim. Sci. 51:652.

Walters, D. L., R. E. Short, E. M. Convey, R. G. Staigmiller, T. G. Dunn and C. C. Kaltenbach. 1982a. Pituitary and ovarian function in postpartum beef cows. II. Endocrine changes prior to ovulation in suckled and nonsuckled postpartum cows compared to cycling cows. Biol. Reprod. 26:647.

Walters, D. L., M. F. Smith, P. G. Harms and J. N. Wiltbank. 1982b. Effect of steroids and/or 48 hr calf removal on serum luteinizing hormone concentrations in anestrous beef cows. Theriogenology 18:349.

Wettemann, R. P. 1980. Postpartum endocrine function of cattle, sheep and swine. J. Anim. Sci. 51(Suppl. II):2.

Whitman, R. W., E. E. Remmenga and J. N. Wiltbank. 1975. Weight change, condition and beef cows reproduction. J. Anim. Sci. 41:387 (Abstr.).

Zolman, J., E. M. Convey and J. H. Britt. 1974. Relationships between the luteinizing hormone response to gonadotropin releasing hormone and endogenous steroids. J. Anim. Sci. 39:355.

PAPER 30

FACTORS AFFECTING FUNCTION OF INDUCED CORPORA LUTEA IN POSTPARTUM ANESTROUS EWES[1]

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Summary

Two experiments were conducted with multiparous, anestrous ewes of mixed breeding that lambed in the autumn. Ewes in each experiment were assigned randomly to treatments in a 2 × 2 factorial arrangement. Ewes in groups 1 and 2 received flurogestone acetate from an intravaginal pessary (containing 20 mg) during days 17 to 21 postpartum, while ewes in groups 3 and 4 received no progestogen. Lambs of ewes in groups 2 and 4 were weaned on day 20 postpartum. All ewes received 150 μg of gonadotropin releasing hormone (GnRH) in a single IM injection on the morning of day 22 postpartum. In Exp. 1, jugular blood was collected just before GnRH and at 30-min intervals over the next 3 hr to monitor release of luteinizing hormone (LH). Jugular blood was collected from each ewe in Exp. 2 just before GnRH and daily for the next 16 days for determination of progesterone. Seven days after treatment with GnRH, all ewes were laparotomized, ovaries were observed and uterine blood samples were collected for assay of prostaglandin $F_2\alpha$ ($PGF_2\alpha$). Ewes in Exp. 2 were checked for estrus at 12-hr intervals beginning the morning after GnRH and were bred when found in standing estrus. Mean concentrations of LH (nanograms/milliliter) during the 3-hr test period were higher ($P<.05$) in progestogen-treated (59.2) than in control (39.5) ewes. Pattern of release of LH after GnRH was altered by progestogen treatment ($P<.01$; progestogen × time interaction) but not by the number of lambs suckling before or after day 20 postpartum. Profiles of progesterone over time after GnRH varied with progestogen treatment ($P<.01$), but not with number of lambs suckling before day 20 or weaning on day 20. Mean concentrations of progesterone (nanograms/milliliter) varied ($P<.05$) with the number of lambs suckling before day 20 (2.3, 1.6 and .6 for ewes suckling one, two and three lambs, respectively). All ewes had corpora lutea (mean = 2.6) at laparotomy. The life of induced corpora lutea in Exp. 2 averaged 11.8 days and did not vary significantly with treatment. Ewes showed estrus and were mated an average of 17.7 days after GnRH, and 82% lambed to this mating. Concentrations of $PGF_2\alpha$ were lower ($P<.05$) in ewes receiving progestogen than in those not receiving progestogen (1.7 *vs* 4.2 ng/ml), but were not affected by number of lambs suckling before or after day 20 postpartum. Weaning of lambs and treatment for 4 days with progestogen did not improve life span or function of corpora lutea induced by GnRH. Variation in duration of luteal function after GnRH could not be explained by the pattern of release of LH after GnRH or by concentrations of $PGF_2\alpha$ in uterine venous plasma 7 days after GnRH.

(Key Words: Postpartum Anestrous, Ewes, Gonadotropin Releasing Hormone, Luteinizing Hormone, Prostaglandin $F_2\alpha$, Corpora Lutea.)

Introduction

Synthetic gonadotropin releasing hormone (GnRH) has been used to induce formation of corpora lutea in anestrous ewes (Reeves *et al.*, 1972; Haresign *et al.*, 1973; Crighton *et al.*, 1975; Frandle *et al.*, 1977; Restall *et al.*, 1977). However, duration of the induced luteal phase during either seasonal or lactational anestrus was short, and progesterone production by the

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induced corpora lutea was reduced (Haresign *et al.*, 1973; Crighton *et al.*, 1973, 1975). Frandle *et al.* (1977) suggested that the function of corpora lutea induced in anestrous ewes with an analog of GnRH, D-Leu^6-desGly NH$_2$^{10}-LHRH ethylamide (D-Leu^6-LHRH), was comparable to that of corpora lutea in the same ewes during spontaneous estrous cycles later in the same year, but their data were not adequate to prove that this was the case (see discussion).

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) is a naturally occurring luteolysin in ewes (Pharriss *et al.*, 1972; Goding, 1974). Uterine release of $PGF_2\alpha$ occurs in response to either suckling (Thorburn *et al.*, 1977) or exogenous oxytocin (Sharma and Fitzpatrick, 1974; Roberts and McCracken, 1976). $PGF_2\alpha$ was elevated in uteroovarian venous plasma of ewes during parturition (Thorburn *et al.*, 1977), as well as during the first few days after parturition (W. Bruce Currie, *personal communication).* Therefore, corpora lutea induced in lactating ewes during the early postpartum period may be exposed to elevated concentrations of $PGF_2\alpha$.

Treatment for 12 days with an intravaginal sponge containing a progestogen, followed by an injection of pregnant mare's serum gonadotropin (PMSG), caused ovulation during seasonal and lactational anestrus in ewes (Cognie and Pelletier, 1976). Ewes lambing in autum began estrous cycles within 34 days after parturition when lambs were weaned at birth (Restall, 1971). Steroid treatment and temporary removal of calves have been effective in producing fertile estrus in lactating beef cattle (Wiltbank and Mares, 1977).

The present study with postpartum, anestrous ewes lambing in the fall was undertaken to ascertain whether weaning of lambs or treatment with progestogen before GnRH would affect the release of LH in response to GnRH (Exp. 1) or the life span of corpora lutea induced by GnRH (Exp. 2). The concentration of $PGF_2\alpha$ in uterine venous plasma 7 days after GnRH was determined in both experiments. In Exp. 2, the relationship of concentrations of progesterone in plasma to $PGF_2\alpha$ was determined.

Materials and Methods

Two experiments were conducted with multiparous, anestrous ewes of mixed breeding that lambed over a 3-week period (from mid-September to the first week of October). Ewes in each experiment were assigned randomly to treatments in a 2 × 2 factorial arrangement with progestogen and weaning as the main factors. Treatments and number of ewes in each group within each experiment are listed in table 1. Each ewe in groups 1 and 2 received a pessary containing 20 mg of a progestogen (flurogestone acetate) from day 17 to 21 postpartum. Ewes in groups 1 and 3 were suckled throughout the experiment, while lambs were weaned on day 20 postpartum from ewes in groups 2 and 4. The numbers of lambs suckling before day 20 were recorded. All ewes received 150 μg of GnRH in a single IM injection on the morning of day 22 postpartum.

Seven days after treatment with GnRH, each ewe was anesthetized with sodium pentobarbital, and the reproductive organs were exposed through a midventral incision. At this time, 10 ml of blood for assay of $PGF_2\alpha$ in plasma were collected by puncture of a uterine branch of the ovarian vein draining each uterine horn. Hydrochloric acid (.1 ml of 1 N/ml of blood) was added immediately to each blood sample (Pexton *et al.*, 1975) to inhibit the synthesis of prostaglandins by platelets (Smith and Willis, 1970). The number and gross appearance of the corpora lutea were recorded.

TABLE 1. ARRANGEMENT OF TREATMENTS AND NUMBER OF EWES ASSIGNED TO EACH GROUP IN EACH EXPERIMENT

Group	Progestogen	Weaning	Exp. 1	Exp. 2
			(No. of ewes)	
1	Flurogestone acetate	Suckled	6	7
2	(20 mg) by pessary	Weaned	6	6
3	None	Suckled	6	10
4		Weaned	6	9

In the first experiment, samples (5 ml) of jugular blood were taken from each ewe with heparinized syringes just before injection of GnRH and again at 30-min intervals over the next 3 hr to monitor release of LH.

In Exp. 2, jugular blood from each ewe was collected into heparinized syringes just before treatment with GnRH and daily for the next 16 days for assay of progesterone. Starting the morning after GnRH, brisket-painted vasectomized rams were pastured with the ewes in order to detect those ewes in estrus. At 12-hr intervals, marked ewes were removed from the flock and rechecked for standing estrus with other vasectomized rams. Except for two ewes selected at random from each treatment group, ewes observed in estrus after the induced luteal phase were pasture-mated to rams of known fertility. Luteal function was evaluated by monitoring concentrations of progesterone in daily samples of jugular plasma during the second spontaneous estrous cycle after parturition in the eight nonmated ewes. Three criteria were used for estimating the life span of corpora lutea induced by GnRH in each ewe: (1) interval from GnRH to estrus, (2) profile of progesterone and (3) gross appearance of corpora lutea at laparotomy 7 days after GnRH.

Hormone Assays. Plasma was collected from all blood samples after centrifugation at 4 C and stored at −20 C until hormones were quantified by radioimmunoassay. Concentrations of LH were measured by the double antibody radioimmunoassay system of Niswender *et al.* (1969). Purified ovine LH (LER 1056 C-2) served as the iodinated preparation. Concentrations are expressed as nanograms/milliliter of NIH-LH-S-19 (1.01 × NIH-LH-S-1). Samples from a pool of plasma from ewes in the midluteal phase and a pool of plasma obtained from ewes during a surge of LH induced with exogenous GnRH were included in each assay. Coefficients of variation were based upon assay of three volumes per pool of plasma and replicated four times per assay. Within and between 12 assays, these coefficients averaged .18 and .16, respectively. The limit of assay sensitivity was .025 ng/tube.

Progesterone was extracted from plasma with anesthesia grade ether and quantified in the unchromatographed extract (Butcher, 1977). The correlation between the concentrations of progesterone in chromatographed and unchromatographed samples (n = 32) was .96. $PGF_2\alpha$ was extracted from each sample of uterine venous plasma, chromatographed and quantified as described by Lewis *et al.* (1978).

Statistical Analyses. Least-squares analyses of variance of a split-plot design were used to test for differences among treatments in patterns of concentrations of LH (Exp. 1) and progesterone (Exp. 2) in jugular plasma. Progesterone, numbers of lambs suckled before (1, 2 or 3) and after (0 *vs* 1 to 3) day 20 and all interactions of these sources were the treatments, and ewe within main effects constituted the error term in the main plot. The continuous, independent variable, time (days or minutes) after GnRH, and its interactions with the sources in the main plot were the treatments in the subplot. In a separate analysis, concentrations of progesterone in jugular plasma were compared by least-squares analysis of covariance of a split-plot design, with uterine venous $PGF_2\alpha$ as the covariate.

The effects of progesterone, number of lambs suckled before and after day 20 and all interactions on number of corpora lutea induced by GnRH and duration of the induced luteal phase were evaluated by least-squares analyses of variance. Values for $PGF_2\alpha$ in the two uterine veins of each ewe were compared, found not to differ and, hence, averaged to yield a single value for each ewe. Variations in these mean concentrations of $PGF_2\alpha$ were evaluated with statistical models similar to those described above, with effects of experiment included.

Results

In Exp. 1, pretreatment with progesterone increased ($P<.05$) the mean concentration of LH in jugular plasma collected during the 180 min after GnRH (59.2 *vs* 39.5 ng/ml in control ewes; table 2). Concentration of LH was not affected by number of lambs suckling before day 20 postpartum or by weaning on day 20. Pattern of release of LH in response to GnRH was altered by progesterone treatment ($P<.01$; progestogen × time interaction); that is, LH peaked at a higher concentration (table 2) in progestogen-treated ewes than in control ewes. Number of lambs suckling before or after day 20 did not affect this pattern.

All ewes had corpora lutea at laparotomy 7 days after treatment with GnRH. Bright red corpora lutea not covered by stroma, found in some ewes in the presence of pale corpora lutea covered with stroma, were judged to indicate

TABLE 2. CONCENTRATIONS OF LH (NANOGRAMS/MILLILITER) IN POSTPARTUM ANESTROUS EWES TREATED AND NOT TREATED WITH PROGESTOGEN BEFORE 150 μg OF GnRH

Time after GnRH, min	Progestogen-treated	Control
0	1.1[a]	2.7
30	24.7	26.7
60	57.5	50.6
90	85.8	53.9
120	84.4	54.5
150	79.5	47.5
180	75.6	40.8
Overall	59.2	39.5

[a] Progestogen treatment affected both mean LH concentration ($P<.05$) and pattern of LH release ($P<.01$; progestogen × time interaction). Error mean square = 2,505.48.

that the corpora lutea induced by GnRH had regressed and the ewe had reovulated. An average of two ewes in each group in each experiment, regardless of treatment, had newly forming corpora lutea in the presence of regressing corpora lutea. When this occurred, the number of regressing corpora lutea was used to determine the number of corpora lutea induced in that ewe by GnRH. In these ewes (Exp. 2), concentrations of progesterone increased from less than .5 ng/ml just before GnRH to about 1.5 ng/ml by day 2, began to decline by day 4, were approximately .5 ng/ml by day 5 or 6 and began to increase again by day 8 or 9. This pattern suggested that the regressing corpora lutea had been induced by GnRH. The number of corpora lutea induced by GnRH did not differ among the groups of ewes, either within or between the two experiments, and did not vary with number of lambs suckling before or after day 20. The mean number of corpora lutea per ewe was 2.6.

In Exp. 2, two to four ewes per group had a luteal phase of normal length (15 to 18 days), while three to seven ewes per group had a short luteal phase (14 days or less). Effects of treatments on the duration of the luteal phase and on the profiles of progesterone after GnRH were determined first for all ewes in each group, regardless of life span of the corpora lutea, then separately for those ewes having either short or normal luteal phases. Duration of induced luteal phases averaged 11.8 days (12.8, 9.3, 12.8 and 11.6 days for ewes in groups 1, 2, 3 and 4, respectively; error mean square = 9.79) and did not differ among the groups or with number of lambs suckled before or after day 20. Progestogen treatment reduced ($P<.05$) the duration of short (14 days) luteal phases (6.6 *vs* 9.8 days for ewes treated and not treated with progestogen, respectively; error mean square = 9.66). Neither weaning lambs on day 20 nor number of lambs suckling before day 20 affected duration of the short luteal phases. Normal (>14-day) luteal phases averaged 16.8 days (error mean square = 1.9), did not vary with treatment and were comparable in length to estrous cycles associated with the second spontaneous corpora lutea in the right nonmated ewes (16.7 days).

Mean concentrations of progesterone (nanograms/milliliter) for all ewes in groups 1, 2, 3 and 4 were 2.1, 1.5, 2.6 and 2.2, respectively, and did not vary with the main effects of progestogen treatment and weaning on day 20 or with their interaction. Number of lambs suckling before day 20 had an effect on the concentration of progesterone ($P<.05$), regardless of progestogen treatment. Overall mean concentrations of progesterone for ewes suckling one (number of ewes [n] = 21; number of blood samples [s] = 294), two (n = 10; s = 96) and three (n = 1; s = 11) lambs were 2.3, 1.6 and .6, respectively.

The profiles of progesterone in jugular plasma after GnRH were modified by pretreatment with progestogen ($P<.05$), but not by number of lambs suckling before or after day 20 postpartum. When profiles of progesterone in jugular plasma of ewes with short and normal luteal phases were considered separately, the profiles in both kinds of ewes were affected by treatment with progestogen before GnRH ($P<.05$; progestogen × day interaction). Among ewes with a short luteal phase (figure 1, panel B), concentrations of progesterone in progestogen-treated ewes declined earlier than they did in the ewes not receiving progestogen. Among ewes with a luteal phase of normal length, the profile of progesterone in ewes that received progestogen was lower throughout the sampling period than it was in control ewes (figure 1, panel C).

Concentrations of $PGF_2\alpha$ (nanograms/milliliter) in uterine venous plasma 7 days after treatment with GnRH did not differ within

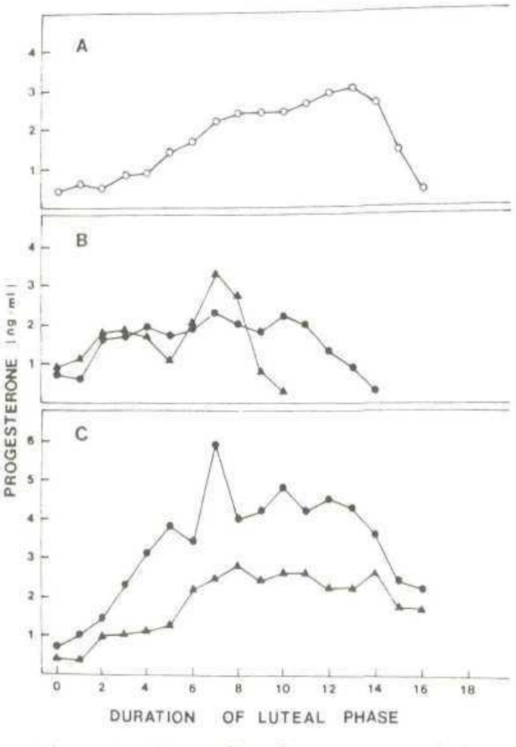

Figure 1. The profile of progesterone during a spontaneous estrous cycle in eight ewes selected at random from the second experiment is depicted in panel A to illustrate the changes that occur during a normal estrous cycle. Profiles of progesterone in anestrous ewes treated (▲—▲) and not treated (●—●) with progestogen before GnRH are shown in panels B (ewes with short luteal phases) and C (ewes with luteal phases of normal duration). In panel B, each point from day 0 to day 5 for the progestogen-treated ewes is the mean progesterone concentration for seven ewes; on days 6, 7, 8, 9 and 10, each point represents three, two, two, two and two ewes, respectively. Each point from day 0 to day 5 for ewes not receiving progestogen (panel B) is the mean of 12 observations; on days 6, 7, 8, 9, 10, 11, 12, 13 and 14, the points represent the means of 11, nine, nine, seven, six, five, four, three and three observations. Error mean square = 8.33. In panel C, each point on each curve from day 0 to day 15 represents the mean concentration of progesterone from six ewes; the points on day 16 are the means for five ewes. Error mean square = 9.23.

treatment groups between the two experiments; therefore, the data from the two experiments were pooled. Treatment with progestogen before GnRH reduced ($P<.05$) the concentrations of $PGF_2\alpha$ in uterine venous plasma (table 3), but number of lambs suckling before or after day 20 did not affect the concentrations of $PGF_2\alpha$. Neither overall mean concentrations nor patterns of progesterone in jugular plasma from ewes in Exp. 2 were related to concentrations of $PGF_2\alpha$ in uterine venous plasma on day 7 post-GnRH.

Of the ewes treated with GnRH in Exp. 2, only one was detected in estrus (on day 5 post-GnRH) before laparotomy, but on the average (not affected by treatment), the ewes were detected in estrus (n = 32) and mated (n = 24) 17.7 days (range of 5 to 40 days) after treatment with GnRH. Eighty-two percent of the ewes lambed to mating during the first estrus after treatment with GnRH.

Discussion

Ewes treated with progesterone released more LH in response to GnRH than did control ewes. This is consistent with the report of Roche *et al.* (1970), who found an increase in concentration of LH in the pituitary that was related temporally to the increase in progesterone in corpora lutea. Jenkin *et al.* (1977) suggested that the quantity of LH released in response to GnRH was related linearly to the content of LH in the pituitary. Further, the release of LH in the progestogen-treated ewes may have been augmented by estrogen, which has been found to be increased within 24 hr after removal of an intravaginal sponge containing progestogen (Echternkamp *et al.*, 1976). Reeves *et al.* (1971) demonstrated that the responsiveness of the pituitary to GnRH was increased in anestrous ewes pretreated with estradiol benzoate.

Several workers (see Wright *et al.*, 1978, for summary) have reported that the pituitary of ewes treated with progesterone released less LH in response to GnRH than did the pituitary in control ewes. Those findings should not be equated with the present work, since ewes in those studies received as much as 100 mg progesterone/day for as long as 3 weeks. Cumming *et al.* (1972) found that physiological amounts of progesterone did not prevent release of LH during infusion of GnRH.

The finding that suckling did not affect the release of LH in response to GnRH is compatible with the work of Mallampati *et al.* (1971). On the other hand, both Pelletier and Thimonier (1973) and Lewis *et al.* (1974) found more LH released after progestogen and LH-releasing treatments in nonlactating (nonlambing) than in lactating ewes during the nonbreeding season. Pelletier *et al.* (1977) reported that seasonally anestrous ewes that had not

TABLE 3. CONCENTRATIONS OF $PGF_2\alpha$ IN UTERINE VENOUS PLASMA OF POSTPARTUM EWES 7 DAYS AFTER TREATMENT WITH GnRH

Group	Treatments		$PGF_2\alpha$, ng/ml[a,b]	
	Progestogen	Weaning	Within subgroup	Pooled across weaning subgroups
1	Flurogestone acetate	Suckled	1.4^c	1.7^c
2	(20 mg) by pessary	Weaned	1.9^c	
3	None	Suckled	4.0^d	4.2^d
4		Weaned	4.5^d	

[a] Error mean square = 11.02.

[b] Mean concentrations of $PGF_2\alpha$ do not include the data on ewes that had luteal phases of less than 7 days (four of 13, five of 12, three of 16 and four of 15 in groups 1, 2, 3 and 4, respectively; pooled over both experiments).

[c,d] Values within a column with different superscripts differ ($P<.05$).

lambed had a greater release of LH after progestogen, PMSG and GnRH than either lactating or nonlactating postpartum ewes, which essentially had equal responses. Thus, postpartum interval may be more important than lactational status in determining the release of LH in response to GnRH. In addition, the high dose of GnRH in the present study may have masked the effects of suckling.

All ewes had corpora lutea at laparotomy 7 days after GnRH in this study, whereas Reeves *et al.* (1972) and Haresign *et al.* (1973) reported that only between 50 and 80% of the ewes treated with GnRH ovulated. These differences may be due to the time of year at which GnRH was given. Ewes in the present study were in lactational anestrus during the normal breeding season, while those in the studies of Reeves *et al.* (1972) and Haresign *et al.* (1973) were treated during seasonal anestrus. At laparotomy 9 days after D-Leu^6-LHRH, Frandle *et al.* (1977) were unable to find corpora lutea on the ovaries of five of the 13 ewes treated and concluded that these ewes had not ovulated. However, progesterone in the five ewes increased to levels above basal concentrations by day 3, peaked at about 1 ng/ml on day 5 and declined steadily until day 11 posttreatment. On the basis of the results of the present study and those reported by Crighton *et al.* (1973, 1975) and Haresign *et al.* (1973, 1975), it can be reasoned that there may have been corpora lutea in those five ewes, the life span and function of which were short and subnormal.

Both early weaning of lambs and treatment with progestogen have been used to shorten the postpartum interval to mating in ewes (Cognie and Pelletier, 1976; Thimonier and Cognie, 1977), but neither treatment alone nor the combination improved luteal function after treatment with GnRH in the present study. In fact, duration of the short luteal phases and the concentration of progesterone in ewes with normal luteal phases after GnRH were reduced in the ewes treated with progestogen (figure 1, panels B,C).

Since both suckling (Thorburn *et al.*, 1977) and exogenous oxytocin (Sharma and Fitzpatrick, 1974; Roberts and McCracken, 1976) cause release of $PGF_2\alpha$ by the uterus of ewes, weaning of lambs on day 20 might have been expected to reduce the concentration of $PGF_2\alpha$ in uterine venous plasma on day 29 postpartum. However, weaning had no effect on concentrations of $PGF_2\alpha$. A significant reduction in the concentration of $PGF_2\alpha$ in uterine venous plasma collected 8 days after a 4-day treatment with progestogen has not been reported previously. Reports of the effects of progesterone on the release of $PGF_2\alpha$ by the uterus vary; usually, the duration of treatment, the time of sampling and the endocrine status of the animals also have varied. The concentrations of $PGF_2\alpha$ soon after treatment with progesterone have been reduced or have remained unchanged (Wilson *et al.*, 1972; Ford *et al.*, 1975; Thorburn *et al.*, 1977). However, Ottobre (1979) found that progesterone administered during estrus hastened the first increases in uterine venous PGF$_2\alpha$ (from day 12 to day 8) in normally cyclic ewes. In the present study, progestogen may have increased the rate of uterine involution, and thus reduced synthesis and release of PGF$_2\alpha$ by the uterus. Foote and Hunter (1964) found that the interval from calving to uterine involution was reduced in cows treated with progesterone. D. S. Eley and W. W. Thatcher *(personal communication)* found a positive relationship between the rate of uterine involution and decline in concentration of 13, 14-dihydro-15-keto-PGF$_2\alpha$ (the major metabolite of PGF$_2\alpha$) in jugular plasma of postpartum, anestrous dairy cows.

Production of progesterone by the induced corpora lutea did not appear to be related to the concentration of PGF$_2\alpha$ in uterine venous plasma at laparotomy. A sample of uterine venous plasma taken at a single point in time may not be adequate for a thorough evaluation of the effects of endogenous PGF$_2\alpha$ on luteal function.

The interval from parturition to mating averaged 39.7 days (17.7 days after GnRH) in the present study, which is shorter than the 70- and 67.7-day intervals reported by Barker and Wiggins (1964) for Rambouillet ewes lambing in September and October, respectively, but comparable to the average of 48.5 ± 14.9 days reported by Wagner (1964) for crossbred ewes lambing in October.

Neither progestogen priming for 4 days nor weaning of lambs for 2 days before GnRH improved longevity or function of induced corpora lutea in postpartum anestrous ewes. Treatment with GnRH 2 to 3 weeks after parturition may shorten the postpartum interval to mating in ewes, but further studies are needed to evaluate this possibility. The short duration of the luteal phase induced with GnRH cannot be explained on the basis of the pattern of release of LH after GnRH or by concentrations of PGF$_2\alpha$ in uterine venous plasma 7 days after treatment with GnRH, although these concentrations of PGF$_2\alpha$ were two to eight times as great as those normally found at this stage after ovulation during the ovine estrous cycle (Ford *et al.*, 1975).

Literature Cited

Barker, H. B. and E. L. Wiggins. 1964. Estrual interval in lactating ewes. J. Anim. Sci. 23:973.

Butcher, R. L. 1977. Changes in gonadotropins and steroids associated with unilateral ovariectomy of the rat. Endocrinology 101:830.

Cognie, Y. and J. Pelletier. 1976. Preovulatory LH release and ovulation in dry and in lactating ewes after progestogen and PMSG treatment during the seasonal anoestrum. Ann. Biol. Anim. Biochem. Biophys. 16:529.

Crighton, D. B., J. P. Foster, W. Haresign, N. B. Haynes and G. E. Lamming. 1973. The effects of a synthetic preparation of gonadotropin releasing factor on pituitary and ovarian function in anoestrous ewes. J. Physiol. (London) 231:98 (Abstr.).

Crighton, D. B., J. P. Foster, W. Haresign and S. A. Scott. 1975. Plasma LH and progesterone levels after single or multiple injections of synthetic LH-RH in anoestrous ewes and comparison with levels during the oestrous cycle. J. Reprod. Fertil. 44:121.

Cumming, I. A., J. M. Buckmaster, J. C. Cerini, M. E. Cerini, W. A. Chamley, J. K. Findlay and J. R. Goding. 1972. Effect of progesterone on the release of luteinizing hormone induced by a synthetic gonadotropin-releasing factor in the ewe. Neuroendocrinology 10:338.

Echternkamp, S. E., D. J. Bolt and H. W. Hawk. 1976. Ovarian and pituitary hormones in blood of progestogen-treated ewes. J. Anim. Sci. 43:893.

Foote, W. D. and J. E. Hunter. 1964. Post-partum intervals of beef cows treated with progesterone and estrogen. J. Anim. Sci. 23:517.

Ford, S. P., C. W. Weems, R. E. Pitts, J. E. Pexton, R. L. Butcher and E. K. Inskeep. 1975. Effects of estradiol-17β and progesterone on prostaglandins F in sheep uteri and venous plasma. J. Anim. Sci. 46:1407.

Frandle, K. A., J. E. Kinder, D. H. Coy, A. V. Schally, J. J. Reeves and V. L. Estergreen. 1977. Plasma progestins in anestrous ewes treated with D-Leu^6-des Gly NH$_2$^{10}-LHRH ethylamide. J. Anim. Sci. 46:486.

Goding, J. R. 1974. The demonstration that PGF$_2\alpha$ is the uterine luteolysin in the ewe. J. Reprod. Fertil. 38:261.

Haresign, W., J. P. Foster, N. B. Haynes, D. B. Crighton and G. E. Lamming. 1975. Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. J. Reprod. Fertil. 43:269.

Haresign, W., N. B. Haynes and G. E. Lamming. 1973. The effects of synthetic gonadotropic-releasing factor on ovarian function in anoestrous ewes. J. Reprod. Fertil. 35:600 (Abstr.).

Jenkin, G., R. B. Heap and D.B.A. Symons. 1977. Pituitary responsiveness to synthetic LH-RH and pituitary LH content at various reproductive stages in the sheep. J. Reprod. Fertil. 49:207.

Lewis, G. S., P. E. Jenkins, R. L. Fogwell and E. K. Inskeep. 1978. Concentrations of prostaglandins E$_2$ and F$_2\alpha$ and their relationship to luteal function in early pregnant ewes. J. Anim. Sci. 47:1314.

Lewis, P. E., D. J. Bolt and E. K. Inskeep. 1974. Luteinizing hormone release and ovulation in anestrous ewes. J. Anim. Sci. 38:1197.

Mallampati, R. S., A. L. Pope and L. E. Casida. 1971.

Effect of suckling on postpartum anestrus in ewes lambing in different seasons of the year. J. Anim. Sci. 32:673.

Niswender, G. D., L. E. Reichert, Jr., A. R. Midgely, Jr. and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84:1166.

Ottobre, J. S. 1979. Regulation of the life span of corpora lutea in ewes. M.S. Thesis. West Virginia Univ., Morgantown.

Pelletier, J., J. P. Signoret, L. Cahill, Y. Cognie, J. Thimonier and R. Ortavant. 1977. Physiological processes in oestrus, ovulation and fertility of sheep. Proc. of Symp. on Management of Reproduction in Sheep and Goats, Madison, WI, p. 1.

Pelletier, J. and J. Thimonier. 1973. Comparison of the induced preovulatory LH discharge in lactating and dry sheep during seasonal anestrus. J. Reprod. Fertil. 33:311.

Pexton, J. E., C. W. Weems and E. K. Inskeep. 1975. Prostaglandins F in uterine venous plasma, ovarian arterial and venous plasma and in ovarian and luteal tissue of pregnant and nonpregnant ewes. J. Anim. Sci. 41:154.

Pharriss, B. B., S. A. Tillson and R. R. Erickson. 1972. Prostaglandins in luteal function. Recent Progr. Horm. Res. 28:51.

Reeves, J. J., A. Arimura and A. V. Schally. 1971. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anestrous ewes pretreated with estradiol benzoate. Biol. Reprod. 4:88.

Reeves, J. J., A. Arimura, A. V. Schally, C. L. Kragt, T. W. Beck and J. M. Casey. 1972. Effects of synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH) on serum LH, serum FSH and ovulation in anestrous ewes. J. Anim. Sci. 35:84.

Restall, B. J. 1971. The effect of lamb removal on reproductive activity in Dorset Horn × Merino ewes after lambing. J. Reprod. Fertil. 24:145.

Restall, B. J., R. D. Kearins and B. G. Starr. 1977. Studies of pituitary function in lactating ewes. J. Reprod. Fertil. 49:291.

Roberts, J. S. and J. A. McCracken. 1976. Does prostaglandin $F_2\alpha$ released from the uterus by oxytocin mediate the ocytocic action of oxytocin? Biol. Reprod. 15:457.

Roche, J. F., D. L. Foster, F. J. Karsch, B. Cook and P. J. Dziuk. 1970. Levels of luteinizing hormone in sera and pituitaries of ewes during the estrous cycle and anestrus. Endocrinology 86:568.

Sharma, S. C. and R. J. Fitzpatrick. 1974. Effect of oestradiol-17β and oxytocin treatment on prostaglandin F alpha release in anoestrous ewe. Prostaglandins 6:97.

Smith, J. B. and A. L. Willis. 1970. Function and release of prostaglandins by platelets in response to thrombin. Brit. J. Pharmacol. 40:545P.

Thimonier, J. and Y. Cognie. 1977. Application of control of reproduction of sheep. Proc. of Symp. on Management of Reproduction in Sheep and Goats. Madison, WI, p. 109.

Thorburn, G. D., J.R.G. Challis and W. Bruce Currie. 1977. Control of parturition in domestic animals. Biol. Reprod. 16:18.

Wagner, J. F. 1964. Hormonal control of reproductive activity in the ewe. Proc. of Conf. on Estrous Cycle Control in Domestic Animals, USDA Misc. Pub. 1005, p. 28.

Wilson, Laird, Jr., R. L. Butcher and E. K. Inskeep. 1972. Prostaglandin $F_2\alpha$ in the uterus of ewes during early pregnancy. Prostaglandins 1:479.

Wiltbank, J. N. and S. E. Mares. 1977. Breeding at a predetermined time following Synchro-Mate B treatment. Proc. 11th Conf. on Artificial Insemination of Beef Cattle, Denver, CO, p. 57.

Wright, P. J., G. Jenkin, R. B. Heap and D. E. Walters. 1978. Pituitary responsiveness to LH-RH and TRH and the effects of progesterone or progesterone and oestradiol treatment in anoestrous sheep. J. Reprod. Fertil. 52:343.

The effect of progestogen and oestradiol priming on luteal function in seasonally anoestrus GnRH-treated ewes

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Seasonally anoestrus Corriedale ewes receiving 1 ml GnRH (0,0042 mg buserelin-acetate) IM, were pretreated as follows: (i) 5 Days with intravaginal progesterone-impregnated sponges removed 24 h prior to GnRH (P-group); (ii) 6 h with subcutaneous *oestradiol silicone rubber implants removed 6 h* prior to GnRH (E-group); or (iii) no pre-GnRH treatment (O-group). The mean E_2 levels of ewes in the P-group (8,18 ± 1,52 pg/ml) and the E-group (8,03 ± 1,74 pg/ml) were elevated by 6 h prior to GnRH injection. Plasma progesterone output of GnRH-induced CL's in sheep primed with progesterone (P-group) were higher ($p < 0{,}01$) than mean values for the control ewes (O-group). The E-group was intermediate. The *life-span of* corpora lutea was not affected. It appears that the trophic effect of progesterone is not mediated via E_2 release, but rather as a direct effect on the ovary or hypothalamo-pituitary axis.

Corriedale-ooie is gedurende seisoenale anestrus óf vir 5 dae behandel met intravaginale progesteroon geïmpregneerde sponsies (P-groep), óf vir 6 uur met E_2-silikoonrubberstafies onderhuids geïmplanteer (E-groep), óf het as kontrole (O-groep) gedien. Die ooie het onderskeidelik 24 h na sponsonttrekking (P-groep) en 6 h na einde van die E_2-behandeling (E-groep). 'n 1 ml intramuskulêre GnVH (0,0042 mg buserelin-asetaat)-inspuiting ontvang. Die O-groep ooie is terselfdertyd met GnVH behandel. Vir die P- en E-groep ooie is plasma-E_2-vlakke van 8,18 ± 1,52 pg/ml en 8,03 ± 1,74 pg/ml onderskeidelik geregistreer 6 h voor GnVH-toediening. Progesteroonsekresie (area onder sekresiekurwe) was hoër ($p < 0{,}01$) vir die P-groep ooie as vir die O-groep. Waardes vir die E-groep was intermediêr. Lewensduur van corpora lutea is nie beïnvloed nie. Die luteotrofiese effek van progesteroon blyk eerder 'n direkte effek te wees, as via E_2.

Robinson (1950) has demonstrated that progesterone plays an important role in hormonally induced ovulations, but that progesterone alone, administered during anoestrus, does not necessarily lead to ovulation. If progesterone is combined with PMSG during postpartum anoestrus, an oestrous cycle of normal duration is experienced (Oldham & Martin, 1979). Available evidence suggests that GnRH treatment combined with progesterone, more often than not, increases luteal activity (Webb, Lamming, Haynes, Hafs & Mann, 1977;

McLeod, Haresign & Lamming, 1982). A progestational phase also prevents the premature regression of ram-induced CL (Oldham & Martin, 1979). Poor luteal function was reported by Hamilton, Lishman & Lamb (1979) after an IM injection of oestrogen followed by GnRH, but pretreatment with E_2 implants eliminated the problem (Walters, Short, Convey, Staigmillar, Dunn & Kaltenbach, 1982). The object of this study was to determine whether the effect of progesterone pretreatment on luteal function was direct or mediated via E_2 release prior to LH release.

Corriedale ewes, checked with vasectomized rams to be in seasonal anoestrus, were randomly allocated to three groups (P, E, and O) of six ewes each. The P-group was pretreated with intravaginal progesterone-impregnated sponges (Repromap, Tuco 60 mg) for 5 days, with sponge removal 24 h prior to a 1 ml GnRH IM injection (0,0042 mg buserelinacetate, Receptal Hoechst). The E-Group was subcutaneously implanted with 8,5 mm-long E_2 silicone rubber rods (Compudose, Elanco) for 6 h (12 h to 6 h prior to GnRH). The implants were previously found to produce blood levels of 12 pg E_2/ml (Liebenberg, 1983). The O-group served as a control, receiving only GnRH. Blood samples to be assayed for E_2 were drawn every 6 h, with the last sample being obtained immediately prior to GnRH. Commencing on the day following GnRH administration, blood samples for progesterone assay were drawn at 48 h intervals over a period of 15 days.

By 6 h prior to GnRH injection the mean E_2 plasma concentration in the ewes of the P-group (in response to progesterone withdrawal) and the E-group had risen to 8,18 ± 1,52 pg/ml and 8,03 ± 1,74 pg/ml respectively (Figure 1). Progesterone pretreatment enhanced luteal function significantly ($p < 0{,}01$ Table 1), with E_2 pretreatment resulting only in a small (NS) effect (Figure 2).

The results of this study are consistent with those of Mc Leod *et al.* (1982) where seasonally anoestrus ewes were studied. These workers recorded a highly significant luteotrophic effect of progestogen priming followed by a multiple injection regime of GnRH. The life-span of corpora lutea induced by HCG was prolonged during postpartum anoestrus in cows pretreated with progesterone implants, but not in cows primed with oestradiol (Pratt, Berardinelli, Stevens & Inskeep, 1982). The results pertaining to progesterone priming are similar to those obtained by Sheffel, Pratt, Ferrel & Inskeep (1982), who concluded that the mechanism by which progesterone increased the subsequent level of luteal function remained unknown.

In this study, the near perfect mimicking of the oestrogen surge in the P and E groups indicates that E_2 implants were

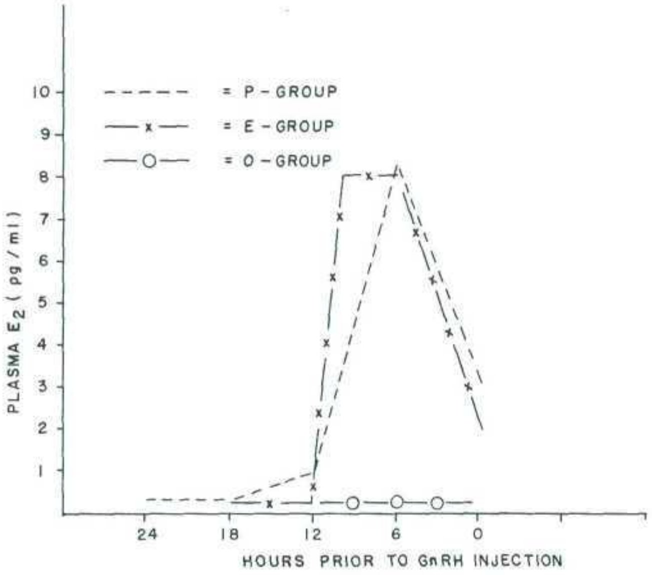

Figure 1 The mean plasma E_2 concentration (pg/ml) of anoestrus ewes treated with intravaginal progestogen sponges for 5 days (P-group), E_2 silicone rubber implants for 6 h (E-group) and control ewes (O-group).

Table 1 Areas under the progesterone curve of anoestrus ewes injected with GnRH after priming for 5 days with intravaginal progestogen sponges (P), for 6 h with E_2 silicone rubber implants (E) and control (O) ewes

Group	n	Treatment mean ± SEM	As % of control
P	6	11,07 ± 0,77	194,2
E	6	7,16 ± 1,98	125,6
O	6	5,7 ± 0,41	100,0

$\bar{x} = 7{,}98 \pm 0{,}85$; P > O ($p < 0{,}01$).

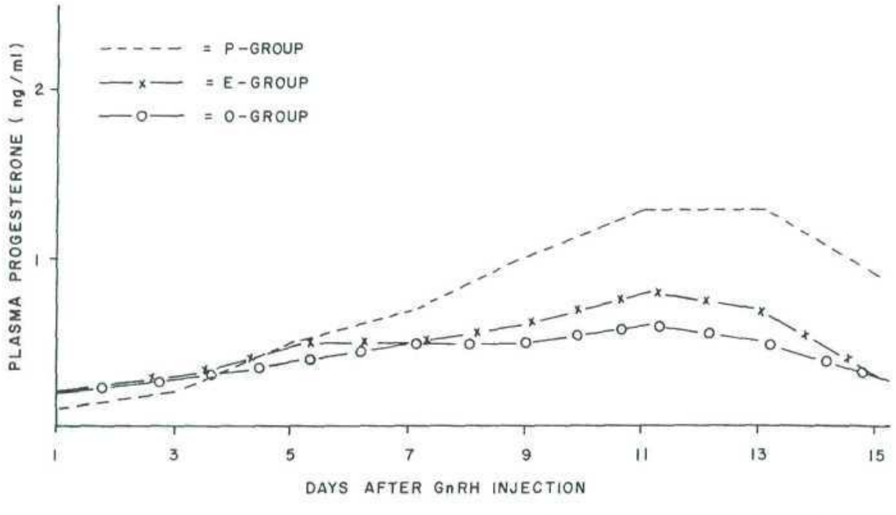

Figure 2 The mean plasma progesterone concentration (ng/ml) of anoestrus ewes injected with GnRH after priming for 5 days with intravaginal progestogen sponges (P), for 6 h with E_2 silicone rubber implants (E) and control (O) ewes.

Melatonin is released from the pineal gland only at night and this gland is involved in mediating day length effects on seasonal breeding. Therefore, it was proposed that the inhibitory effect of night-time suckling might be dependent on suckling occurring while melatonin levels are high. This proposal formed the basis of the trial described in Paper 50.

Paper 50

The purpose of the experiment was to evaluate the effect, on lactation anoestrus, of artificially raising day-time levels of melatonin and of abolishing the night-time rise. The former was "achieved" by twice-daily injections of melatonin in oil and the latter by providing lights at night.

Findings :-

1. The mean interval to, and pattern of occurrence of first oestrus after calving, were similar for cows not suckled at 12:00h, for cows exposed to lights at night and for cows suckled once daily.
2. The rate at which cows showed first oestrus was different for cows suckled at 01:00h and for cows injected with melatonin than seen in cows exposed to the treatments mentioned in paragraph 1 above.
3. Cows suckled once daily had higher plasma progesterone concentrations after first oestrus than for any of the other treatments.

Implications/Significance :-

1. Abolishment of the night-time rise in melatonin counteracts the prolonging effect on post-partum anoestrus of suckling late at night.
2. When melatonin levels are artificially raised during daylight and suckling occurs at this time then anoestrus is prolonged.
3. Restricted suckling may improve luteal function through its effect on episodic LH release.

PAPER 40

17]

Factors Affecting Postpartum Anestrus in Beef Cattle

by E. K. INSKEEP AND A. W. LISHMAN*

ABSTRACT

The length of postpartum anestrus in cattle varies with breed, age, plane of nutrition, and intensity of the suckling stimulus. Yet the mechanisms by which these factors exert their effects are poorly understood. Recent studies have been made with cows and ewes to determine whether pituitary and ovarian responsiveness to hormonal stimuli during the postpartum period vary with plane of nutrition, intensity of suckling, or prior treatment with progestogen. For example, the pattern of release of LH after injection of GnRH varied with plane of nutrition, short-term removal of a calf, and size of the largest follicle in the ovaries. The probability of formation of a corpus luteum after treatment did not differ significantly with these three factors. The concentration of estradiol-17β in jugular plasma, eight hours after GnRH, was greater with short-term calf removal. The life span of corpora lutea induced by GnRH has almost invariably been short and has not varied in any consistent manner with treatments studied.

INTRODUCTION

Long postpartum anestrus is widely recognized as a limiting factor in efficiency of production of beef cattle. In this paper, some major factors affecting the length of interval from calving to first postpartum estrus in beef cows will be presented and consideration will be given to experimental attempts to determine how some of these factors may affect pituitary and ovarian function.

The magnitude of the problem becomes apparent when one attempts to apply a method for synchronization of estrus that requires the presence of a corpus luteum (CL) in order to be effective, for example, treatment with prostaglandin $F_2\alpha$ ($PGF_2\alpha$). During a four-year period in West Virginia, 1164 cows in 24 herds were examined between 30 and 99 days postpartum. Only 57% of these cows, on a single day of examination, were either in estrus or had a palpable CL in either ovary. Estrus was synchronized effectively in 74% of cows which were 40 days or most postpartum and possessed a palpable

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Suzuki, O. and Sato, M., 1979. Post-partum oestrus and serum progesterone and oestradiol-17-beta levels in beef cows whose calves were weaned early. Jpn. J. Anim. Reprod., 25: 183—188.

Trail, J.C.M., Buck, N.G., Light, D., Rennie, T.W., Rutherford, A., Miller, M., Prachett, D. and Capper, B.S., 1977. Productivity of Africander, Twsana, Tuli and crossbred beef cattle in Botswana. Anim. Prod., 24: 57—62.

Walters, D.L., Songster, B., Valencia, M., Burrel, W.C. and Wiltbank, J.N., 1977. Steroids in conjunction with 48 hour calf removal or early weaning in thin anoestrous cows. J. Anim. Sci., 45 (Suppl. 1): 215 (abstr.).

Wells, P.L., Hopley, J.D.H. and Holness, D.H., 1981. Fertility in the Afrikaner cow. 1. The influence of concentrate supplementation during the post-partum period on ovarian activity and conception. Zim. J. Agric. Res., 19: 13—21.

Wetteman, R.P., Turman, E.J., Wyatt, R.D. and Totusek, R., 1978. The influence of suckling intensity on reproductive performance of range cows. J. Anim. Sci., 47: 342—346.

Wiltbank, J.N. and Cook, A.C., 1958. The comparative reproductive performance of nursed cows and milked cows. J. Anim. Sci., 17: 640—649.

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FERTILITY IN THE AFRIKANER COW. 3. ONCE A DAY SUCKLING AND ITS EFFECT ON THE PATTERN OF RESUMPTION OF OVARIAN ACTIVITY AND CONCEPTION RATE IN EARLY LACTATION

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ABSTRACT

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Earlier studies in the Afrikaner cow indicated that suckling exerts a profound influence on the resumption of cyclic ovarian activity and conception post partum. The aim of this study was to assess the effect of once a day suckling on the resumption of ovarian activity and conception within 100 days of calving.

Partial weaning significantly ($P < 0.01$) reduced the number of cows which were anovulatory for 100 days post partum from 13/32 of normally suckled (NS) cows to 2/33 in partially weaned (PW) cows. Conception rates were significantly ($P < 0.001$) improved from 16/32 in NS cows to 30/33 in PW cows. Within the cows which ultimately ovulated, reduced suckling intensity did not significantly shorten the post-partum interval to first rise (0.27 ng/ml) in plasma progesterone concentration (55 ± 5 days in NS cows to 52 ± 4 days in PW cows). Considering all cows in the study, partial weaning reduced ($P < 0.01$) the mean post-partum interval to first ovulation by at least 20 days. Within cows which conceived, the mean interval to conception was 72 ± 5 days in NS cows and 69 ± 3 in PW cows; once again if all cows are considered in the analysis, partial weaning shortened ($P < 0.01$) the mean post-partum interval to conception by a minumum of 21 days. The probability of conception appeared to be highest 55—65 days post partum, regardless of whether or not this was preceded by an oestrous cycle of normal duration. Total progesterone output during a shortened luteal phase was estimated to be 10% of the output during a normal luteal phase (5.9 ng/ml·days ± 1.1 vs. 51.8 ng/ml·days ± 5.8, respectively). A reduction in the suckling stimulus had no significant effect on any parameter of luteal function measured in this study. However, the resumption of ovarian activity in Afrikaner cows appears to be suppressed to varying degrees by the constant attentions of a suckling calf. In this study post-partum anovulation was the primary cause of conception failure within 100 days of calving.

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INTRODUCTION

The absence of ovulation within 100 days of calving is a major cause of low conception rates and long calving intervals in tropical and sub-tropical areas. Factors influencing post-partum anoestrus have been comprehensively reviewed by Inskeep and Lishman (1981).

Suckling has proved to be an important factor in the resumption of ovarian activity post partum. Although conception rates improve when suckling intensity is reduced, the mechanics of this improvement are not fully understood at present (Wiltbank and Cook, 1958; Oxenreider, 1968; Laster et al., 1973). With early weaning, early resumption of cyclic ovarian activity (Wetteman et al., 1978) and a decrease in the proportion of cows which are anovulatory for 100 days post partum are important components of the improvement in conception rates. Early first ovulation has not been highly correlated with short conception intervals (Wheeler et al., 1982) and the pattern of ovarian activity prior to conception has proved to be highly variable (Wells et al., 1985). Short luteal phases at the onset of ovarian activity provide the hypothalamo–pituitary axis with its first exposure to progesterone. Progesterone exposure has been proposed as an essential prerequisite for a fertile ovulation (Webb et al., 1977; La Voie et al., 1981; A.W. Lishman, unpublished, 1981).

By day 10, calf removal will have a substantial influence on serum Luteinizing Hormone (LH) concentrations (Forrest et al., 1979). Reduced suckling intensity induces an early increase in frequency of LH release (Carruthers et al., 1980. Riley et al., 1980; Forrest et al., 1981), causing higher plasma LH concentrations than in normally suckled cows. Not only does suckling delay the release of gonadotropin-releasing hormones from the hypothalamus but it modifies the responsiveness of the pituitary to exogenous steroids (Short et al., 1979; Forrest et al., 1980; Stevenson et al., 1982) and both endogenous (Carruthers et al., 1980) and exogenous-releasing hormones (Carter et al., 1980; Troxel et al., 1980). Thus it is not surprising that progesterone levels are elevated within 14 days of calving in cows weaned within 3 days post partum (Williams et al., 1983).

In the Afrikaner cow with a condition score of 2.5–3.0 (on a scale of 1 = extremely thin to 5 = excessively fat) at calving, conception rates in nursing cows are not expected to rise above 65% with a 90-day breeding period. Calving percentages reported in the literature for this breed range from 54–75%, depending on environment (Trail et al., 1977; Ward et al., 1978; Holness et al., 1980; Thorpe and Cruikshank, 1981).

In order to assess the feasibility of applying weaning routines to breeding management systems it is necessary to establish the minimum reduction in suckling stimulus necessary to achieve the maximum improvement in conception rates. Most Afrikaner cows weaned 3 days after calving conceived before 60–65 days post partum (Wells et al., 1985); therefore, any weaning routine should be applied to induce the first ovulation 40–50 days post partum. This study was designed specifically to investigate the effects of once a day suckling on cow fertility at first ovulation and its temporal relationship to weaning treatment.

MATERIALS AND METHOD

Twenty-eight days after calving, mature multiparous Afrikaner cows with a condition score of 2.5–3.0 were blocked according to calving date and randomly allocated to either a normally suckled (NS) group or a partially weaned (PW) group. Calves in the NS group had continuous access to their dams for the duration of the trial. PW calves were confined to a small pen within the paddock where their dams were grazing. Partial weaning commenced with calves being allowed access to their dams for 30 min at 08.00 h and 16.00 h each day. From days 42–100 post partum, calves were allowed to suckle once each day at 16.00 h. At 100 days post partum, calves were returned to their dams where they had continuous access until complete weaning at 7 months of age.

From 28 days to 100 days post partum, blood samples were collected three times each week by jugular venipuncture. Within 2 h of collection, samples were centrifuged and plasma stored at -12°C. Progesterone content of plasma samples was determined according to the method of Holness and Hale (1980).

Entire bulls were included in cow herds from 28 days post partum. All bulls used in the herds were physically sound and semen tests showed that sperm motility was satisfactory. Bulls were rotated weekly to minimize sire effects on conception rates in each group. Cows were observed for 14 h each day.

Calves in the PW group were offered a creep feed from days 28 to 100 post partum. Experimental cows were grazed on star grass pasture of sufficient quality to ensure that none of the cows in the NS group lost livemass over the experimental period.

Elevated progesterone levels for not less than 26 days were taken as the criterion for conception to have occurred. Pregnancies were confirmed by the birth of a live calf.

Progesterone assay

Progesterone concentration of all plasma samples was determined in two batches of 12 assays each. Details of the method have been reported earlier by Holness and Hale (1980). Within each assay 10 control plasma samples were included. Variation between assays was 18% and 22% for the two batches of assays and was based on the standard deviations of the mean values of all control plasmas within each assay. Standard errors of the means of the control samples within each assay were 0.17 ng/ml and 0.13 ng/ml, giving coefficients of variation of 8% and 11%, respectively. The standard errors for a single observation in each batch assay of assays were ± 0.54 ng/ml and ± 0.42 ng/ml, respectively.

Definitions

Based on parameters determined from the progesterone profiles of each cow on trial, two forms of ovarian activity have been distinguished. Both forms of activity have been reported in the literature, where reference has been made to the occurrence of "short luteal phases" frequently observed at the onset of ovarian activity in a high proportion of dairy cows at 10—20 days post partum and suckled beef cows at 35—55 days post partum (Henricks et al., 1972; Carter et al., 1980; Williams and Ray, 1980; Hinshelwood et al., 1982; Wells et al., 1985).

Primary ovulation. Following a primary ovulation, plasma progesterone concentrations exceed 0.27 ng/ml for a minimum period of 72 h. This figure was derived from the mean of basal levels of plasma progesterone concentrations plus 5 standard deviations from this mean. Minimal oestrous behaviour precedes this progesterone surge and coitus is seldom observed. Conception does not accompany a primary ovulation.

Full ovulation. Following a full ovulation, plasma progesterone concentrations exceed 0.27 ng/ml for a period of not less than 11 days. Following a full ovulation, a further ovulation will not occur within the next 19 days. Conception can only accompany a full ovulation.

RESULTS

Primary ovulation

Significantly more ($P < 0.001$) NS cows 13/32 than PW cows 2/33 were anovulatory for 100 days post partum (Table 1). Post-partum interval to primary ovulation was not affected by weaning (59 ± 5 days amongst the NS cows and 52 ± 4 days in PW cows (Table 1). Following primary ovulations, the duration of the shortened luteal phases (progesterone 0.27 ng/ml) was unaffected by a reduction in suckling intensity, and had a mean duration of 4.9 ± 0.6 days in NS cows and 6.1 ± 0.6 days in PW cows (Table 2). A shortened luteal phase was exhibited by more than 85% of cows in each group which ovulated within the experimental period (18/19 NS cows and 27/31 PW cows).

Full ovulation

Partial weaning significantly ($P < 0.01$) improved the occurrence of full ovulations within 100 days of calving from 19/32 in NS cows to 31/33 in

TABLE 1

Characteristics of the resumption of ovarian activity post partum in normally suckled (NS) and partially weaned (PW) Afrikaner cows

	Normally suckled	Partially weaned	Signif. diff.
Cows anovulatory for 100 days post partum	13/32	2/33	$P < 0.001$
Primary ovulation			
Proportion of group exhibiting short cycles	18/32	27/22	$P < 0.01$
PPI to primary ovulation	59.0 ± 5.2[a]	52.2 ± 4.17	NS
Full ovulation			
Proportion of group exhibiting full ovulation	19/32	31/33	$P < 0.01$
PPI to full ovulation excluding anovulatory cows	64.4 ± 4.6	53.1 ± 3.8	NS
PPI to full ovulation including all cows[b]	77.6 ± 4.1	56.9 ± 3.9	$P < 0.01$
Proportion of full ovulations accompanied by conception	16/19	30/31	NS

[a] Mean ± standard error of the mean in days.

[b] Cows which failed to ovulate within 100 days of calving were allocated a post-partum interval to full ovulation of 100 days for this comparison.

PPI = Post-partum interval in days.

NS = Treatment means are not significantly different.

TABLE 2

Characteristics of luteal function in suckled and partially weaned Afrikaner cows; parameters derived from the progesterone profile of each cow

	Normally suckled	Partially weaned	Signif. diff.
Short cycle			
Sample number	18	27	
Area under curve (ng/ml·days)	4.5 ± 1.1[a]	7.2 ± 1.3	NS
Duration of shortened luteal phase (days)	4.9 ± 0.6	6.1 ± 0.6	NS
Peak progesterone conc. (ng/ml)	1.6 ± 0.3	2.6 ± 0.4	NS
First normal cycle			
Sample number	8	10	
Area under curve (ng/ml·days)	52.1 ± 4.6	50.4 ± 6.7	NS

[a] Mean ± standard error of the mean.

PPI = Post-partum interval in days.

NS = Treatment means not significantly different.

Area under the progesterone curve calculated for the days that plasma progesterone concentrations were above 0.27 ng/ml.

PW cows (Table 1). Excluding cows which failed to ovulate, the mean post-partum interval to first full ovulation was shortened by 11 days to 53 ± 4 days in PW cows, although this difference was not significant (Table 1). As a comparison and for the purpose of analysis, cows which failed to ovulate within the experimental period were assigned a post-partum interval of 100 days to their first full ovulation. From this analysis, it is evident that partial weaning significantly reduced ($P < 0.01$) the mean post-partum interval to first full ovulation by 20 days to 57 ± 4 days (PW cows). The probability of conception accompanying a full ovulation was not changed by reducing suckling to once each day (16/19 in NS cows to 30/31 in the PW cows; Table 1).

Characteristics of luteal activity

Plasma progesterone profiles indicate that luteal activity was not altered significantly by partial weaning. During the short luteal phase preceding a full ovulation there was no difference between the two treatment groups in the peak in plasma progesterone concentration, the post-partum interval to this peak, or the area beneath the progesterone profile (4.5 ng/ml·days ± 1.1 in NS cows and 7.2 ng/ml·days ± 1.3 in PW cows; Table 2). Similarly, following a full ovulation luteal function was not altered by a reduction in the suckling stimulus (52.1 ng/ml·days ± 4.6 in NS cows and 50.4 ng/ml·days ± 6.7 in PW cows; Table 2, Fig. 1).

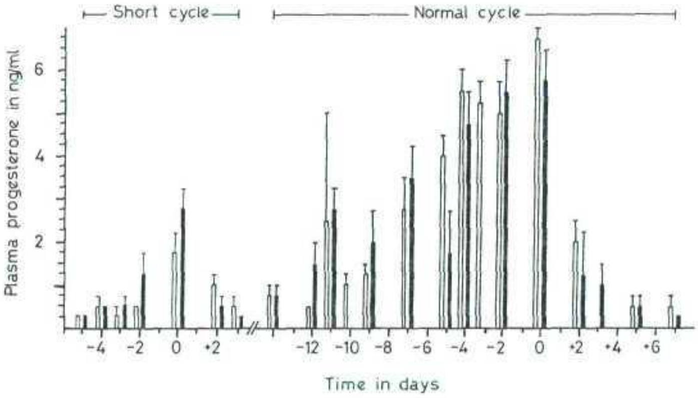

Fig. 1. Plasma progesterone concentrations during short cycles and during normal cycles in partially weaned and normally suckled Afrikaner cows. Day 0 marks the day of highest plasma progesterone concentration. Open bars = normally suckled; solid bars = partially weaned.

Conception rate

Conception rates were significantly improved by partial weaning ($P < 0.001$) from 16/32 in the NS group to 30/33 in the PW group (Table 3).

TABLE 3

Aspects of reproductive performance post partum in normally suckled and partially weaned Afrikaner cows

	Normally suckled	Partially weaned	Signif. diff.
Conceptions	16/32	30/33	$P < 0.001$
Full-term pregnancies	16/32	27/33	$P < 0.01$
PPI to conception excluding anovulatory cows	71.8 ± 4.9[a]	61.8 ± 3.2	NS
PPI to conception including all cows[b]	85.9 ± 3.5	65.3 ± 3.5	$P < 0.01$
Gestation	291.2 ± 1.2	290.6 ± 1.2	NS

[a] Mean ± standard error of the mean.

[b] For this comparison, a post-partum interval of 100 days was assigned to all cows which failed to conceive within the period of the trial.

NS = Treatment means are not significantly different.

PPI = Post-partum interval in days.

However, weaning did not significantly shorten the mean interval to conception (72 ± 5 days in NS cows and 62 ± 3 days in PW cows; Table 3). Calving rate in the PW cows dropped to 27/33 whilst all conceptions in the NS group were carried to full term (16/32). Assigning a mean value of 100 days to cows which failed to conceive, this analysis demonstrated how partial weaning significantly ($P < 0.01$) improved reproductive performance, shortening this interval by a minimum of 20 days from 86 ± 4 days in NS cows to 65 ± 4 days in PW weaned cows (Table 3).

Timing of conception in relation to first ovulation

Within each group considerable variation was observed in the post-partum intervals to first full ovulation. Two cows in the NS group and one cow in the PW group exhibited symptoms of either a retained corpus luteum or, conception followed by embryonic death followed by reconception, within 100 days of calving; their data therefore have been excluded from this analysis. The majority of cows in each group conceived to their first full ovulation (11/14 NS cows and 20/29 PW cows; Table 4). Cows which conceived to their second ovulation ovulated for the first time 43 ± 10 days post partum, whereas cows which conceived to their first ovulation ovulated 63 ± 20 days post partum (Table 4). Partial weaning did not exert any significant effects on the timing of these events.

Pattern of resumption of ovarian activity

A reduction in the suckling intensity failed to alter the form of resumption of ovarian activity prior to conception (data not presented). The ma-

TABLE 4

Post-partum intervals in days to first full ovulation as affected by weaning and timing of conception

	Conception at first ovulation	Conception at second ovulation
Normally suckled	68.8 ± 21.0[a] (11)	46.0 ± 2.65 (3)
Partially weaned	59.2 ± 19.0 (20)	41.8 ± 10.6 (8)
Signif. diff.	NS	NS
Mean	62.6 ± 20.0	42.9 ± 9.2

[a] Mean ± standard deviation of the mean in days.

NS = Treatment means are not significantly different.

jority of conceptions in this trial followed a single short cycle (13/16 NS cows and 25/30 PW cows); conception following one short cycle and one normal cycle accounting for virtually all of the remaining conceptions (3/3 in NS cows and 3/5 in PW cows).

DISCUSSION

Conception rate amongst Afrikaner cows was increased by 40% by reducing the intensity of suckling to twice each day from 28 days post partum and to once each day from 42 days until 100 days post partum. This was achieved by stimulating the resumption of ovarian activity in approximately 33% of cows which, under normal suckling intensities, would have remained anovulatory for at least 100 days post partum. Since a conception rate of 60–67% is expected for the unselected Afrikaner herd under near-optimal conditions of nutrition and management, it would seem that the breed is particularly sensitive to the suppressive effects of suckling on the resumption of ovarian activity after calving. Similar improvements have been achieved in Brahman cows, suckling their first calf for 1.5 h/day. Conception rates were 30% higher than those of their normally suckled contemporaries (Velasquez et al., 1980).

Prior to the resumption of ovarian activity, the pulsatile discharge of LH commences 20–30 days post partum (Carruthers and Hafs, 1980. Peters et al., 1981). As the post-partum interval increases, LH pulse frequency increases, raising mean plasma LH concentrations (Garcia–Winder et al., 1984). Hinshelwood et al. (1985) suggested that suckling acts directly on the hypothalamo–pituitary axis to limit the release of LH.

A reduction in suckling intensity increases pituitary responsiveness to exogenous GnRH (Inskeep et al., 1977; Carter et al., 1980. Troxel et al.,

1980) and presumably to endogenous GnRH. Walters et al. (1982a) reported an increase in pituitary responsiveness to GnRH within 24 h of weaning. Thereafter the steady increase in plasma LH concentrations noted by Short et al. (1972), Forrest et al. (1979, 1980), Peters et al. (1981) and Walters et al. (1982b) may be ascribed to an increase in pulsatile GnRH release rather than any further change in pituitary responsiveness (Walters et al., 1982b).

In the Afrikaner cow once a day suckling did not significantly advance the mean post-partum interval to primary ovulation. This is contrary to reports by Randel and Welker (1976) who noted a decrease in post-partum interval to first oestrus with once a day suckling in Brahman cows. However, reports by Edwards (1985) would seem to indicate that a threshold in LH activity may be necessary before reduced suckling intensity will enhance pulsatile release of LH activity and enable a full resumption of ovarian activity. However, since significantly more cows ovulated in the partially weaned group, the reduction in the suckling stimulus was clearly sufficient to increase LH activity in anovulatory cows and induce full ovarian function.

We suggest that although partial weaning will enhance hypothalamic activity, its application must be timed to coincide with and amplify the unprompted endogenous increase in gonadotropin concentrations 20—40 days post partum. Since the mean interval to primary ovulation was similar in the two groups, the timing of treatment application in this study appears to have coincided with natural changes in gonadotropin activity prior to ovulation.

In a previous study where calves were weaned completely 3 days post partum, the interval to primary ovulation was advanced by 34 days to 18 ± 5 days (Wells et al., 1985). Thus, the degree of change in gonadotropin activity may be directly proportional to suckling intensity.

Once ovarian activity commenced, all parameters used to describe luteal function measured in this study did not differ between the two treatment groups. Shortened luteal phases have been reported by others (Robertson, 1972; Corah et al., 1974; Suzuki and Sato, 1979; Odde et al., 1980; Hinshelwood et al., 1982) and were observed in most cows in this study (18/19 in NS cows and 27/31 in PW cows). Shortened luteal phases would seem to occur at the resumption of ovarian activity in all cows. Webb et al. (1977) and La Voie et al. (1981) considered this luteal activity essential for the resumption of full oviarian activity. These shortened luteal phases were 4.9—6.1 ± 0.6 days long and progesterone output was estimated to be about 10% of that measured during a normal luteal phase in this study.

As a consequence of the increased proportion of cows ovulating in the partially weaned group, the higher conception rate achieved was not surprising. However, cell numbers were too small to determine whether or not a reduction in suckling stimulus at and around oestrus improved the probability of conception per se. Post-partum intervals to conception in this study and in a previous study (Wells et al., 1985) indicate that the probability of conception is highest 58—70 days after calving. In this study conception at first ovulation occurred 62 days post partum whilst conception to second ovulation occurred at about the same time, 64 days post partum (Table 4). This situation was unaffected by reduced suckling intensity and would indicate that, in general, ovulations occurring earlier than 50 days post partum are likely to be sub-fertile. Uterine environment is the factor most likely to limit conception in cycling post-partum cows within 50 days of calving.

CONCLUSION

Partial weaning commencing at 28 days post partum significantly ($P < 0.001$) improved conception rates by 40%, without altering the mean post-partum interval to conception. Post-partum ovarian recovery, particularly conception, appears to have the highest probability of success later than 55 days post partum. This trait appears to be independent of the occurrence of the first indications of the resumption of ovarian activity. In Afrikaner cows in this trial, the major hurdle to the resumption of ovarian activity was the occurrence of any luteal activity. Having achieved this threshold, the probability of conception was not improved by the continued reduction in suckling intensity. The logical follow-up to this trial is to establish whether it is necessary to partially wean cows prior to or in conjunction with breeding as a means of stimulating the resumption of ovarian activity.

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REFERENCES

Carruthers, T.D. and Hafs, H.D., 1980. Suckling and four times daily milking: influence on ovulation, estrus and serum luteinizing hormone, glucocorticoids and prolactin in post-partum Holsteins. J. Anim. Sci., 50: 919—925.

Carruthers, T.D., Convey, E.M., Kesner, J.S., Hafs, H.D. and Cheng, K.W., 1980. The hypothalamo—pituitary gonadotrophic axis of suckled and non-suckled dairy cows post-partum. J. Anim. Sci., 51: 949—957.

Carter, M.L., Dierschke, D.J., Rutledge, J.J. and Hauser, E.R., 1980. Effect of gonadotropin releasing hormone and calf removal on pituitary ovarian function and reproductive performance in post-partum beef cows. J. Anim. Sci., 51: 903—910.

Corah, L.L., Quealy, A.P., Dunn, T.G. and Kaltenbach, C.C., 1974. Prepartum and postpartum levels of progesterone and estradiol in beef heifers fed two levels of energy. J. Anim. Sci., 39: 380—385.

Edwards, S., 1985. The effects of short term calf removal on pulsatile LH secretion in the postpartum beef cow. Theriogenology, 23: 777–785.

Forrest, D.W., Moseley, W.M., Kaltenbach, C.C. and Dunn, T.G., 1979. Serum LH response to estrone or short-term calf removal in postpartum beef cows. J. Anim. Sci., 49 (Suppl. 1): 297 (Abstract).

Forrest, P.K., Rhodes, R.C., III and Randel, R.D., 1980. Effect of variable suckling intensity and estrogen administration upon serum luteinizing hormone in Brahman cows. Theriogenology, 13: 333–339.

Forrest, P.K., Rhodes, R.C., III and Randel, R.D., 1981. Effect of bleeding stress and variable suckling intensity upon serum luteinizing hormone in Brangus heifers. J. Anim. Sci., 51 (Suppl. 1): 3 (Abstract).

Garcia-Winder, M., Imakawa, K., Day, M.L., Zalesky, D.D., Kittok, R.J. and Kinder, J.E., 1984. Effect of suckling and ovariectomy on the control of luteinizing hormone secretion during the post-partum period in beef cows. Biol. Reprod., 31: 771–778.

Henricks, D.M., Dickey, J.F., Hill, J.R. and Johnston, W.E., 1972. Plasma oestrogen and progesterone levels after mating and during late pregnancy and post-partum in cows. Endocrinology, 90: 1336–1344.

Hinshelwood, M.M., Dierschke, D.J. and Hauser, E.R., 1982. The effect of suckling and time post partum on GnRH-induced LH release in ovariectomized cows. J. Anim. Sci., 55 (Suppl. 1): 359 (Abstract).

Hinshelwood, M.M., Dierschke, D.J. and Hauser, E.R., 1985. The effect of suckling on the hypothalamic–pituitary axis in postpartum beef cows, independent of ovarian secretions. Biol. Reprod., 32: 290–300.

Holness, D.H. and Hale, D.H., 1980. The response of lactating Africander cows to treatment with a progesterone releasing intravaginal device or injections of synthetic GnRH. Anim. Reprod. Sci., 3: 181–188.

Holness, D.H., Hale, D.H. and Hopley, J.D.H., 1980. Ovarian activity and conception during the post-partum period in Afrikaner and Mashona cows. Zim. J. Agric. Res., 18: 3–11.

Inskeep, E.K. and Lishman, A.W., 1981. Factors affecting post-partum anestrus in beef cattle. Anim. Reprod. (Barcelona Symp. 3): 277–289.

Inskeep, E.K., Lishman, A.W., Butcher, R.L. and Allison, S.M.J., 1977. Effect of short term removal of the calf on release of LH, ovulation and CL function following GnRH in beef cows. Am. Soc. Anim. Sci. Annu. Meet., Abstr., No. 431.

Laster, D.B., Glimp, H.A. and Gregory, K.E., 1973. Effects of early weaning on post-partum reproduction of cows. J. Anim. Sci., 36: 734–742.

La Voie, V., Han, D.K., Foster, D.B. and Moody, E.L., 1981. Suckling effects on estrus and blood plasma progesterone in post-partum beef cows. J. Anim. Sci., 52: 802–812.

Odde, K.G., Ward, H.S., Kiracofe, G.H., McKee, R.M. and Kittok, R.J., 1980. Short oestrous cycles and associated progesterone levels in beef cows. Theriogenology, 14: 105–112.

Oxenreider, J.L., 1968. Effects of suckling and ovarian functions on post-partum reproductive activity in beef cows. Am. J. Vet. Res., 29: 2099–2107.

Peters, A.R., Lamming, G.E. and Fisher, M.W., 1981. A comparison of plasma LH concentrations in milked and suckling post-partum cows. J. Reprod. Fertil., 62: 567–573.

Randel, R.D. and Welker, G.A., 1976. Once daily suckling effect on cow–calf performance. J. Anim. Sci., 43: 301 (Abstract).

Riley, G.M., Peters, A.R. and Lamming, G.E., 1980. Multiple GnRH injections in post-partum acyclic beef cows. Winter Meeting 1980, Society for the Study of Fertility: 17 (Abstract).

Robertson, H.A., 1972. Sequential changes in plasma progesterone in the cow during the oestrous cycle, pregnancy, at parturition and post partum. Can. J. Anim. Sci., 52: 645–658.

Short, R.E., Bellows, R.A., Moody, E.L and Howland, B.E., 1972. Effects of suckling and mastectomy on bovine post-partum reproduction. J. Anim. Sci., 34: 70–74.

Short, R.E., Randel, R.D., Staigmiller, R.B. and Bellows, R.A., 1979. Factors affecting estrogen induced LH release in the cow. Biol. Reprod., 21: 683–689.

Stevenson, J.S., Spire, M.F. and Britt, J.F., 1982. Estradiol induced luteinizing hormone (LH) in postpartum suckled (S) and milked (M) cows: ovarian dependence. J. Anim. Sci., 55 (Suppl. 1): 394 (Abstract).

Suzuki, O. and Sato, M., 1979. Postpartum oestrous and serum progesterone and oestradiol-17β levels in beef cows whose calves were weaned early. Jpn. J. Anim. Reprod., 25: 183–188.

Thorpe, W. and Cruikshank, D.K.R., 1981. Genetic and environmental influences on beef cattle production in Zambia. 4. Weaner production from purebred and reciprocally crossbred dams. Anim. Prod., 33: 165–177.

Trail, J.C.M., Buck, N.G., Light, D., Rennie, T.W., Rutherford, A., Miller, M., Pratchett, D. and Capper, B.S., 1977. Productivity of Africander, Tswana, Tuli and crossbred beef cattle in Botswana. Anim. Prod., 24: 57–62.

Troxel, T.R., Kesler, D.J., Noble, R.C. and Carlin, S.E., 1980. Ovulation and reproductive hormones following steroid pretreatment, calf removal and GnRH in post-partum suckled beef cows. J. Anim. Sci., 51: 652–659.

Velasquez, J., Beltran, J., Troconiz, J., Silva, O., Bastidas, P. and D'Enjoy, G., 1980. Effect of monensin and restricted suckling on lactating Brahman heifers. Anim. Breed. Abstr., 53: 725 (Abstract).

Walters, D.L., Kaltenbach, C.C., Dunn, T.G. and Short, R.E., 1982a. Pituitary and ovarian function in postpartum beef cows. I. Effects of suckling on serum and follicular fluid hormones and follicular gonadotropin receptors. Biol. Reprod., 26: 640–646.

Walters, D.L., Short, R.E., Convey, E.M., Staigmiller, R.B., Dunn, R.B. and Kaltenbach, C.C., 1982b. Pituitary and ovarian function in post partum beef cows. II. Endocrine changes prior to ovulation in suckled and non-suckled post partum cows compared to cyclic cows. Biol. Reprod., 26: 647–654.

Ward, H.K., Tawonezvi, H.P.R., Davidson, J., Prentice, A., Tiffin, J. de W., Bennet, S., Brownlee, J.W.I., Arrowsmith, S.P., Harvey, H.R., Le Cordeur, A., Sairai, J.N., Dube, I., Moyo, G. and La Grange, R., 1978. Improvement of beef cattle productivity through crossbreeding. Ministry of Agriculture, Zimbabwe, Annual Report of the Division of Livestock and Pastures 1977–1978, pp. 105–121.

Webb, R., Haynes, N.B., Lamming, G.E. and Waters, R., 1977. Hormone status during the early post-partum period. University of Nottingham School of Agriculture, Report 1975–1976. Anim. Breed. Abstr., 45(5): 260.

Wells, P.L., Holness, D.H., Freymark, P.J., McCabe, C.T. and Lishman, A.W., 1985. Fertility in the Afrikaner cow. 2. Ovarian recovery and conception in suckled and non-suckled cows post-partum. Anim. Reprod. Sci., 8: 315–326.

Wetteman, R.P., Turman, E.J., Wyatt, R.D. and Totusek, R., 1978. Influence of suckling intensity on reproductive performance on range cows. J. Anim. Sci., 47: 342–346.

Wheeler, M.B., Anderson, G.B., Bondurant, R.H. and Stabenfeldt, G.H., 1982. Postpartum ovarian function and fertility in beef cattle that produce twins. J. Anim. Sci., 54: 589–593.

Williams, G.L. and Ray, D.E., 1980. Hormonal and reproductive profiles of early postpartum beef heifers after prolactin suppression or steroid induced luteal function. J. Anim. Sci., 50: 906–918.

Williams, G.L., Talavera, F., Petersen, B.J., Kirsch, J.D. and Tilton, J.E., 1983. Coincident secretion of Follicle-Stimulating Hormone and Luteinizing Hormone in early post-partum beef cows: Effects of suckling and low level increases of systemic progesterone. Biol. Reprod., 29: 362–373.

Wiltbank, J.N. and Cook, A.C., 1958. The comparative reproductive performance of nursed cows and milked cows. J. Anim. Sci., 17: 640–648.

PAPER 44

THE RESUMPTION OF OVARIAN ACTIVITY AND CONCEPTION IN AFRIKANER AND MASHONA COWS AFTER PARTIAL WEANING EITHER BEFORE OR DURING THE BREEDING PERIOD

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ABSTRACT

Wells, P.L., Holness, D.H. and Lishman, A.W., 1997. The resumption of ovarian activity in Afrikaner and Mashona cows after partial weaning either before or during the breeding period. *Anim. Reprod. Sci.*, (to be submitted).

The effect of once daily suckling (partial weaning), without introduction of bulls, on resumption of post partum ovarian activity and oestrus was investigated in Afrikaner and Mashona cows. Partial weaning was applied either prior to (for 35 days) or only after joining with breeding bulls. Cows suckled normally served as controls.

In Afrikaner cows, PW on its own did not reduce the proportion of Afrikaner cows remaining anovulatory. Reduction in suckling frequency, together with introduction of bulls, reduced number of anovulatory cows ($P < 0.05$), increased proportion of cows ovulatory for the first time ($P < 0.05$), reduced interval to conception in Mashona cows and increased progesterone secretion after conception($P < 0.01$) for both breeds. Conception rates were 47 and 90% for Afrikaner and Mashona cows, respectively, and interval from start of brreding to conception for Mashona cows was decreased ($P < 0.01$) by PW after joining with bulls.

Partial weaning without introduction of bulls has limited potential for inducing ovulation in anovulatory Afrikaner cows.

INTRODUCTION

A considerable degree of improvement in post-partum reproduction (particularly conception rate, +30%) was achieved in the Afrikaner cow with either an elimination of suckling (Wells *et al*.. 1985) or a reduction in the frequency of suckling to once a day (Wells *et al*., 1986). Normal suckling intensities delayed the onset of ovarian activity post partum by more than 100 days in some cows, despite the reproductive system of the Afrikaner cow being fully capable of functioning at acceptable levels (75 to 85% conception rates at 65 days post partum) in the absence of suckling (Wells *et al*., 1986).

Both of the previous studies in the Afrikaner cow included the introduction of bulls at the time reduced suckling treatments were applied. Since cows in the control group were exposed to bulls at the same intervals post partum, the improvement in post partum reproduction was attributed entirely to the reduction in suckling stimulus. The term biostimulation has been used by Fraser (1968) to describe the stimulatory effect males can have on oestrus and ovulatory responses in females (Baker, 1984). Although the neuro- and endocrine mechanisms are unknown, biostimulation may be caused by either direct genital stimulation allelomimetic cues or pheromones (Baker, 1984). The ram effect in sheep is well documented (Schinckel, 1954; Watson and Radford, 1960; Knight *et al*., 1978). In cattle the existence of a "bull effect" has not been so clearly demonstrated. The presence of either entire or vasectomized bulls has been shown to improve oestrus synchronisation in cows synchronised with progestogens (Pexton *et al*., 1977), shorten the interval to first oestrus post partum (Fraser, 1968; Kiddy *et al*., 1978; Zalesky *et* *al.*, 1984) and increase the proportion of cows displaying oestrus during early lactation (MacMillan *et al.*, 1979). Puberty has been advanced by exposing heifers to the urine of mature bulls (Izard and Vandenbergh, 1982).

If a reduction in suckling stimulus *per se* was responsible for the improvements in reproductive performance observed in the research reported earlier (Wells *et al.*, 1985,1986), then partial weaning in the absence of bulls should enable the hypothalamo-pituitary axis to escape from the suppressive effects of suckling and induce ovulation in the anovulatory post-partum cow. If, on the other hand, gonadotropin activity was only stimulated after the synergistic action of early (3 or 25 days post partum) introduction of bulls and a reduction in suckling intensity, then partial weaning alone should not be as effective in improving post-partum reproduction in the Afrikaner.

Regular ovarian activity has always followed the induced ovulations (Well *et al.*, 1985,1986). If partial weaning alone proved to be effective in inducing ovulation in the anovulatory Afrikaner cow the technique could be applied prior to the breeding season as a method of improving ultimate conception rates and compacting the subsequent calving period.

Calf removal has increased gonadotropin activity and induced ovulation in 50% of anovulatory cows (Edwards, 1985). Unfortunately, restoration of normal suckling levels after a period of weaning has depressed gonadotropin activity within four hours of calf return (Walters *et al.*, 1982; Edwards,1985). However, Edwards (1985) reported that this occurred only in cows which were anovulatory. In cyclic cows no such reduction in gonadotropic activity was observed. Thus, the effect of returning calves to their dams, when bulls were introduced, could be expected to have unpredictable results.

This study was designed to investigate the value of partial weaning (suckling once a day) on its own as a technique for inducing ovulation in the anovulatory Afrikaner cow. For the first time the technique was to be used as a blanket treatment. With the variation between cows in post-partum intervals at the start of the trial the effectiveness of the technique was not expected to be as great as when partial weaning was applied to each cow at a fixed interval after calving. The stimulating effect of cows that are cycling on anoestrous herdmates should not be ignored (Wright *et al.*, 1994). Nevertheless, if partial weaning is to be used commercially there can be no question of weaning each calf at a specific age.

MATERIALS AND METHODS

Experimental animals

Mature (range 5 to 10 years), multiparous (range 3 to 6) Afrikaner and Mashona cows were blocked according to calving date within each breed and then randomly allocated to one of three treatment groups. The 62 Afrikaner cows and 39 Mashona cows used in this study were in fair to good condition (condition score range 2.5 to 3.0). The calving season was unnaturally protracted from experimental work in previous years. Consequently, long mean post-partum intervals at the start of the trial, in early calvers, was a result of delaying the trial until sufficient experimental cows were available.

Manipulation of suckling

A reduction in suckling intensity was achieved by partial weaning as before, with cows being allowed access to the calf enclosure within their grazing paddock. Cows could still see their calves and could reach over the fence to lick their calves, although this was seldom observed. Cows were allowed to suckle their calves at 1600 hours and were removed at 1630 hours. Most suckling was completed within 17 minutes of starting. This suckling treatment was applied without any adaption period (twice a day suckling for two weeks) as used in the previous study Wells *et al.*,1986).

Three treatment groups were used in this trial. The two groups in which partial weaning was applied differed in the timing of partial weaning application in relation to the breeding period. In the control group cows were normally suckled for the duration of the trial.

A...Partial weaning during the breeding period:

Twenty two Afrikaner cows and 16 Mashona cows which were respectively 59 ± 29 days and 72 ± 24 days post partum were partially weaned when the bulls were introduced into the herd on 25 January. Normal suckling resumed in this group at the end of the breeding period (end of the trial) on 5 March.

B...Partial weaning before the breeding period:

Twenty five Afrikaner cows and 13 Mashona cows which were respectively 61 ± 23 days and 77 ± 9 days post partum on 25 January were partially weaned from 21 December to 25 January. Thereafter, and for the duration of the breeding period (25 January to 5 March) calves had continuous access to their dams.

C....Control:

Fifteen Afrikaner cows and ten Mashona cows which were, respectively 63 ± 27 days and 74 ± 5 days post partum (on 25 Jan) had continuous access to their calves for the duration of this study from 21 December until 5 March. Bulls were run with these cows from 25 January until 5 March.

Bulls

All bulls used in this study had been examined and declared sound prior to the commencement of the trial. From previous years these bulls were known to be fertile and have satisfactory libido. A total of six bulls (two per treatment group) were run with the cows from 25 January until 5 March. Bulls were rotated between the treatment groups at weekly intervals.

Observation of oestrous behaviour

All three treatment groups were observed for a limited period each day from the start of the trial on 21 December until 5 March. Trained observers recorded oestrous behaviour between 0500 and 0900 hours and between 1500 an 1900 hours. The results of oestrous observation are be presented in detail elsewhere (Paper ??).

Progesterone assay

Three times each week (at 0800 hours) from the 30 November until the 5 March blood samples were collected, by jugular venipuncture. Plasma was extracted from these samples and stored at -20 C for determination of plasma progesterone concentration. Determinations were carried out according to the method of Holness *et al.* (1980). Within assay variation was 22% and the between assay variation was 15%. The standard error for a single observation in an assay was ± 0.42ng/ml.

RESULTS

Anovulatory cows

Compared to normal suckling, partial weaning before the breeding period did not significantly reduce the incidence of Afrikaner cows which were anovulatory for the duration of the trial. However, when applied with the introduction of the bulls this treatment significantly ($P < 0.05$) decreased the proportion of anovulatory cows (Table 1). There were no instances of anovulatory Mashona cows in this study.

TABLE 1

The number of Afrikaner and Mashona cows in each treatment group which ovulated within the experimental period from 21 December to 5 March

Treatment	Breed	
	Afrikaner	Mashona
Partial weaning with breeding	$18/22^a$	16/16
Suckled normally + Partial weaning before breeding	$20/40^b$	23/23
Total for breed	$38/62^x$	$39/39^y$

a,b; x,y Proportions differ, $P < 0.05$

* In the absence of any significant differences between cows normally suckled throughout the trial and cows partially weaned prior to the breeding period these two groups of cows have been combined.

Resumption of ovarian activity

By the start of the trial on 21 December, 19% of Afrikaner cows and 80% of Mashona cows had ovulated with no difference between the treatment groups (Table 2). Thus, too few cows remained to assess whether partial weaning alone would have stimulated ovulation in the anovulatory Mashona cow (Table 2).

Amongst Afrikaner cows, once a day suckling in the absence of exposure to bulls failed to stimulate (based on the number of cows ovulating for the first time) the onset of ovarian activity compared to that observed in normally suckled cows over the same period. By the end of the trial a similar proportion of cows in each of these two groups had ovulated (Table 2).

In contrast to this lack of response to partial weaning in the absence of bulls, partial weaning, at the same time as introduction of bulls, significantly ($P < 0.05$) increased the proportion of cows which ovulated for the first time during the breeding period by 40% (Table 2).

TABLE 2

The distribution of the resumption of ovarian activity in relation to the application of partial weaning and the introduction of bulls

Breed of cow	Treatment group	Number in group	Number of cows commencing ovarian activity:-			
			Before 21 Dec	21 Dec - 24 Jan	25 Jan - 5 Mar	Anoestrus
Afrikaner	PW during breeding	22	4	3	11^a	4^a
	PW before breeding	25	5	5	3^b	12^b
	Normal suckling	15	3	2	2^b	8^a
Mashona	PW during breeding	16	12	1	3	0
	PW before breeding	13	11	2	3	0
	Normal suckling	10	9	1	0	0

^{a,b} Proportions differ, $P < 0.05$

Ovulations during the pre-breeding and breeding periods

Figure 1 clearly illustrates the difference in ovarian activity between Afrikaner and Mashona cows at the start of this trial. Although only 14 days separated the mean post-partum interval of these two breeds at the start of the trial, nearly all of the Mashona cows had ovulated before the 21 December.

All of the Afrikaner cows ovulated for the first time after the start of the experiment. Although not significantly different, there was a tendency for Afrikaner cows partially weaned for 35 days before the breeding period to have a slightly higher mean ovulation rate during this time than their normally suckled contemporaries. This trend was also observed in the Mashona cows weaned before the introduction of the bulls (Fig. 1).

During the breeding period, partial weaning and the introduction of bulls significantly ($P < O.05$) increased the mean ovulation rate per cow (Fig. 1). Both in control cows and cows weaned before breeding there were a number of cows which failed to ovulate after the introduction of the bulls.

In the Mashona cows, the mean ovulation rate appeared to decline when cows were partially weaned with the introduction of bulls (Fig. 1). This apparent anomaly was due to earlier conceptions in cows weaned during the breeding period compared to their normally suckled contemporaries.

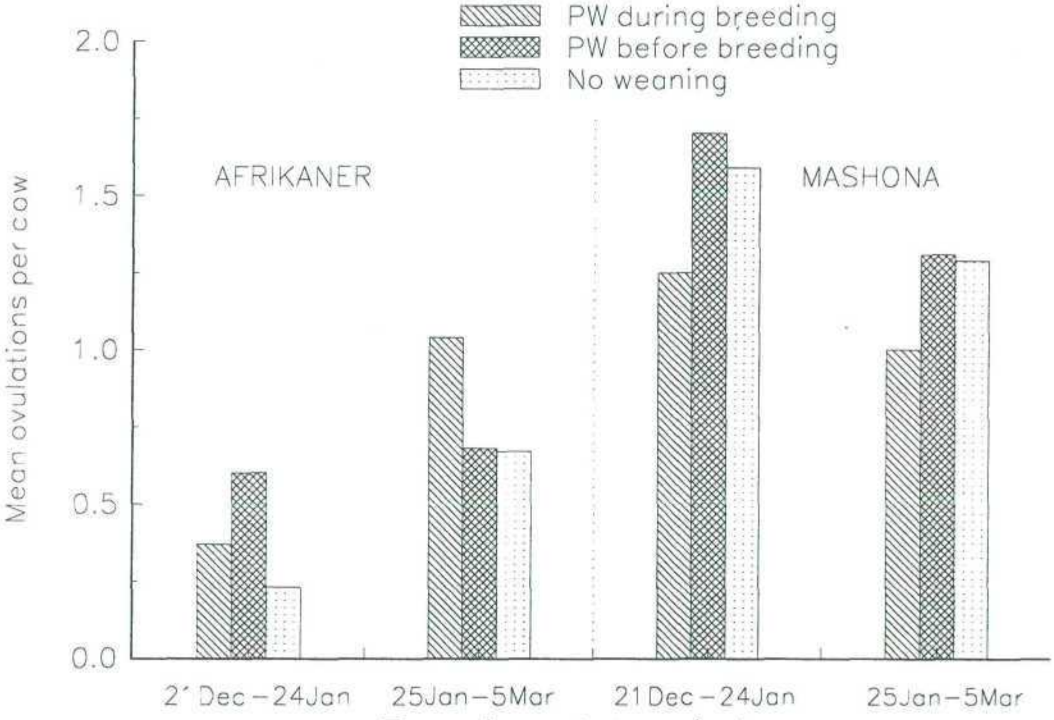

Fig. 1. Ovulation rate for Afrikaner and Mashona cows for pre-breeding (21 Dec - 24 Jan) and breeding periods (25 Jan - 5 Mar)

Conception rates

During the breeding period of 39 days 47% of Afrikaner cows and 90% of Mashona cows conceived. No significant differences in conception rate were observed between the three treatment groups within each breed (Table 3).

Amongst Afrikaner cows, calving rates were lower than in Mashona cows for two reasons. Firstly 39% of Afrikaner cows were anovulatory for the duration of the trial while all of the Mashona cows ovulated during the same period. Secondly, not all cows which ovulated before the end of the breeding period conceived. In only 76% of ovulations in Afrikaner cows was conception successful, compared to 89% in the Mashona cows (Table 3).

TABLE 3

Conception rates following suckling once each day either before or during the breeding period in Afrikaner and Mashona cows (no significant differences observed)

	Afrikaner			Mashona		
Time of weaning	With breeding	Before breeding	None	With breeding	Before breeding	None
Number in group	22	25	15	16	13	10
Number ovulating	18	13	7	16	13	10
Number pregnant	13	9	7	15	11	9
Breed totals		29/62			35/39	

Of the 18 Afrikaner cows stimulated by partial weaning during the breeding period to ovulate only 72% (13/18) conceived. In the control group the figure was lOO% (Table 3). Sample numbers were too small to test whether the probability of conception per ovulation was higher in normally suckled cows.

Luteal activity

Luteal activity was assessed by estimating the total plasma progesterone within prescribed sectors of the progesterone profile for each cow in the experiment. Three categories of luteal activity were analyzed for each cow when they occurred: short luteal phase following primary ovulation; 21 day oestrous-cycle following a full ovulation; and the first 14 days after conception.

An analysis of up to four successive cycles within breed and treatment group, indicated that the total plasma progesterone during a normal oestrous cycle did not differ significantly from one cycle to the next. For this reason data from successive oestrous cycles prior to conception were pooled for the analysis of treatment effects on luteal activity (Table 4).

Total estimated plasma progesterone in the Afrikaner cow did not differ during either short luteal phases or during the first four oestrous cycles (Table 4), although the occurrence of short luteal phases was higher ($P < 0.01$) in cows PW with breeding than in control animals. During the breeding period, however, cows which were suckling their calves once each day, had significantly ($P < 0.01$) higher total plasma progesterone concentrations for the 17 days after conception than those cows in either of the remaining two groups.

TABLE 4

Estimated total plasma progesterone (ng/ml * days) following primary, full ovulations and conception in Afrikaner and Mashona cows which were either normally suckled or partially weaned before breeding or with breeding

Luteal type:-	Partially weaned		
	With breeding	Before breeding	Control
Short luteal phase:-			
Afrikaner	5.5 ± 0.7	4.7 ± 2.2	2.3 ± 1.3
n	15	10	3
Mashona	5.4 ± 1.2	4.8 ± 0.3	7.6 ± 3.3
n	9	3	3
First 4 oestrous cycles (pooled)			
Afrikaner	54.4 ± 4.5	50.5 ± 3.3	56.7 ± 6.6
n	23	26	10
Mashona	77.2 ± 4.6^{a}	60.5 ± 3.6^{b}	78.5 ± 3.8^{a}
n	37	43	28
Conception + 17 days			
Afrikaner	77.4 ± 7.2^{a}	50.9 ± 5.4^{b}	58.8 ± 4.1^{b}
n	10	9	7
Mashona	94.9 ± 9.4^{c}	64.5 ± 6.6^{d}	93.0 ± 5.0^{c}
n	15	9*	7

[a,b] Figures differ, $P < 0.01$ [c,d] Figures differ, $P < 0.05$

* Conception occurred near the end of the breeding period and insufficient plasma samples remained in certain cows to be able to estimate total progesterone for 17 days after conception.

Total estimated plasma progesterone during the short luteal phase did not differ significantly between treatment groups. Similarly, total plasma progesterone during all oestrous cycles prior to conception did not differ significantly between control cows or cows suckled once a day during the breeding period. However, the resumption of normal suckling after 35 days of partial weaning, depressed total progesterone ($P < 0.01$) during subsequent cycles prior to conception and for the first 14 days after conception ($P < 0.05$).

Post-partum intervals to first ovulation and conception

Post-partum intervals to either primary ovulations or first full ovulations were not significantly altered in either breed by any of the treatments imposed (Table 5).

TABLE 5

The mean interval (days) from calving until the start of each category of luteal activity and the mean interval (days) from the introduction of bulls until conception in Afrikaner and Mashona cows. Figures in brackets denote number of observations

Luteal type	Partial weaning:-		
	With breeding	Before breeding	None
Short luteal phase:-			
Afrikaner	89.8 ± 7.6 (15)	89.5 ± 7.8 (10)	94.7 ± 12.7 (3)
Mashona	46.2 ± 5.0 (9)	72.7 ± 3.0 (3)	54.0 ± 15.0 (3)
First oestrous cycle:-			
Afrikaner	79.6 ± 8.9 (9)	73.6 ± 7.1 (10)	63.5 ± 9.5 (2)
Mashona	49.8 ± 3.0 (12)	57.6 ± 4.5 (12)	57.1 ± 5.9 (8)
Conception:-			
Afrikaner	108.2 ± 8.3 (13)	118.0 ± 3.2 (9)	121.1 ± 9.3 (7)
Mashona	107.5 ± 5.5^c (15)	128.5 ± 4.0^d (11)	123.7 ± 6.9^d (9)
Bulls in to conception:-			
Afrikaner	15.6 ± 2.4^a (13)	8.2 ± 5.4^b (9)	12.9 ± 1.6^a (7)
Mashona	7.8 ± 1.6^c (15)	16.9 ± 3.6^d (11)	17.2 ± 3.0^d (9)

Treatment means differ ^{a,b} $P < 0.05$ ^{c,d} $P < 0.01$

Mean intervals to conception in Afrikaner cows, did not differ significantly between treatment. However in the Mashona, partial weaning together with the introduction of bulls, significantly ($P < 0.01$) shortened the mean interval from calving to conception (Table 5).

Mean intervals from start of breeding to conception

In the Afrikaner cow, once a day suckling before the breeding period significantly shortened ($P < 0.05$) the interval from the start of breeding to conception by 4 to 7 days compared to the remaining two treatment groups. Partial weaning with breeding stimulated ovulation in 50% of the cows in this group which were anovulatory at the start of the breeding period. Since conception rates to these first ovulations were low, most cows did not conceive until their second ovulation. Thus, in the Afrikaner cows in this study there was no change in the mean interval from the introduction of bulls to conception compared to the control group.

Reducing the suckling stimulus during breeding in Mashona cows significantly ($P < 0.01$) shortened the interval from bull introduction to conception by 9-10 days compared to the remaining two groups. These results confirm that partial weaning during the breeding period in fertile breeds can increase the probability of conception to first ovulations after breeding has commenced.

DISCUSSION

Need for introduction of bulls

In the Afrikaner cow, twice a day suckling followed by once a day suckling at 40 days post partum was as effective as complete weaning three days post partum in improving conception rates by more than 25% (Wells *et al.*, 1986). Since bulls were introduced into the control herds at the same time, treatment differences reported in those studies were attributed entirely to the effects of reduced suckling intensity.

The results of this present study confirm that the application of once a day suckling without the introduction of bulls will not induce ovulation in the majority of anovulatory Afrikaner cows. Since a reduction in suckling intensity is not a stimulus, but rather the removal or decrease of a suppressive stimulus, the response to any weaning treatment will depend upon the current endocrine status of the cow.

If the hypothalamo-pituitary axis is poised to escape from the suppressive effects of suckling, then ovulation may follow a reduction in suckling intensity. Thus, before partial weaning can stimulate primary or first full ovulations, an unprompted increase in LH pulse frequency and LH concentration followed by limited follicular development must have occurred (Edwards, 1985).

Perhaps if partial weaning alone had been applied later, the response in anovulatory cows might have been more favourable. However, this would have delayed breeding and would have been unrepresentative of the commercial situation. If partial weaning cannot be used soon after calving then it will be of little use in cows where it is needed most (late calvers).

In contrast to the above, when suckling intensity was reduced simultaneously with the introduction of bulls, significantly ($P < 0.05$) more cows ovulated and although the conception rate was higher, than in both groups of normally suckled cows, the increase was not significant.

Partial weaning and ovarian activity

These results support the findings of (Well *et al.*, 1986) where partial weaning in conjunction with breeding increased the proportion of cows resuming ovarian activity, but failed to shorten the post-partum interval to primary ovulation. With complete weaning during breeding (Wells *et al.*, 1985), however, the mean interval from calving to primary ovulation was significantly ($P < 0.001$) shortened by 35 days in the Afrikaner. The results obtained here confirm earlier findings (Well *et al.*, 1986) that calving rates in Afrikaner cows were reduced by the occurrence of anovulatory cows plus cows not conceiving to the first breeding.

In the absence of suckling and in the presence of the bull, the pituitary of the cow appears to increase its release of gonadotropins as early as 5 to 15 days post partum (Hinshelwood *et al.*, 1985) with first ovulation occurring approximately 14 days later. Once a day suckling is not quite as effective as complete weaning because, although partial weaning will increase LH activity within 48 hours (Edwards, 1985), the increase will not induce ovulation. Only when this increase in LH activity, due to weaning, is supported by the endogenous increase in LH activity due to post-partum interval (Echternkamp and Hansel, 1973; Kesler *et al.*, 1977; Webb *et al.*, 1980) will ovulation be induced.

There are numerous instances where reduced suckling has been employed in an effort to improve reproductive efficiency. Although temporary calf removal is used routinely in synchronised breeding programmes, the results reported in the literature are variable. Smith *et al.* (1979); Pace and Sullivan (1980) and Betts *et al.* (1932) reported that calf separation for up to 48 hours had no effect on ultimate pregnancy rates. Kiser *et al.* (1980) and Peterson *et al.* (1980) recorded improved pregnancy rates with calf removal following synchronisation. In both of these last two studies cows and calves were still separated at or within 6 hours of insemination. Since the return to normal suckling after a period of temporary weaning is known to depress gonadotropin concentrations in acyclic cows (Edwards, 1985) the duration of weaning in relation to insemination has, as yet received little attention.

Luteal function

Following 35 days of partial weaning, normal suckling would have simulated an increase in the intensity of suckling, relative to once daily. In the Afrikaner this had no detectable effect on luteal function, but in the Mashona this treatment significantly lowered total plasma progesterone during the oestrous cycle by approximately 28%. This apparent increase in suckling stimulus may have depressed LH and progesterone concentrations relative to concentrations during the period of reduced suckling.

In the Afrikaner cow, plasma progesterone concentrations following conception were significantly increased (± 40%) when cows were suckled once a day in conjunction with the introduction of bulls. This finding concurs with the results of La Voie *et al.* (1981). Since reduced suckling has been shown to induce higher plasma LH levels (Randel *et al.*, 1976 and Carruthers *et al.*, 1980) changes in steroid levels in the Afrikaner are almost certain to have been mediated by changes in gonadotropin concentrations. These findings support Lishman *et al.*, (19) and Stewart *et al.*, (1997).

Conception rates

Considering the interval from the introduction of bulls until conception in each group, there appears to be some evidence of a breed by treatment group interaction. Amongst Mashona cows 36/39 cows had ovulated when the breeding period began. Partial weaning during this time enabled a higher proportion of weaned cows compared to control cows to conceive to the first ovulation during the breeding period. This is the first strong evidence that partial weaning during oestrus has improved the probability of conception. A study of the behaviour at oestrus has eliminated the possibility that conception rates were reduced because cows failed to be mated (Wells et al., 1997b). Consequently, it seems that the improved conception rates were due to cows being more fertile at the first ovulation. This is supported by the improved luteal activity recorded.

Amongst Afrikaner cows, where only 35% had ovulated at the start of breeding, once a day suckling during the breeding period stimulated the resumption of ovarian activity in 54% of the remaining anovulatory cows in this group. Since conception could only occur after a short cycle lasting 6.6 ± 1.1 days the mean interval to conception was not significantly shortened in this group but the conception rate was significantly improved.

Reduced suckling during breeding has proved to be a powerful stimulus to the reproductive system, capable of stimulating ovulation in anovulatory cows which are within 20 to 40 days of spontaneous ovulation. However the importance of reduced suckling in the presence of bulls is stressed and agrees with the findings of Lishman and Harwin (1985).

This study conclusively demonstrated that plasma progesterone concentrations in fertile Afrikaner cows are significantly lower than in Mashona cows. There is however no evidence that this characteristic is implicated in the lower fertility of the Afrikaner compared to the Mashona. Since the Afrikaner is almost certainly more sensitive to changes in suckling intensity and steroid environment (lower concentrations of circulating progesterone) than the Mashona, perhaps the sub-fertility of the Afrikaner is an active state determined by as yet unknown factors of the environment.

REFERENCES

Baker, A.A., 1984. Modern reproductive techniques in cattle breeding. Proc. Aust. Soc. Anim. Prod., 15: 24-37.

Betts, J.G., Forrest, D.W., Beverley, J.R. and Huston, J.E., 1982. Effect of calf removal regimes on pregnancy rates in a controlled breeding program. J. Anim. Sci., 55 (Suppl. 1): 176 (abstr.).

Carruthers, T.D., Convey, E.M., Kesner, J.S., Hafs, H.D. and Cheng, K.W., 1980. The hypothalamo-pituitary gonadotrophic axis of suckled and nonsuckled dairy cows post partum. J. Anim. Sci., 51: 949-957.

Echternkamp, S.E. and Hansel. W., 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first post-partum estrous cycle. J. Anim. Sci., 6: 1362-1370.

Edwards, S., 1985. The effects of short term calf removal on pulsatile LH secretion in the postpartum beef cow. Theriogenology, 23: 777-785.

Fraser, A.F., 1968. Reproductive behaviour in Ungulates. New York: Academy Press.

Hinshelwood, M.M., Dierschke, D.J. and Hauser, E.R., 1985. Effect of suckling on the hypothalamo-pituitary axis in postpartum beef cows, independent of ovarian secretions. Biol. Reprod., 32: 290-300.

Holness, D.H., Hale, D.H. and Hopley, J.D.H., 1980. Ovarian activity and conception during the post-partum period in Afrikaner and Mashona cows. Zim. J. Agric. Res., 18: 3-11.

Izard, M.K. and Vandenbergh, J.G., 1982. Priming pheromones from estrous cows increase synchronisation of estrus in dairy heifers. J. Anim. Sci., 55: 1160-1166.

Kesler, D.J., Gaverick, H.A., Younquist, R.G., Elmore, R.G. and Bierschwal, C.J., 1977. Effect of days postpartum and endogenous reproductive hormones on GnRH-induced Lh release in dairy cows. J. Anim. Sci., 46: 797-803.

Kiddy, C.A., Mitchell, D.S., Bolt, D.J. and Hawk, H.W., Detection of estrus-related odors in cows by trained dogs. Biol. Reprod., 19: 389-395.

Kiser, T.E., Dunlap, S.E., Benyshek, L.L. and Mares, S.E., 1980. The effect of calf removal on estrous response and pregnancy rate of beef cows after Synchro-mate-B treatment. Theriogenology, 13: 381-389.

Knight, T.W., Peterson, A.J. and Payne, E., 1978. The ovarian and hormonal response of the ewe to stimulation by the ram early in the breeding season. Theriogenology, 10: 343-347.

La Voie, V, Han, D.K., Foster,D.B. and Moody, E.L., 1981. Suckling effect on oestrus and blood plasma progesterone in post-partum beef cows. J. Anim. Sci., 52: 802-812.

Lishman, A.W., Louw, B.P., van Niekerk, A. and Raciborska, C., 1988. Once daily suckling after GnRH to improve luteal function in beef cows. 29th Congr. S. Afr. Soc. Anim. Prod.

Lishman, A.W. and Harwin, G.O., 1985. Failure to induce ovulation by short-term calf removal in lactating beef cows on dry-lot. S. Afr. J. Anim. Sci., 15: 21-22.

MacMillan, K.L., Allison, A.J. and Struthers, G.A., 1979. Some effects of running bulls with suckling cows or heifers during the premating period. N. Z. J. Exp. Agric., 7: 121-124.

Pace, M.M. and Sullivan, J.J., 1980. Effect of SYNCRO-MATE-B (SMB) and calf separation on beef cattle estrus and pregnancy rates. J. Anim. Sci. 51 (Suppl. 1): 312 (Abstr.).

Peterson, L.A., Mares, S.F., Henderson, E.A. and Davenport, M.E., 1980. Effect of calf separation time on pregnancy rates of cows synchronised with SYNCHRO-MATE-B. J. Anim. Sci., 49 (Suppl.1) : 326(Abstr.).

Pexton, J.E.and Chenoweth, P.J., 1977. Using bulls to breed heifers at synchronised estrus. J. Anim. Sci., 45(Suppl. 1) : 195(Abstr.).

Randel, R.D., Short, R.E. and Bellows, R.A., 1976. Suckling effects on LH and progesterone in beef cows. J. Anim. Sci. 42; 267 (Abstr.).

Schinckel, P.G., 1954. The effect of the ram on the incidence and occurrence of oestrus in the ewe. Austr. Vet. J., 30: 189-195.

Smith, M.F., Burrell, W.C. Ship, L.D., Sprott, L.R., Songster, W.N. and Wiltbank, J,N., 1979. Hormone treatments and use of calf removal in postpartum beef cows. J. Anim. Sci., 48: 1285-1294.

Stewart, I.B., Lishman, A.W., Morgenthal, J.C. and Levin, J., 1997. Interaction between melatonin and suckling in prolonging post partum oestrous activity in suckled cows. S. Afr. J. Anim. Sci. (to be submitted).

Walters, D.L., Kaltenbach, C.C., Dunn, T.G. and Short, R.E., 1982. Pituitary and ovarian function in postpartum beef cows. 1. Effects of suckling on serum and follicular fluid hormones and follicular gonadotropin receptors. Biol, Reprod., 26: 649-646.

Watson, R.H. and Radford, H.M., 1960. Them influence of rams on onset of oestrus ın Merino ewes in the spring. Aust. J. Agric. Res., 11: 65-71.

Webb, R., Lamming, G.E., Haynes, N.B. and Foxcroft, G.R., 1980. Plasma progesterone and gonadotropin concentrations and ovarian activity in post-partum dairy cows. J. Repro. Fert., 59: 133-143.

CL. The conception rate averaged 46% in these synchronized cows. The net result (*48, 58*) was that less than 20% of the average herd could be expected to conceive to artificial insemination (AI) on a single day (57% treated × 74% synchronized × 46% conceived = 19% pregnant). An injection of estradiol benzoate 40 to 48 hours after the $PGF_{2}\alpha$ increased synchronization to 92%. Conception rate averaged 48%, so that net proportion of the herd pregnant to AI on a single day was still only 25%.

Effects of breed, age, plane of nutrition, and suckling intensity on the postpartum interval to estrus in beef cows have been established and can be illustrated from either recent data or classical literature. For the present purpose, the effects of these factors will be discussed briefly to provide a background upon which to consider the possible physiological mechanisms or deficiencies in function associated with long intervals from calving to estrus.

EFFECTS OF BREED AND AGE

Data on the occurrence of estrus or a palpable CL on a single day were obtained in 1164 cows that were 30 to 99 days postpartum in 24 herds in West Virginia. These data were subjected to least squares analysis, considering the interval from parturition to examination as a covariate. The pattern of return to a functional reproductive status after parturition was linear and varied with breed, age, year, herd (all $P<.01$), and the interaction of age and breed ($P<.05$; Fig. 17.1). At two years of age, while rearing their first calf, Angus cows and crossbreds (crosses of Angus or Hereford with Charolais, Simmental, Brown Swiss or Holstein; designated exotic crosses) were more likely to have a CL at a given stage postpartum than Herefords or various crosses of the British breeds. At three years of age, the overall mean pattern of occurrence of CL or estrus was similar to that at two years, but no breed differences were apparent. Mature cows, four years of age and older, were reproductively active earlier than the two- and three-year olds; Angus were again superior, but the exotic crosses ranked lower than the Herefords.

The weighted means for data from the literature summarized by Casida et al. (*5*) and the data they presented (Table 17.1) show only a four-day advantage for Angus over Herefords in interval from parturition to first estrus. Similarly, Laster et al. (*24*) found no effect of breed on this interval in small numbers of Hereford, Angus and Charolais-cross cows. Mean advantages in interval for Angus over Herefords suckling calves were 4, −2 and 7 days for two-, three-, and four-year-olds, respectively. In another study (*25*), Hereford cows showed a greater incidence of calving difficulty than Angus, especially as two-year-olds, and calving difficulty decreased the proportion of cows detected in estrus during a 45-day period by 9, 17, and 9 percentage points in two-, three-, and four- and 5-year-olds, respectively. More two-year-old Herefords (30%) than Angus (9%) failed to show estrus on a low energy ration after calving (*12*).

In the present study, more Angus than Herefords were in purebred herds. Thus patterns of management common to either purebred or commercial

Wells, P.L., Holness, D.H., Freymark, P.J., McCabe, C.T. and Lishman, A.W., 1985. Fertility in the Afrikaner cow. 2. Ovarian recovery and conception in suckled and non-suckled cows *post partum*. Anim. Reprod. Sci., 8: 315-326.

Wells, P.L., Holness, D.H., McCabe, C.T. and Lishman, A.W., 1986. Fertility in the Afrikaner cow. 3. Once a day suckling and its effect on the pattern of resumption of ovarian activity and conception rate in early lactation. Anim. Reprod. Sci., 12: 1-12.

Wright, I.A., Rhind, S.M. Smith, A.J. and Whyte, T.K., 1994. Female-female influences on the duration of the post-partum anoestrous period in beef cows. Anim. Prod., 59: 49-53.

Zalesky, D.D., Day, M.L., Garcia-Winder, M., Imakawa, K., Kittok, R.J., D'Occhio, M.J. and Kinder, J.E., 1984. Influence of exposure to bulls on resumption of estrous cycles following parturition in beef cows. J. Anim. Sci., 59: 1135-1139.

PAPER 45

THE PATTERN OF BEHAVIOUR PRIOR TO AND DURING OESTRUS IN AFRIKANER AND MASHONA COWS AND THE EFFECT OF CALF REMOVAL AND ABSENCE OF BULLS

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ABSTRACT

Wells, P.L., D.H. Holness and A.W. Lishman., 1997. The pattern of behaviour prior to and during oestrus in Afrikaner and Mashona cows and the effect of calf removal and absence of bulls. *Anim. Reprod. Sci. (to be submitted).*

In an attempt to account for low calving rates of Afrikaner cows, the patterns of behaviour prior to and during oestrus and the modifying effect of calf removal were studied in three experiments. Mashona cows were included for comparative purposes as they were noted for high calving rates in the same environment as Afrikaners. Experiment 1 utilised 60 Afrikaner and 20 Mashona cows and calf removal involved weaning at three days of age. In experiment 2 the Afrikaner cows calves were allowed to suckle twice daily from 28-42 days of age and thereafter once daily (partial weaning). For experiment 3, Afrikaner and Mashona cows were suckled once daily (partial weaning) for 35 days prior to onset of the breeding period, while a second group had this treatment applied only after introduction of bulls. In all three experiments cows that suckled their calves normally served as controls. Observations focussed on oestrous related behaviour of both cows and bulls. Progesterone profiles derived from samples drawn three times per week were used to denote primary (levels exceeded .27ng/ml for 3 days) and full ovulations (levels above .27ng/ml for 11 days).

Consideration was given primarily to oestrous periods at which cows were seen to be served by bulls. In general, cows that suckled their calves normally exhibited low levels of homosexual activity with cows subsequently observed to be served by bulls mounting other cows or standing for mounting by herdmates in less than 30% of the oestrous periods. In Afrikaner, cows partial weaning of calves during the breeding period significantly increased the incidence of homosexual behaviour, while in both breeds the incidence of multiple mounting was increased by this treatment.

When attention was focussed on heterosexual behaviour, although mounting the bull was the most commom component of oestrous behaviour after service, this behaviour was seen in less than 40% of the oestrous periods. No consistent effect of calf weaning was seen, except in Experiment 3 where partial weaning at start of breeding increased the incidence by 27% in Mashona and by 50% in Afrikaner cows.

Observation of behavioural components initiated by bulls showed that chin-resting occurred in 82 and 90% of the oestrous periods in Experiments 2 and 3, respectively. Nosing of the perineum occurred only once in 31% (Exp. 2) and 21 % (Exp. 3) of the oestrous periods, while the incidence of Flehmen was below expectation ($< 44\%$). Not more than 60% of the full ovulations were seen to be accompamied by service, but both complete and partial weaning increased the incidence of observed service. The proportion of oestrous periods at which cows were served more than once was not increased by weaning treatments, except for partial weaning during the breeding period (increased from 24% to 63%).

In the Afrikaner cow oestrous behaviour is low-key with the service in many instances being limited to a single event.

INTRODUCTION

For conception to occur, essential cow-bull interactions mustprecede fertilization. Cows and bulls should pair prior to ovulation and initiate behaviour which will culminate in insemination of the cow by the bull.

Studies in bovine reproduction, particularly in beef cattle, have been aimed primarily at understanding the physiological mechanisms which culminate in ovulation. Patterns of oestrus-expression have received limited attention and the lack of data on the behavioral signs of oestrus in tropical cattle has been emphasised by Galina and Arthur (1990). These authors maintain that since AI has a great potential for improving local breeds, mostly zebus, attention has recently been focused on characterising the behavioral signs of oestrus. This is especially important because problems associated with "heat spotting" are often givenas the reason why farmers do not use AI more widely in beef cows, particularly under ranching conditions (Boulle, 1986).

Reproductive rates of some breeds of beef cattle that are indigenous to tropical and sub-tropical areas are limited by prolonged lactation anoestrus. However, in such breeds complete (Wells*et al.*, 1985) temporary (Symington and Hale, 1967; Holness *et al.*, 1978) or partial (Randel, 1981; Wells *et al.*, 1986) weaning of the calf has been successfully employed to induce early resumption of ovarian activity (Randel, 1981; Wells *et al.*, 1985, 1986). The delay to conception was not always shortened in Afrikaner cows since the probability of conception to early ovulations (prior to day 50 post-partum) was considerably lower than that associated with later ovulations (Wells *et al.*, 1986).

The possibility existed that the low incidence of successful conceptions to early ovulations was due to an absence of mating. This was supported by the more frequent observation ofservice in cows not suckling their calves (weaned at three days of age), although a shift in the time of service to the hours of darkness may have occurred in cows which suckled their calves (Wells *et al.*, 1985). There was also the possibility that suckling a calf either markedly reduced the behavioral responses to the endocrine signals (a neural effect) or the strength of these signals was reduced thereby reducing the intensity of oestrus expression.

The objectives of this study were to observe (i) the patterns of behaviour which signalthe approach of oestrus and those which occur during oestrus and (ii) to determine whetherpresence of the suckling calf or of a bull would modify these responses.

MATERIALS AND METHOD

Animals

Experiment 1

Sixty Afrikaner (age 3 to 9 years; parity 1 to 5) and 20 Mashona (age 2 to 8 years; parity 4 to 8) cows were blocked according to calving date and allocated to a group that had continuous access totheir calves (normally suckled = NS) and a group in which the calves were weaned at 3 days of age (weaned group). The cows were drafted into these groups 3 days after calving and the groups remained intact until the last cow that calved had reached 100 days post-partum. The two treatment groups were grazed in separate paddocks. Two Mashona bulls (physically sound and showing satisfactory sperm motility) were joined with each group of cows at calving and they were rotated between treatment groups weekly.

Experiment 2

At 28 days after calving for each cow, 65 Afrikaner cows (age 4 to 10 years; parity 4 to 9) were allocated to either a normally suckled (NS) group or a partially weaned (PW) group (suckled twice daily until 42 days post-partum and once daily thereafter). When the last cow to calve had reached 100 days post-partum all calves were returned to their dams for continuous access until weaning.

Mashona bulls (two per treatment group) were included in the cow-herds from 28 days post-partum and treated as in Experiment 1.

Experiment 3

Afrikaner (n=47, age 3 to 9 years; parity 1 to 5) and Mashona (n=29, age 2 to 8 years; parity 4 to 8) cows were blocked according to calving date and randomly allocated to one of three treatment groups. One group (25 Afrikaner, 61 ± 23 days post-partum; Mashona, 74 ±15 days post-partum) was partially weaned (once daily suckling) for 35 days (21 Dec to 24 Jan) prior to the onset of the breeding period. The second group (22 Afrikaner, 59 ± 29 days post-partum; 16 Mashona, 74 ± 24 days post-partum) was partially weaned during the 39 day breeding period which commenced on 25 January. Thecontrol group (15 Afrikaner, 63 ± 27 days post-partum; 10 Mashona, 74 ± 15 days post-partum) suckled their calves throughout the trial.

Six fertility-tested Mashona bulls were run with the cows (two per group) and rotated weekly.

Observations of oestrous behaviour

Cows were monitored between 04.30 h and 18.00 h each day in Experiment 1 and between05.00 h and 19.00 h in Experiment 2. To avoid bias in the opportunity for interactive behaviour cows were not removed from the treatment groups until the last cow to calve had reached 100 days post-partum. Only data pertaining to oestrus that occurred up to 100 days post-partum were used for each cow. For each treatment group, two observers recorded oestrous related behaviour, including cows associating with bullsor other cows prior to oestrus (cow-bull and cow-cow pairing), cows mounting other cows (homosexual mounting), cows standing to be mounted by other cows (homosexual standing), cows mounting the bull (heterosexual mounting) and service by the bull. Certain of the findings have been reported previously (Wells *et al.*, 1985).

Since pairing of cows and bulls also occurs at times not related to the approach ofoestrus, chin-resting by the bull and nosing of the cow's perineum were also recorded in Experiment 2in an effort to more accurately define the transition from di-oestrus to oestrus.

Nosing of the perineum involved investigation of the cow's urino-genital area by the bull. Simultaneous cow-bull pairing was required for the investigation to be regarded as an oestrus-related component. Chin-resting in conjunction with cow-bull pairing was recorded when the bull placed his head and neck on the back of the cow, flexed his neck, simulating the beginning of a mount, without raising his feet from the ground.

In Experiment 3, due to the shortage of skilled observers andthe requirements for three observation groups, behaviour was monitored for a limited period each day. Beginning on 21 December, all cows were observed from 05.00 h until 09.00 h and again from 15.00 h to 19.00 h.

In Experiments 1 and 2 the formation of homosexual groups had not been monitored. This was included in Experiment 3, particularly as the formation of such groups may have been preceeding oestrus in the absence of mounting activity. In addition, the flehmen response was also recorded to support data onmale initiated behaviour during oestrus. In this experiment a total of nine components of oestrous-related behaviour was recorded. Homosexual behaviour, including mounting and standing to be mounted by cows was recordedas for Experiments

1 and 2. The periods between instances of homosexual mounting, when cows were associated with other cows in synchronous oestrus, were noted as well as homosexual pairing.

All components of heterosexual activity which had been recorded previously, including chin-resting, nosing of the perineum, heterosexual pairing, heterosexual mounting by cows and service were noted.

Detection of ovulation

Blood samples were collected on days 7, 14 and 21 (Experiment 1) and three times per week from day 23 (Experiment 1) or day 28 (Experiment 2) post-partum and from 30 November (Experiment 3) for assay of progesterone content as described by Wells *et al.* (1985, 1986). Based on the progesterone profiles of each cow, primary and full ovulations were defined as ones where progesterone concentrations exceeded 0.27ng/ml for minimum periods of 72 h and 11 days, respectively (Wells *et al.*, 1986).

Estimation of day of ovulation

If service was observed during a nadir in plasma progesterone concentration, that day was designated the day of ovulation. Where service was not in evidence, the day of greatest activity and longest cow-bull pairing was assumed to be the day of ovulation. Behaviour records related to luteal phase anovulatory periods (i.e. more than two days prior to an estimated day of ovulation) were not included in the analysis of oestrous behaviour.

Statistical analysis

One-way analysis of variance was employed to assess treatment effects on continuous variates such as duration of specific components of oestrous behaviour and intervals between the onset of one type of behaviour and another. Chi-square test and Fisher's exact test were used to assess treatment effects on the incidence of specific components of oestrous behaviour (Rayner, 1967). Generally, comparisons between treatments, within experiments, were limited to the same breed.

RESULTS

In the data presented in Tables 1 to 8 attention has been focused on oestrous related behaviour in cows actually seen to be served by the bull. In Figures 1 to 5 the results from ovulations at which the cows were not seen to be served were also included. Where cows were subjected to partial weaning only at the time of introduction of bulls (Expt. 3) the results recorded prior to this event for this group were combined with those for cows suckled normally.

Homosexual activity

The frequency with which cows that were subsequently seen to be served, mounted or stood to be mounted by other cows, the period over which such behaviour occurred and the interval to service for Experiments 1 and 3 are presented in Tables 1 and 2, respectively. In these tables, the term incidence depicts the number of oestrous periods at which a particular behavioural event was observed. Only 46 homosexual events were observed at the 50 full ovulations accompanied by service in Experiment 2. Consequently, attention was concentrated on heterosexual behaviour in that experiment.

TABLE 1

Characteristics of homosexual behaviour in normally suckled or weaned (3 days post partum) Afrikaner and Mashona cows

	Afrikaner		Mashona	
	Suckling treatment:			
Characteristic	Normal	Weaned	Normal	Weaned
No. in group	30	30	10	10
Full ovulations	46	56	16	20
Mounting:				
Incidence	7	9	2	5
Mean mounts/oestrus	2.1 ± 0.7	1.8 ± 0.4	1.5 ± 0.5	1.8 ± 0.2
Incidence of multiple mounts[1]	4	4	1	4
Mean hours 1st to last mount	2.4 ± 0.9	2.1 ± 0.5	-	5.0 ± 2.3
Interval (h) to service[2]	1.3 ± 1.2	1.8 ± 0.7	1.0 ± 0.7	1.0 ± 0.5
Standing:				
Incidence	7	14	3	7
Mean stands/oestrus	1.6 ± 0.3	2.0 ± 0.4	2.0 ± 0.6	2.5 ± 0.6
Incidence of multiple stands[1]	3	5	2	4
Mean hours 1st to last stand	2.5 ± 1.3	4.5 ± 1.7	1.5 ± 0.5	3.5 ± 1.5
Interval (h) to service[3]	0.2 ± 0.3	0.4 ± 0.8	+0.3 ± 1.9	2.7 ± 1.4

[1] = Number of oestrous periods during which more than one event occurred

[2] = Interval from first homosexual mount to first service

[3] = Interval from first homosexual stand to first service

TABLE 2

Homosexual activity during the breeding period in Afrikaner and Mashona cows seen to be served and either partially weaned prior to or during the breeding period or suckled normally throughout

	Afrikaner			Mashona		
	Partially weaned:					
Characteristic	During breeding	Prior to breeding	None	During breeding	Prior to breeding	None
No. in group	22	25	15	16	13	10
Full ovulations	23	15	7	17	17	3
Observed service	17	14	3	13	15	9
Mounting:						
Incidence	7^a	2^b	1^b	7	3	0
Mean mounts/ oestrus	3.7 ± 0.6	2.0 ± 1.0	3.0	2.7 ± 0.4	4.3 ± 2.8	-
Incidence of multiple mounts[1]	7^a	0^b	0^b	6^a	0^b	0^b
Mean hours first to last mount	2.0 ± 0.2	-	-	0.8 ± 0.2	-	-
Interval (h) to service[2]	2.0 ± 0.3	0.5 ± 0.1	7.0	.07 ± 0.3	2.9 ± 1.7	-
Standing:						
Incidence	7^a	1^b	0^b	8	4	0
Mean stands per oestrus	1.7 ± 0.4	2.0	-	1.9 ± 0.4	3.8 ± 2.4	-
Incidence of multiple stands[1]	3	0	0	5	2	0
Mean hours first to last stand	1.1 ± 0.3	-	-	3.9 ± 2.0	-	-
Mean hours first stand to first mount	1.2 ± 0.4	0.7	-	3.6 ± 1.8	0.2 ± 0.7	-

[a,b] Figures within the same breed and same row having different superscripts differ ($P < 0.05$)

[1] = Number of oestrous periods during which more than one event occurred

[2] = Interval from first homosexual mount to service

[3] = Interval from first homosexual stand to first service

Only 20 (Experiment 1) and 28.2% (Experiment 3) of observed services were accompanied by homosexual mounting and oestrous cows stood for other cows in only 26.9 (Experiment 1) and 28.2% (Experiment 3) of the oestrous periods. In Afrikaner cows weaning of the calves had no significant effect (Experiment 1), but partial weaning during the breeding period (Experiment 3) significantly ($P < 0.05$) increased the incidence of homosexual mounting and standing compared to cows suckled normally (Table 2). In both Afrikaner and Mashona cows the proportion of oestrous periods during which more than one homosexual mount was recorded was also increased ($P < 0.05$) by this treatment. Partial weaning prior to onset of breeding had no positive effect on the incidence or duration of homosexual behaviour (Table 3).

The frequency of occurrence of each component of oestrous-related behaviour, per full ovulation (including ovulations not accompanied by observed service), was used to describe the intensity of oestrus (Figures 1, 2, 3 and 4). The generally low level of homosexual activity in cows that suckled their calves normally is evidentfrom the data depicted in Figures 1 to 4. Oestrous-related activity was increased by total weaning (Fig. 1), partial weaning (Fig. 2) and by partial weaning prior to the onsetof the breeding period (Fig. 4). The component most affected by manipulation of suckling was observed service by bulls (Figs. 1 and 3).

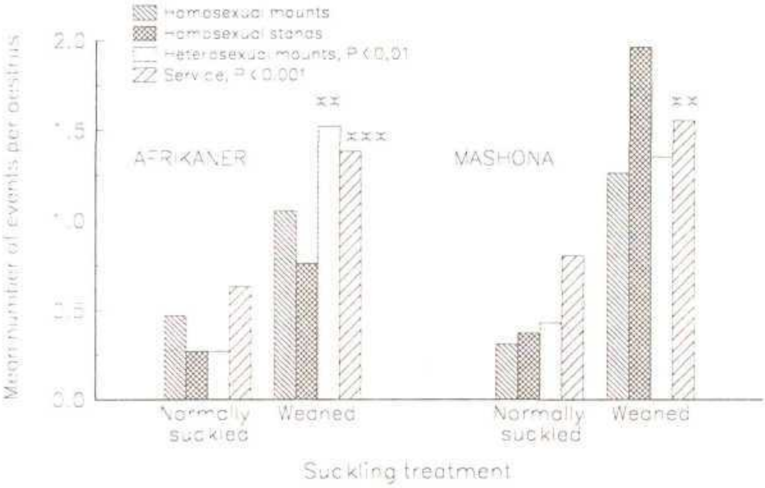

Fig. 1. The frequency of occurrence of different components of oestrus over all full ovulations in two breeds of cows either suckling their calves normally or weaned at three days of age.

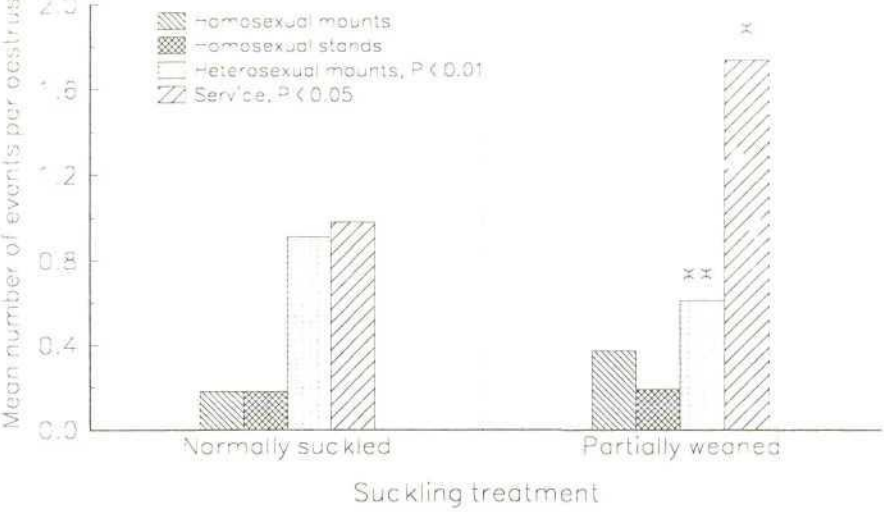

Fig. 2. Homosexual behaviour at oestrus in Afrikaner cows suckled normally or in which the calves were partially weaned.

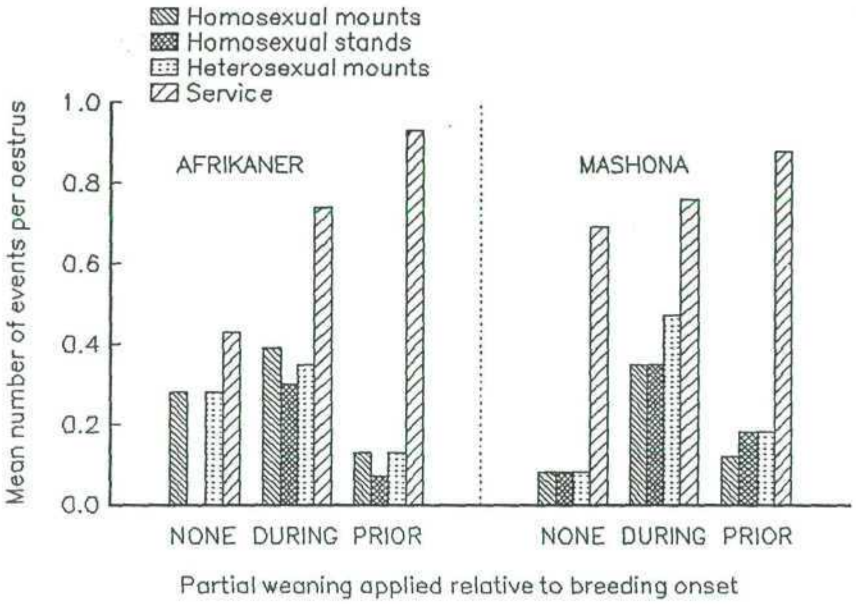

Fig. 3. Oestrous-related behaviour during the breeding period in normally suckled cows and in cows partially weaned either prior to or during the breeding period.

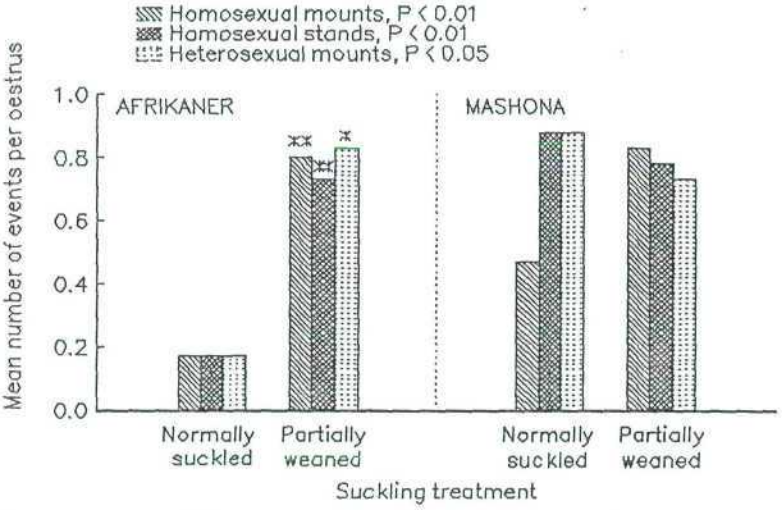

Fig. 4. Influence of suckling treatment on homosexual behaviour during the 35 days prior to the breeding period in Afrikaner and Mashona cows which suckled their calves normally or where the calves were partially weaned.

During the 35-day period prior to initiation of breeding, when bulls were absent in Experiment 3, partial weaning significantly increased the incidence of homosexual pairing, mounting and standing in Afrikaner cows (Table 3; Fig. 4) as compared to cows which suckled normally during this time. The homosexual activity of the Mashona and Afrikaner cows suckling once daily during this non-breeding period was similar (Fig. 4), but the activity was much lower among Afrikaner cows that suckled their calves normally.

The generally low level of homosexual activity in cows which suckled their calves normally is evident from the data depicted in Figures 1, 2, 3 and 4.

TABLE 3

Homosexual behaviour in Afrikaner and Mashona cows during the 35 day period prior to onset of the breeding period

Characteristic	Afrikaner		Mashona	
	Suckling treatment:-			
	Normal	Once daily	Normal	Once daily
No. in group	37	25	26	13
Full ovulations	12	15	36	23
Pairing:				
Incidence	2^{c}	12^{d}	17^{c}	19^{d}
Mean duration (h)	2.25 ± 0	1.9 ± 0.4	1.5 ± 0.2	1.3 ± 0.2
Mounting:				
Incidence	2^{c}	11^{d}	15^{e}	18^{f}
Mean mounts per oestrus	12.5 ± 1.2^{a}	4.7 ± 0.5^{b}	6.3 ± 1.5	4.0 ± 1.4
Standing:				
Incidence	2^{e}	10^{f}	15^{e}	17^{f}
Mean stands per oestrus	8.5 ± 1.0	9.7 ± 2.4	6.6 ± 0.7	6.7 ± 1.0

Means within the same column, within a breed having different superscripts differ

^{a,b} $P < 0.001$

^{c,d} $P < 0.01$

^{e,f} $P < 0.05$

Heterosexual behaviour

Cows mounting the bull

Apart from service, heterosexual mounting by the cow was the most common component of oestrous behaviour observed, but occurred in less than 40% of the oestrous periods seen to be accompanied by service (Table 4). Neither complete or partial weaning consistently increased the number of oestrous periods at which mounting of the bull was seen. In Experiment 3, partial weaning, when applied at the start of the breeding period increased the incidence of cows mounting the bull by 27% ($P < 0.05$) in Mashona cows and by50% ($P < 0.10$) in Afrikaner cows (Table 4).

TABLE 4

Mounting of bulls by Afrikaner and Mashona cows in oestrus (heterosexual behaviour) as influenced by suckling treatment

Expt	Breed	Suckling treatment	Incidence of mounting bull	Mean mounts/oestrus	Oestrous periods with > 1 mount	Hours 1st to last mount	Hours 1st mount to service
1	Afrikaner	Normal	7^a	1.3 ± 0.2	2	5.8 ± 4.8	4.5 ± 2.2
		Weaned	25^b	1.8 ± 0.3	12	2.8 ± 0.4	2.9 ± 0.5
	Mashona	Normal	4	1.8 ± 0.2	3	1.3 ± 0.4	2.1 ± 1.0
		Weaned	12	1.7 ± 0.4	4	5.1 ± 2.2	3.3 ± 1.2
2	Afrikaner	Normal	8	1.9 ± 0.5	7	5.8 ± 2.9	1.9 ± 1.5
		1x daily	11	1.8 ± 0.4	15	1.9 ± 1.5	1.7 ± 0.9
3	Afrikaner	Normal	2	1.5 ± 0.5	1	10.7	8.8 ± 2.0
		1x daily with breeding	7	3.0 ± 1.2	5^a	1.7 ± 0.5	1.4 ± 0.5
		1x daily before breeding	2	1.0 ± 0	0^b	-	0.4 ± 0.3
	Mashona	Normal	1	1.0	0	-	0.8
		1x daily with breeding	8^a	2.6 ± 0.7	7	0.8 ± 0.3	0.5 ± 0.4
		1x daily before breeding	3^b	1.3 ± 0.3	1	0.1	5.8 ± 2.4

^{a,b} Figures within the same column for the same breed with different superscripts differ ($P < 0.05$)

Activity initiated by bulls

Chin-resting

The component of oestrus, initiated by bulls, that was most frequently recorded in association with service was chin-resting by bulls (82 and 90% of oestrous periods in Experiments 2 and 3, respectively; Table 5).

Nosing of the perineum

Surprisingly, nosing of the perineum occurred only once in as many as 31% (Experiment 2) and 21% (Experiment 3) of the oestrous periods. In Experiment 2 partial weaning did not increase the incidence (Table 5), but Afrikaner cows that suckled their calves once daily prior to onset of the breeding period stimulated a greater incidence ($P < 0.05$) of nosing by the bulls than where this treatment was delayed by 35 days (Table 5). The opposite effect occurred in the Mashona cows ($P < 0.01$).

TABLE 5

Bull-initiated mating behaviour around the time of oestrus in Afrikaner and Mashona cows

Characteristic	Experiment 2		Experiment 3					
	Afrikaner		Afrikaner			Mashona		
			Partial weaning :-					
	None	From day 28 post partum	With breeding	Prior to breeding	None	With breeding	Prior to breeding	None
Chin-resting:								
Incidence	14	27	15	13	2	13	14	7
Mean number per oestrus	6.5 ± 1.3	5.5 ± 0.9	8.0 ± 1.4	5.9 ± 1.5	10.0 ± 5.7	8.1 ± 1.9	8.2 ± 1.6	5.3 ± 1.3
Hours from 1st to last chin-rest	8.6 ± 1.3	6.8 ± 0.9	3.5 ± 1.0^{a}	5.5 ± 1.5^{a}	10.6 ± 1.3^{b}	5.5 ± 1.3^{a}	5.2 ± 1.3^{ab}	2.5 ± 1.3^{b}
Hours from 1st chin-rest to first service	4.2 ± 1.2	3.2 ± 0.7	3.2 ± 1.0^{a}	3.1 ± 1.3^{a}	10.1 ± 1.4^{b}	3.9 ± 1.4	4.1 ± 1.2	3.8 ± 4.0
Nosing of perineum:								
Incidence	10	25	7^{c}	11^{d}	2	13^{a}	8^{b}	6
Mean number per oestrus	3.1 ± 0.5	2.7 ± 0.3	2.9 0.8	3.4 ± 1.0	5.0 ± 3.0	2.6 ± 0.4^{a}	6.5 ± 1.1^{b}	5.7 ± 0.7^{b}
Mean duration (h) of multiple nosings	8.3 ± 1.2	7.0 ± 0.9	5.3 ± 2.5^{a}	7.8 ± 1.7	10.8 ± 1.8^{b}	4.5 ± 1.3	6.3 ± 3.9	4.7 ±1.5
Hours from 1st nosing to first service	5.4 ± 1.2^{a}	2.4 ± 1.0^{b}	4.5 ± 2.0	4.0 ± 1.7^{a}	9.9 ± 1.4^{b}	3.8 ± 1.4	7.0 ± 1.7	4.4 ± 1.6
Flehmen:								
Incidence			5	9	2	6	5	4
Mean number per oestrus			1.2 ± 0.2	1.7 ± 0.3	1.5 ± 0.5	1.3 ± 0.2^{a}	2.8 ± 0.5^{b}	2.3 ± 0.8^{ab}
Mean duration (h) of multiple flehmen			1.8*	3.9 ± 3.6	11.4*	1.2 ± 0.6	3.8 ± 2.1	1.6 ± 0.5
Mean hours 1st flehmen to service			5.1 ± 2.7	2.2 ± 2.5^{a}	9.8 ± 1.4^{b}	3.7 ± 2.3	6.8 ± 2.0^{a}	1.2 ± 0.8^{b}

Figures within the same row for the same breed with different superscripts differ
^{a,b} $P < 0.01$ ^{c,d} $P < 0.05$ * $n = 1$

Flehmen

No significant treatment effects were recorded (Table 5), although a higher incidence was again observed in Afrikane cows partially weaned before joining with bulls. The number of events per oestrus was surprisingly low (Table 5).

Service

Amongst the cows suckled normally (breeds pooled) in Experiments 1, 2 and 3 only 53.2, 53.3 and 60.0%, of the respective full ovulations were seen to be accompanied by service (Table 6). Both complete and partial weaning of the calves increased the proportion of ovulations at which service was observed. Thus, in Experiment 1 significantly more cows were seen to be served at both the primary ($P < 0.01$) and at subsequent full ($P < 0.05$) ovulations (Figure 5). Similarly, in Experiment 2, partial weaning increased the incidence of observed service over all full ovulations ($P < 0.01$) and at conception ($P < 0.05$; Figure 5).

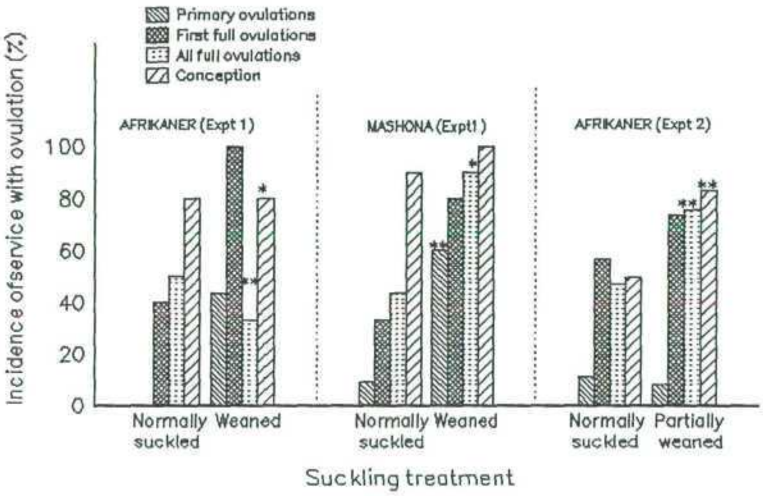

Fig. 5. The incidence of observed service at primary, first full, at all full ovulations and at conception, as influenced by suckling treatment. * $P < 0.05$; ** $P < 0.01$.

Partial weaning before bulls were introduced (Experiment 3) also increased ($P < 0.05$) the proportion of full ovulations (93.3%) and conceptions (100%) associated with observed service (Table 6) compared to Afrikaner cows suckled throughout the experiment (42.8%). This positive response was absent when the cows were partially weaned at the time of joining with bulls (Table 6).

TABLE 6

Characteristics of observed service in Afrikaner and Mashona cows as influenced by suckling treatment (PW = Partial weaning)

Experiment	Breed	Suckling treatment	No. in group	Full ovulations	Observed service with oestrus	Mean services per oestrus	Incidence of multiple services	Duration (h) of multiple services	Confirmed conceptions	Observed conceptions
1	Afrikaner	Normal	30	46	23^a	0.6 ±	8	2.0 ± 0.4	15^c	11
		Weaned	30	56	50^b	1.2 ±	23	2.4 ± 0.5	24^d	20
	Mashona	Normal	10	16	10^c	0.9 ±	5	2.0 ± 1.0	9	6
		Weaned	10	21	21^d	1.1 ±	4	0.9 ± 0.2	10	10
2	Afrikaner	Normal	32	30	16	1.1 ±	7	2.0 ± 0.7	16^a	11
		PW	33	42	34	1.5 ±	15	2.7 ± 0.8	30^b	25
3	Afrikaner	Normal	22	23	17	1.5 ± 0.2	10	0.8 ± 0.2	13	10
		PW before breeding	25	15	14c	1.3 ± 0.2	3	0.6 ± 0.3	9	9c
		PW during breeding	15	7	3^d	2.0 ±0.6	2	1.1 ± 0.6	7	3^d
	Mashona	Normal	16	17	13	2.1 ± 0.2	9^c	1.2 ± 0.4	15	10
		PW before breeding	13	17	15	1.3 ± 0.2	4^d	1.2 ± 0.6	11	10
		PW during breeding	10	13	9	1.2 ± 0.2	1	2.2 ± 0.0	9	5

[a,b; c,d] Figures within the same column for the same breed and for the same experiment with different superscripts differ

[a,b] $P < 0.001$ [c,d] $P < 0.05$

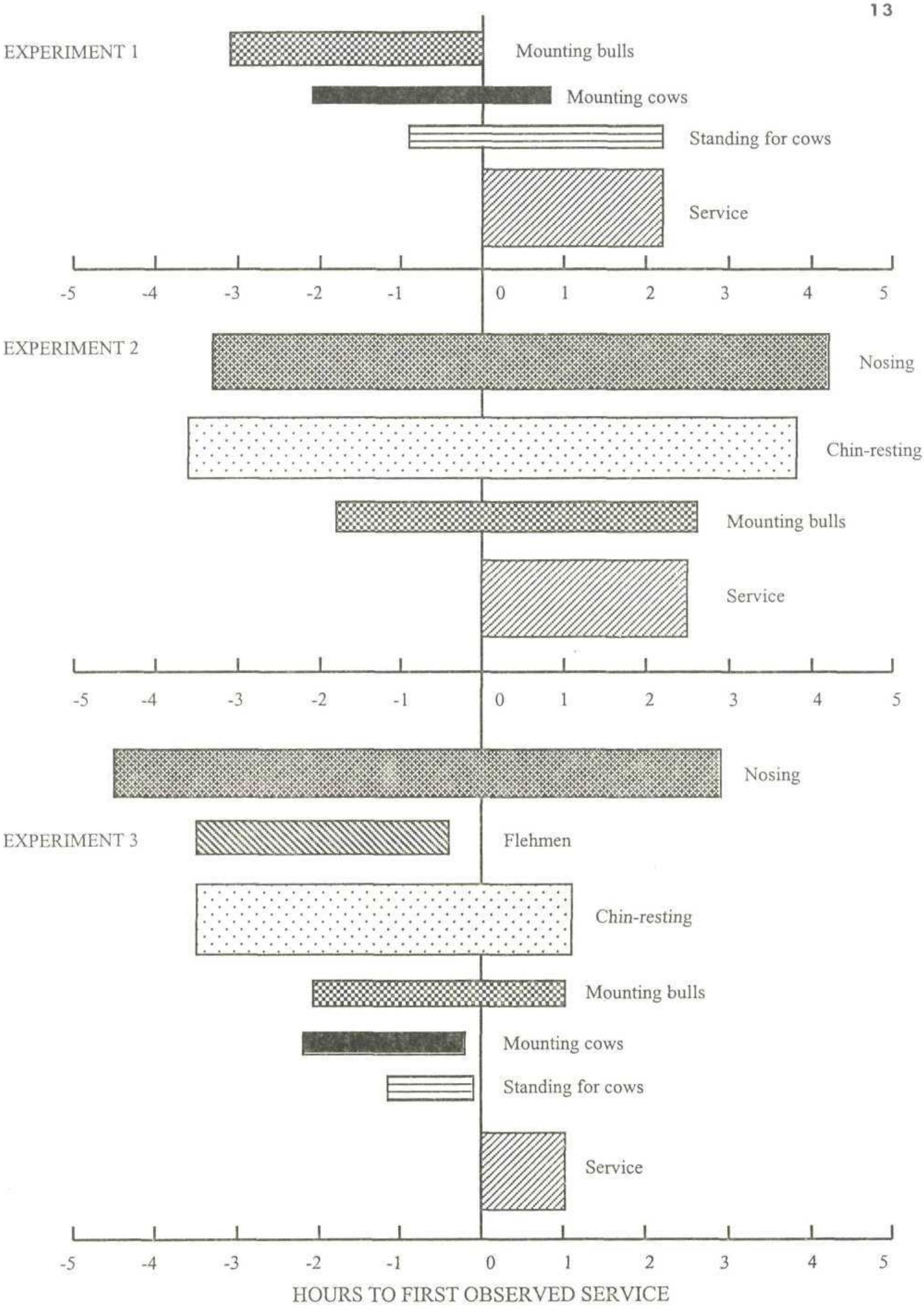

Fig. 6. Summary of the incidence, time of onset and duration of each component of oestrous behaviour. The height of each bar indicates the proportion of the cows in which a component was observed, relative to cows served.

The proportion of oestrous periods at which cows were served more than once was not increased by complete (suckled cows = 8/21, weaned cows = 26/60) or partial (suckled cows = 7/21, PW cows = 15/31) weaning of calves in Experiments 1 and 2. However, partial weaning during the breeding period (Experiment 3) increased ($P < 0.01$) the incidence of multiple services from 24% in cows PW before bulls were joined to 63%. This response was significant only for the Mashona cows.

General pattern of oestrus

The temporal relationship between the components of oestrus recorded and analysed for both breeds in detail is illustrated in Fig. 6. The relatively major role played by bull-initiated behaviour is evident from Fi. 6. Although the pattern was not exactly duplicated in the three experiments, usually the approach of first service was initiated by bull-initiated behaviour (nosing of the perineum, chin resting and flemen). These events preceded service by no more than 5h, on the average. In Experiment 1 homosexual mounting was initiated prior to homosexual standing (Figure 6) whereas in Experiment 3 the reverse occurred. The low level of homosexual behaviour in Experiment 2 was not analysed.

Multiple services during a single oestrus occurred in only 28.8% of the oestrous periods and on average cows were served over an interval lasting no more than 2.5 h. Mounting of the bull ceased before (Experiment 3) or at the time of (Experiment 1) of first service, while in Experiment 2 heterosexual mounting by the cows continued even after the last service.

Bulls continued to display interest in oestrous cows for up to 2h after the last service (Fig. 6), but this attention did not continue after last service in Experiment 3 (Fig. 6).

DISCUSSION

Characteristics of oestrus

Two striking features of oestrus in the Afrikaner cow were observed in this study. These were, firstly, the low incidence of interactive behaviour between cows (Tables 1, 2 and 3) and between cows and bulls prior to service (Table 4) and secondly, the short duration (3 to 4 h) of heterosexual activity (Tables 4 and 5). Of a total of 84 ovulations where service was seen amongst Afrikaner cows in Experiment 1, 34 (41%) were not accompanied by any form of interactive behaviour other than service and on 47% of these occasions only one service was seen. Sixty four percent of the remaining 50 ovulations included only one additional form of interactive behaviour. This agrees with observations in Boran cattle where Llewelyn *et al.* (1987) noted that only 27% of the cows were detected by mounting behaviour and di-oestrous cows did not readily participate in the sexually active group. Galina *et al.* (1982) and Gutierrez *et al.* (1993) have also reported a low incidence of homosexual standing during oestrus in Zebu cows. Based on the findings of Hurnik *et al.* (1975), it is reasonable to assume that the small number of cows in oestrus on any given day probably contributed to the low level of sexual activity seen in the present study. the low level of sexual activity

In spite of the variation in timing of courtship activities depicted in Fig. 6, the results support the finding that oestrous cows are ridden by herdmates several hours before being mounted by bulls (Kilgour *et al.*, 1977). The duration of oestrus was no longer than 4 h which is shorter than the result obtained by others for *Bos indicus* cows (Anderson, 1936; Quinlan *et al.*, 1941; De Alba *et al.*, 1961; Plasse *et al.*, 1970). Oestrus in the Afrikaner and Mashona cow, and possibly many *Bos indicus* breeds, thus appears to be a subtle affair with a minimum of unnecessary activity during oestrus. This is supported by the reports of a low intensity oestrus in Brahman (Randel, 1983), Boran (Llewelyn *et al.*, 1987) and White Fulani (Johnson and Gambo, 1979) cows. These findings contrast sharply with reports on dairy cows where oestrus lasted 14 h and cows were mounted more than 50 times (Esselemont and Bryant, 1976).

The daytime temperatures and small number of lactating cows likely to be oestrus at the same time undoubtedly played a role since it is known that expression of oestrus is affected by temperature (Gangwar *et al.*, 1965; Bond and McDowell, 1972) and the number of animals in oestrus (Hurnik *et al.*, 1975; Esselemont *et al.*, 1980; Glencross et al., 1981).

Definition of oestrus

In view of the foregoing, the classic definition of oestrus as being the **period** when cows will stand to be mounted is inadequate for three reasons viz., (i) with treatment groups of no more than 40 cows and with such cows in various stages of prolonged post-partum anoestrus, the possibility of two or more cows being in standing oestrus at the same time was low, (ii) the number of services per oestrus was usually two or less and (iii) homosexual activity occurred in less than 20% of ovulations. Although homo- and heterosexual mounting remain the only conclusive signs of oestrus in the Afrikaner, their low incidence (Figs. 1,2,3 and 4) makes consistent detection of oestrus, using these as guide in the absence of bulls, impossible. Furthermore, a precise definition of oestrus, under free-mating conditions, is unlikely because of the wide variation in patterns of behaviour between animals.

The question then arises of how to define oestrus in the Afrikaner cow since in many instances it appears to be an **event** rather than a period. At the same time, it should be borne in mind that in this study the focus was on ovulations which were associated with at least one observed service. This excluded instances in which cows may have been served at night or not at all. Based on the subsequent calving records 15, 28 and 26% of the pregnancies resulted from service which occurred outside the surveillance period in Experiments 1, 2 and 3, respectively. Clearly, a less specific definition than the classical one is required and observed service remains the only consistently reliable indicator of oestrus in the Afrikaner cow.

Signs of impending oestrus

One of the principal objectives of Experiment 2, and to a lesser extent Experiment 3, was to record the changes in behaviour with the transition from pro-oestrus to oestrus in cows. This was expected to be accompanied by a measurable increase in oestrus-related behaviour. An increase in mounting activity at the transitional stage has been observed in dairy cows (Kilgour *et al.*, 1977; Esselemont *at al.*, 1980;

Glencross *et al.*, 1981; Helmer and Brit, 1985).

Guastafson *et al.* (1986) have described the passage from pro-oestrus to oestrus as the time when cows spontaneously lowered their backs in response to the advances of a teaser bull, without standing to be mounted.

In the reports referred to above there were obviously more than two homosexual mounts per oestrus. Thus, a marked change in mounting rate could be used as a reasonable estimate of the onset of oestrus. In the Afrikaner and Mashona cows however, this was not the case (Table 8, Tables 8 and 9). Clearly, homosexual behaviour was an unreliable indicator of oestrus in these breeds.

With large variations in patterns of behaviour observed in the oestrous periods of Afrikaner cows in this study, unambiguous evidence of oestrus did not exist. The results obtained generally concur with those of Kilgour and Dalton (1983) who reported that cows will mount other cows, prior to standing to be mounted themselves. However, there was no fixed progression of one form of behaviour to the next, with the approach of time of service. Rather, service was most likely to be the last form of interactive behaviour, but it may be preceded by any of the other forms of social behaviour. In certain case service was followed by such other forms of oestrous behaviour.

Chin-resting and nosing of the perineum figured prominently in oestrus in the Afrikaner cows, being the only two components of oestrus that had a high probability of being observed in every oestrus. However, these behavioural characteristics were not clearly related to the peak of oestrus (service) and also featured prominently in pro-oestrus. Nosing of the perineum also occurred in cows not in oestrus. Although these two behavioural characteristics of the bull were seen to be directed at cows up to 4 days before oestrus, they undoubtedly represented investigatory activity, not specifically related to oestrus and were thus too imprecise to indicate a definite transition to oestrus.

Causes of conception failure

In Afrikaner cows that suckled their calves normally, service was observed in only about 50% of the full ovulations (Table 4) and this figure was supported by subsequent calvings without service having been observed. This suggested that in as many as half the number of full ovulations, service occurred outside the surveillance period and was unlikely to have been missed during daylight hours. In Afrikaner cows where the calf was permanently or partially weaned, 80% of the conceptions were associated with an observed service (Table 4). These results do not support the conclusion that low conception rates at early full ovulations (Wells *et al., 1985, 1986)* are due to cows failing to stand for service. These findings imply that conception rates to ovulations around 30 days post partum might be limited by uterine and/or endocrine factors rather than by behavioural deficiencies. Whether the low incidence of homosexual activity limited the ability of bulls to locate oestrous cows (Blockey, 1976, 1979) remains open to speculation.

It is tempting to attribute the change in preferred mating time (Wells *et al.*, 1985; Table 4 ,Figures 4 and 5) to the absence of the calf during daylight hours. However, this explanation does not account for high incidence (93%) of observed service in cows partially weaned only before onset of the breeding period (Table 4). The data available also does not indicate whether the time of oestrus and of ovulation were both shifted or only the time of peak oestrus. In addition, no cognisance has been given to possible preferred mating times in bulls. It is common knowledge amongst farmers that *Bos indicus*, particularly Brahman bulls, seldom mate during daylight hours.

Increasing the intensity of oestrus

In addition to a change in the time of day when service occurred, limited suckling also enhanced the intensity of expression of oestrus (Figures 1, 3, 4 and 5) and attractiveness of the cow to the bull (Table 5). Removal of the suckling stimulus increased the incidence of all components of oestrus-related activity (Figures 1) and increased the intensity of oestrus at the resumption of ovarian activity to a point where cows would stand to be mounted (Experiment 1). Thus, while 50% of the primary ovulations in weaned cows were accompanied by service, this did not occur during the surveillance period at any of the 21 primary ovulations in normally suckled cows. Apparently, reduction in the frequency of suckling from an estimated 3.8 times per 24 h to once per 24 h in Experiment 2 was not a sufficiently strong stimulus to promote service at a primary ovulation (Figure 5). At full ovulation such limitation in suckling frequency heightened the chances of observing service (Figure 5) and may have increased the intensity of oestrus slightly (Table 10). In Experiment 3 partial weaning enhanced the expression of homosexual behaviour (Figures 5), but only as long as the calves were not returned to normal suckling. It appears that PW *per se* enhanced the expression of homosexual behaviour during oestrous. In contrast, bulls appeared to be stimulated more by Afrikaner cows weaned 35 days earlier (service seen in 93% of the ovulations) than cows weaned at joining with bulls (73.9% of ovulations accompanied by service). This was in spite of a greater stimulation of heterosexual mounting in cows PW at the onset of breeding (Table 4) which should have, in turn, aroused interest by the bulls.

PW and NS cows in Experiment 2 spent about the same amount of time with the bull, yet limited suckling induced more chin-resting (Table 5). Thus, cows which had limited access to their calves appeared to stimulate more interest by the bulls. The similarity in frequency of nosing of the perineum in NS and PW cows (Table 5) suggested that perhaps the increased bull interest in PW cows was not due to an augmentation in pheromone production. The production of substances which attract bulls at oestrus (pheromones) has been demonstrated (Hart *et al.*, 1946; Hradecky *et al.*, 1983; Garcia *et al.*, 1986; Denhard *et al.*, 1991). An alternative explanation is that the behaviour of the PW cows was more arousing (immobility of the cow being solicited) than that of NS cows. There also appeared to be a breed difference as regards attractiveness of cows in oestrus. In Experiment 1, weaned Mashona cows in oestrus were mounted more frequently by herd-mates (probably not in oestrus) than NS cows (Figure 1). Since both breeds were run in the same herd the results indicate that the Afrikaner cows in oestrus mounted Mashona cows in oestrus in preference to other Afrikaner cows. Galina *et al.* (1982) have reported that whereas Brahman cows rarely mounted Charolais cows almost 40% of the mounts seen in Brahmans were by Charolais cows.

The results in Table 5 regarding chin-resting appear to contradict Figure 5. However, the former relates only to heats in which service was seen whereas the latter refers to all oestrous periods, even where no service was recorded. The apparent contradiction does demonstrate that in PW cows there was a high incidence of chin-resting on days preceding oestrus and on days when service was not noticed. This supports the conclusion that PW facilitates the stimulus that induces bulls to chin-rest.

Presence of bulls

When Afrikaner and Mashona cows were joined with bulls, after having been suckled only once daily for 35 days, the incidence of homosexual mounting declined from a mean of 7.3 to one of 3.3. This agrees with the results of Orihuela *et al.* (1988) who found that more riding activity occurred at night when bulls were absent. Their conclusion does however not take account of the possible influence of cooler night-time temperatures. Arave and Albright (1980) have also reported that presence of the bull inhibits homosexual mounting.

REFERENCES

Anderson, J., 1936. Studies on reproduction in cattle. II. The influence of environmental factors on reproduction. Emp. J. Exp. Agric., 4: 197-209.

Arave, C.W. and Albright, J.L., 1981. Cattle behaviour. J. Dairy Sci., 64: 1318-1329.

Blockey, M.A., 1976. Sexual behaviour of bulls at pasture. Theriogenology, 6: 387-392.

Blockey, M.A., 1979. Sheep and cattle mating behaviour. Rev. Rural Sci., 4: 53-62.

Bond, J. and McDowell, R.E., 1972. Reproductive performance and physiological responses of beef females as affected by a prolonged high environmental temperature. J. Anim. Sci., 35: 820-829.

Boulle, J.P., 1986. A survey of management factors affecting production in Natal beef breeding herds. M.Sc. Thesis. University of Natal.

DeAlba, J., Villa Corta, E. and Ulloa, G., 1961. Influence of natural service on length of oestrus in the cow. Anim. Prod., 3: 327-330.

Denhard, M., Claus, R., Pfeiffer, S. and Schopper, D., 1991. Variation in estrus-related odors in the cow and its dependency on the ovary. Theriogenology, 35: 645-652.

Esselmont, R.J. and Bryant, M.J., 1976. Oestrous behaviour in a herd of dairy cows. Vet. Rec. 99: 472-475.

Esselmont, R.J., Glencross, R.G. Bryant, M.J. and Pope, G.S., 1980. A quantitative study of pre-ovulatory behaviour in cattle (British Friesian heifers). Appl. Anim. Ethol. 6: 1-17.

Galina, G.S. and Arthur, G.H., 1990. Review on cattle reproduction in the tropics. Part 4. Oestrous cycles. Anim. Breed. Abstr. 58: 697-707.

Galina, G.S., Calderon, A. and McCloskey, M., 1982. Detection of signs of estrus in the Charolais cow and its Brahman cross under continuous observation. Theriogenology, 17: 485-499.

Gangwar, P.C., Branton, C. and Evans, D.L., 1965. Reproductive and physiological responses of Holstein heifers to controlled and natural climatic conditions. J. Dairy Sci., 48: 222-227.

Glencross, R.G., Esselmont, R.G., Bryant, M.J. and Pope, G.S., 1981. Relationships between the incidence of pre-ovulatory behaviour and the concentrations of estradiol. Appl. Anim. Ethol., 7: 141-148.

Gutierrez, C., Galina, C.S. and Rubio, I., 1993. The influence of social structure of a Zebu herd on the manifestation of signs of oestrus. Wld. Rev. Anim. Prod., 28: 58-70.

Gustafsson, H., Larsson, K., Kindahl, H. and Madej, A., 1986. Sequential endocrine changes and behaviour during oestrus and metoestrus in repeat breeder and virgin heifers. Anim. Reprod. Sci., 10: 261-273.

Hart, G.H., Mead, S.W. and Regan, W.M., 1946. Stimulating the sex drive of bovine males in artificial insemination. Endocrinology, 39: 221-223.

Hradecky, P., Sis, R.F. and Klemm, W.R., 1983. Distribution of flehmen reactions of the bull throughout the bovine estrous cycle. Theriogenology, 20: 197-204.

Helmer, S.D. and Britt, J.H., 1985. Mounting behaviour as affected by stage of estrous cycle in Holstein heifers. J. Dairy Sci., 68: 1290-1296.

Holness, D.H., Hopley, J.D. and Hale, D.H., 1978. The effects of plane of nutrition, liveweight, temporary calf weaning and breed on the occurrence of oestrus in beef cows during the post-partum period. Anim. Prod., 26: 47-54.

Hurnik, J.F., King, G.J. and Robertson, H.A., 1975. Estrus and related behaviour in postpartum Holstein cows. Appl. Anim. Ethol., 2: 55-68.

Johnson, A.O. and Gambo, A., 1979. Oestrous behaviour in Burnaji (White Fulani) heifers. J. Agric. Sci., 92: 689-694.

Kilgour, R. and Dalton, C., 1984. Livestock Behaviour. A practical guide. Granada. London.

Kilgour, R., Skarsholt, B.H., Smith, J.F., Bremmer, K.J. and Morrison. M.C.L., 1977. Observations on the behaviour and factors influencing the sexually active group in cattle. Proc. N. Z. Soc. Anim. Prod., 37: 128-135.

Llewelyn, C.A., Munro, C.D., Luckins, A.G., Jordt, T., Murray, M. and Lorenzini, E., 1987. Behavioural and ovarian changes during the oestrous cycle in the Boran (*Bos indicus*). Brit. Vet. J., 143: 75-82.

Orihuela, A., Galina, C.S., Escobar, F.J. and Riquelme, E., 1983. Estrous behaviour following prostaglandin $F_{2?}$ injection in Zebu cattle under continuous observation. Theriogenology, 19: 795-808.

Plasse, D., Warnick, A.C. and Koger, M., 1970. Reproductive behaviour of *Bos indicus* females in a sub-tropical environment. IV. Length of estrous cycle, duration of estrus, time of ovulation, fertilisation and embryo survival in grade Brahman heifers. J. Anim. Sci., 30: 63-72.

Quinlan, J., Bisschop, J.H.R. and Adelaar, T.F., 1941. Bionomic studies on cattle in the semi-arid regions of the Union of South Africa. IV. The ovarian cycle of heifers during summer. Onderstepoort J. Vet. Sci., 16: 213-239.

Randel, R.D., 1981. Effect of once-daily suckling on postpartum interval and cow-calf performance of first calf Brahman X Hereford heifers. J. Anim. Sci., 53: 755-757.

Randel, R.D., 1983. Reproductive endocrinology of Brahman cattle (1). Presented to "Reproduction des ruminants en zone tropicale". 8 - 10 June 1983. INRA, 1984.

Rayner, A.A., 1967. A first course in Biometry for agriculture students. University of Natal Press. Pietermaritzburg. South Africa.

Symington, R.B. and Hale, D.H., 1967. Stimulation of sexual activity in ranch cows. Proc. S. Afr. Soc. Anim. Prod., 6: 181-184.

Wells, P.L., Holness, D.H., Freymark, P.J., McCabe, C.T. and Lishman, A.W., 1985. Fertility in the Afrikaner cow. 2. Ovarian recovery and conception in suckled and non-suckled cows *post partum*. Anim. Reprod. Sci., 8: 315-326.

Wells, P.L., Holness, D.H., McCabe, C.T. and Lishman, A.W., 1985. Fertility in the Afrikaner cow. 3. Once daily suckling and its effect on the pattern of resumption of ovarian activity and conception rate in early lactation. Anim. Reprod. Sci., 12: 1-12.

SUCKLING BEHAVIOUR DURING DAYLIGHT IN AFRIKANER, MASHONA AND HEREFORD COWS

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ABSTRACT

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In an attempt to explain differences in reproductive performance of Afrikaner, Mashona and Hereford cows five cow-calf pairs of each breed were studied at three stages of the suckling period. Hereford cows and calves were included for comparative purposes. Attention was focussed on udder stimulation by the calf and included teat changes and udder butting during each suckling bout. Observations were made only during daylight hours (05.00h to 19.00h).

Removal of milk during suckling occurred in two distinct phases viz., an extraction and a stripping phase, with the transition being marked by a rapid increase in the rate of teat changes. All components of suckling behaviour varied widely within breeds. The stripping phase lasted about 50% longer than the extraction phase (8.5 vs 5.9 mins), but the 135 □ 4 teat changes occured during the stripping phase compared to a mean of only 24 □ 2 changes during the extraction phase. Afrikaner and Mashona calves showed more teat changes during the stripping phase than Hereford calves and for the Afrikaner more udder butts occurred ($P < 0.05$).

No consistent trend in behavioural changes were evident as the age of the calves increased and no significant breed differences occurred in the proportion of calves suckling from one to six times each day.

Since virtually the same effort was exerted by calves at each suckling bout, the stimulation (stripping phase teat changes and udder butts) experienced by the cows depended more ($P < 0.001$) on the number of suckling bouts per day rather than on variation in individual patterns.

In a second experiment an attempt was made to find an association between milk yield of the cows and suckling behaviour of the calves. When milk intake varied between 0 and 4.2 kg at the observation following overnight starvation, the duration of suckling increased ($P < 0.01$) only when milk intake rose above 1kg. Significant increases in time between teat changes ($P < 0.01$) and the duration ($P < 0.01$) of the extraction phase increased as milk intake increased. Components of suckling behaviour during the stripping phase did not change significantly with change in milk consumption.

Afrikaner and Mashona cows appear to differ little in their patterns of suckling activity.

INTRODUCTION

In contrast to the Afrikaner cow, with its low (60%) calving rate (Wells *et al.*, 1985, 1986) and strong maternal instincts (aggressive defence of new-born calf), the Mashona is more fertile (Ward and Dlodlo, 1985; Wells, 1986) and yet exhibits the same strong bonding with its calf. In the absence of suckling (calf weaned at three days of age), the mean post-partum interval to conception and the conception rate did not differ significantly between these breeds (Wells, 1986). This would suggest that the temporal control of ovarian activity in these two breeds is similar, but that in the Afrikaner cow suckling prolongs post-partum anoestrus to a greater degree than in the Mashona. This promted the question of whether or not suckling behaviour differed between these two breeds and whether this would explain the difference in duration of anoestrus?

Whilst there is a paucity of studies that relate suckling behaviour to reproductive function in beef cows, the behaviour patterns have been described for both Bos taurus (Drewry *et al.*, 1959; Odde *et al.* (1985) and Bos indicus (Hutchinson *et al.*, 1962; Reinhardt and Reinhart, 1981) cows and calves. Since in some studies lactation in beefs cow peaked four weeks post-partum (Cole and Johansson, 1933; Heyns, 1960a; Kress & Anderson, 1974) a change in frequency of suckling per 24 hours might be expected with increasing age of calf. Whilst Hutchinson *et al.* (1962), Ewbank (1969) and Reinhardt and Reinhardt (1981) observed a reduction in suckling frequency as calf age increased, Walker (1962) and Somerville & Lowman (1979) could detect no such relationship. The contradiction may be explained by the finding (Odde *et al.*, 1985) that milk production of the cow and live weight of the calf were related to suckling incidence whereas age of the calf was not. They proposed that high milk production increased the interval to the next nursing, provided that the calf had the capacity to drink the milk available at each suckling bout. The milk yield of the cows may thus have differed in the studies mentioned.

None of the published works available contained any reference to the specific activities of the calf during each suckling bout. The primary objective of this study was to observe, in detail, levels of udder stimulation, specifically teat changes and butting of the udder during each suckling bout. It was hoped that such information would explain the difference in reproductive performance between the Afrikaner and Mashona cow. From published results it appeared that the duration of suckling bouts and frequency of suckling during a 24 hour period were not likely to differ sufficiently between Afrikaner and Mashona cows to account for the marked breed difference in calving rate. However, the possibility that calf activity during each suckling bout might be related to breed of cow and also to milk yield of the dam needed investigation and formed the second objective of this study.

MATERIALS AND METHOD

Experiment 1

A total of 15 mature (range from 3 to 9 years), multiparous (range 1 to 5 calves) cows that calved within a seven-day period were utilised for this study and comprised five cow-calf pairs from each of three breeds viz. Afrikaner, Mashona and Hereford. With one observer per cow-calf pair able to compile detailed records only limited numbers could be accommodated. Hereford cows were included in the study to provide an indication of suckling behaviour in a *Bos taurus* breed under similar conditions. Calving dates were closely matched for the Afrikaner and Mashona cows, but the Herefords had calved a month earlier and their data are included essentially for comparative purposes only.

Behaviour records were compiled for observations made between the hours of 0500 and 1900h for each day on which observations were made (observation day). The cows were grazed (fertilized *Cynodon nlemfluensis*) over an 8 hectare area, sub-divided into 2 hectare paddocks. Cows were supplemented with maize meal and cotton seed meal to increase live weight by 2% of calving mass over the experimental period.

Preliminary observation during each single suckling event indicated that the removal of milk by the calf occurred in two phases, an extraction phase and a stripping phase. The transition between the two phases could be estimated within 10 to 30 seconds by a marked increase in the rate of teat changes. Data recorded at each suckling event included the starting time, the number of teat changes during the extraction phase, time of phase change, the number of teat changes during the stripping phase, the total number of udder butts and the time at the end of suckling.

All cows were observed at least three times at approximately one, two and 2.5 months post-partum, for a total of 56 observation days. The order of observation of cow-calf pairs at each stage post-partum was determined randomly, prior to the commencement of the trial.

Experiment 2

Following the initial analysis of the data relating to suckling behaviour and as a result of an independent study on the milk yield of suckled Mashona cows, a further study was initiated. At approximately 30 days post partum, teat changes and udder butting rate within the extraction and stripping phases were related to the estimated milk intake of each of 20 calves at the suckling following overnight-separation. Milk yield was estimated by the calf weigh-suckle-weigh procedure.

Statistical analysis

All data pertaining to suckling events were analyzed by a simple one way analysis of variance (Rayner, 1967). Comparisons of proportions were by Chi-square analysis.

RESULTS

Experiment 1

General characteristics of a single suckling event

When observations at the three stages post partum were combined within each breed, cows varied widely in all components of suckling behaviour recorded (Table 1). Although the stripping phase lasted (mean = 8.5 ± 0.3 minutes) only about 50% longer than the extraction phase (mean = 5.9 ± 0.3 minutes) nearly seven times as many teat changes occurred during stripping (mean = 135 ± 4) than during extraction (24 ± 2) teat changes. Thus, during the extraction phase, calves changed teats every 15 seconds whilst during the stripping phase teat changes occurred every 4 seconds. There was a tendency ($P < 0.01$) for Afrikaner and Mashona calves to have a slightly higher number of teat changes than Hereford calves during stripping (Table 1). In the Afrikaner the total number of udder butts per suckling was significantly ($P < 0.05$) higher than in the other two breeds (Table 1).

Changes in suckling behaviour with increase in post partum interval

Between the first and second month post-partum the total number of teat changes during the extraction phase did not differ significantly between breed or between these two observation periods for the same breed (Table 2). Only amongst the Afrikaner cows did the number of teat changes during the stripping phase differ significantly ($P < 0.05$) with increase in post-partum interval, but the trend was not consistent.

The number of udder butts per suckling bout showed a consistent decline with increasing post-partum interval only amongst the Mashona cows (Table 2). Although there were significant differences ($P < 0.05$) over time in Hereford cows, no consistent trend was again evident.

During the first observation period (approximately one month after calving) Afrikaner and Mashona cows did not differ significantly in any of the three components of suckling behaviour (Table 2). A month later, significantly fewer stripping phase teat changes and udder butts occurred in the Mashona cows than in the Afrikaners (Table 2). At the third observation period significantly more udder butts again occurred in the Afrikaner cows than for Mashona or Hereford cows (Table 2).

TABLE 1

Characteristics of suckling behaviour during a single event in Afrikaner, Mashona and Hereford cows. Means and SEM are for three stages postpartum combined

Suckling component	Afrikaner	Mashona	Hereford	Mean
Number of sucklings observed	65	60	48	-
Extraction phase:				
Teat changes	25 ± 2.1	25 ± 2.0	22 ± 3.2	24 ± 2.1
Range	5-80	5-75	6-110	-
Duration (minutes)	6.3 ± 0.5	5.6 ± 0.3	5.8 ± 0.6	5.9 ± 0.3
Range	1-27	2-25	0-25	-
Stripping phase:				
Teat changes	145 ± 6.8^{a}	$133 \pm 5.9^{a,b}$	123 ± 7.7^{b}	135 ± 4.0
Range	26-305	18-240	35-266	-
Duration (minutes)	8.9 ± 0.5	8.3 ± 0.7	8.4 ± 0.6	8.5 ± 0.3
Range	3-21	2-30	3-20	-
Udder butts	34 ± 2.9^{a}	22 ± 2.3^{b}	$27 \pm 3.3^{a,b}$	28 ± 1.7
Range	0-102	0-89	0-94	-

^{a,b,} Means in the same row with different superscripts differ ($P < 0.01$)

TABLE 2

Characteristics of suckling behaviour with advance in post-partum interval

Observation period	Characteristic	Afrikaner	Mashona	Hereford
First	P.p. interval	29 ± 2.4	36 ± 2.6	-
	Teat changes:			
	Extract. phase	$26 \pm 4.4^{a}_{x}$	26 ± 3.4^{a}	-
	Stripp. phase	$139 \pm 12.7^{a}_{x}$	$142 \pm 7.9^{a}_{x}$	-
	Udder butts	$43 \pm 7.8^{a}_{x}$	$34 \pm 5.3^{a}_{x}$	-
	Sucklings observed	17	19	0
Second	P.p. interval	53 ± 1.0	64 ± 3.4	63 ± 0.6
	Teat changes:			
	Extract. phase	$22 \pm 3.0^{a}_{x}$	$23 \pm 3.0^{a}_{x}$	$28 \pm 7.2^{a}_{x}$
	Stripp. phase	$181 \pm 16.0^{a}_{y}$	$134 \pm 10.1^{b}_{x}$	$142 \pm 12.8^{b}_{x}$
	Udder butts	$42 \pm 6^{a}_{x}$	$21 \pm 3^{b}_{x}$	$34 \pm 5^{a}_{x}$
	Sucklings observed	17	18	19
Third	P.p. interval	70 ± 2.6	80 ± 3.2	91 ± 1.0
	Teat changes:			
	Extract. phase	$29 \pm 5.6^{a}_{x}$	$22 \pm 5.4^{a}_{x}$	$14 \pm 1.5^{a}_{x}$
	Stripp. phase	$133 \pm 9.9^{a}_{z}$	$113 \pm 13.2^{ab}_{x}$	$98 \pm 10.5^{b}_{y}$
	Udder butts	$29 \pm 5^{a}_{x}$	$13 \pm 2^{b}_{x}$	$15 \pm 2^{b}_{y}$
	Sucklings observed	16	15	14
Fourth	P.p interval	-	-	111 ± 0.7
	Teat changes:			
	Extract. phase	-	-	$20 \pm 4.5_{x}$
	Stripp. phase	-	-	$122 \pm 14.0_{z}$
	Udder butts	-	-	$28 \pm 6.1_{z}$
	Sucklings observed	-	-	15

P.p interval = post-partum interval
Extract. phase = extraction phase
Stripp. phase = stripping phase

^{a,b,c} Means for the same item in the same row with different superscripts differ ($P < 0.05$)

$_{x,y,z}$ Means for the same item in the same column with different subscripts differ ($P < 0.05$)

Number of suckling bouts per day

Analysis of observations at all sucklings (Figure 1) indicated that only for cows suckled three times per day did the proportion of Hereford cows exceed ($P < 0.05$) that seen for Afrikaner cows. All other breed differences were not

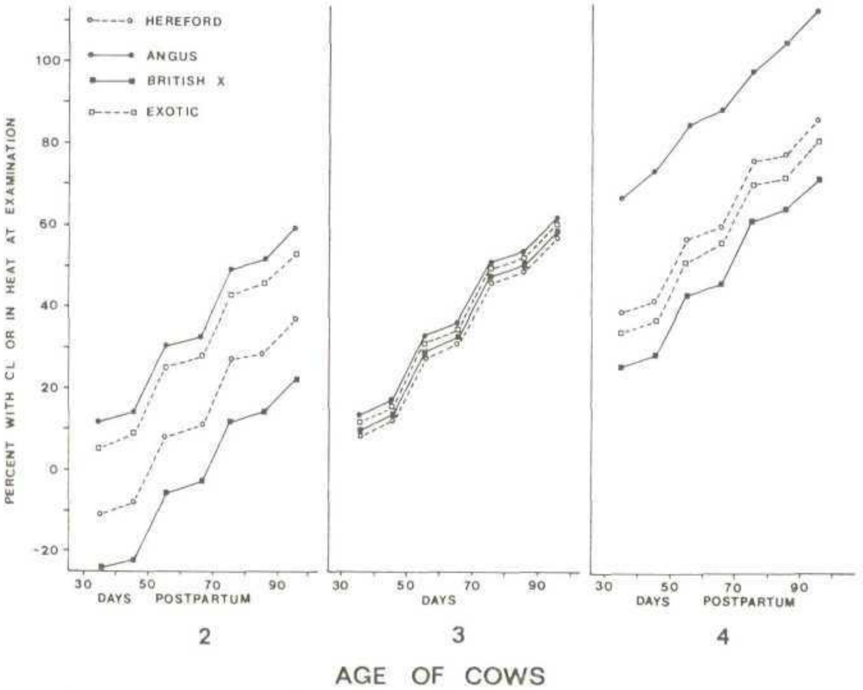

Figure 17.1. Effect of age by breed interaction (least squares means) on proportion of lactating beef cows with a corpus luteum at intervals from 30 to 99 days postpartum.

herds may have contributed to the breed difference even though herd differences were included in the model. The performance of crosses of British breeds in this study contrasts to the positive heterosis for reproductive performance and longevity observed in Angus × Milking Shorthorn crosses (*42*). Like Herefords, these cows were in commercial herds and there were few of them. Planned breed comparisons within experiments may help to explain the mechanisms that control the return from postpartum anestrus to reproductive cyclicity, but these comparisons will require large numbers of cows.

The need to be concerned with genetic differences in reproductive function might be reinforced by an example from studies within the Holstein breed. In 1935, Chapman and Casida (*7*) found that interval to conception after calving was positively associated with butterfat production during the first two months of lactation. Today, dairy producers expect high producing cows to have longer postpartum intervals to estrus. Casida et al. (*5*) and Whitmore et al. (*51*) found this to be true in comparing Holsteins with low or high genetic potentials for milk production. Selection for milk production for over 30 years, with no concern for early conception, apparently has changed the relationship between these traits, at least in the Holstein breed. It is only logical to expect that selection for 205-day-weight in beef cattle may lead to greater milk production, more vigorously suckling calves, and significant. On 14/56 and 22/56 days on which sucklings were observed, cows nursed their calves two and three times per day, respectively, between the hours of 0500 and 1900 (Figure 1).

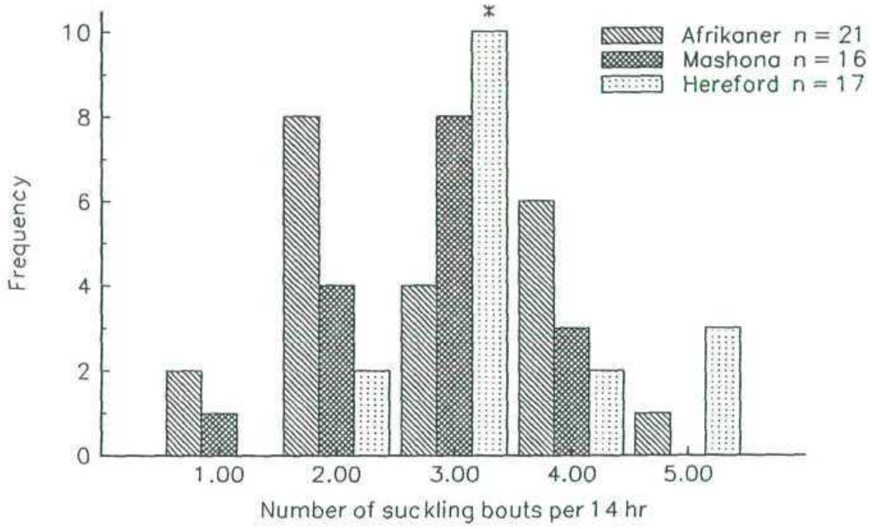

Fig. 1. Influence of breed of dam on the number of suckling bouts between 0500 and 1900hrs.
* Indicates frequency significantly greater ($P < 0.05$) than for Afrikaner cows.

Diurnal distribution of suckling bouts

The interval between suckling events varied according to the number of times a calf suckled each day. Consequently, mean starting times for each suckling event were fairly regularly distributed over the day, but considerable individual variation was observed between cows with the same suckling frequency. Since extraction phase teat changes and udder butts appeared to be the two components of suckling most likely to change under different circumstances, only these two facets were assessed further (Table 3). Regardless of the number of times a calf suckled during each 14 hour observation period, virtually the same amount of effort was exerted by the calf in each bout. Thus, the intensity of suckling stimulus to which the cow was exposed was more dependant upon the frequency of suckling (bouts/day) than variation in individual patterns of suckling at each event.

Over the course of a day, number of teat changes at each suckling did not differ regardless of the number of sucklings per day (Table 3). Significant changes did occur in the total number of udder butts per suckling where suckling occurred more than twice, but not five times per day (Table 3). Udder butting commonly occurred at the highest frequency at the first suckling of the day and declined thereafter.

TABLE 3

Distribution of suckling bouts and patterns of behaviour with cows suckled one to five times per day

			Sequence of suckling events				
Bouts /day	Cows/ breed	Pattern	First	Second	Third	Fourth	Fifth
One	Afrk = 2	Start(Hour)	13h12 ± 108m				
	Mash = 1	Range (hrs)	6				
	Here = 0	Teat changes	129 ± 15				
		Udder butts	38 ± 9				
Two	Afrh = 8	Start (Hour)	10h06 ± 48m	15h06 ± 40m			
	Mash = 4	Range (hrs)	7	9			
	Here = 2	Teat changes	127 ± 16^{a}	158 ± 20^{a}			
		Udder butts	26 ± 6^{a}	34 ± 9^{a}			
Three	Afrk = 4	Start (Hour)	06h42 ± 20m	11h12 ± 21m	16h00 ± 24m		
	Mash = 8	Range (hrs)	4	6	8		
	Here = 10	Teat changes	142 ± 9^{a}	119 ± 1^{b}	128 ± 10^{ab}		
		Udder butts	38 ± 5^{a}	23 ± 5^{ab}	20 ± 4^{b}		
Four	Afrk = 6	Start (Hour)	05h48 ± 12m	09h54 ± 36m	14h06 ± 30m	17h18 ± 15m	
	Mash = 3	Range (hrs)	2	6	5	3	
	Here = 2	Teat changes	137 ± 16^{a}	127 ± 16^{a}	121 ± 11^{a}	140 ± 12^{a}	
		Udder butts	47 ± 9^{a}	31 ± 5^{a}	20 ± 3^{b}	104 ± 23^{c}	
Five	Afrk = 1	Start (Hour)	05h18 ± 12m	09h00 ± 36m	11h12 ± 68m	15h06 ± 24m	17h48 ± 13m
	Mash = 0	Range (hrs)	1	3	3	2	1
	Here = 3	Teat changes	122 ± 11^{a}	167 ± 21^{a}	130 ± 30^{a}	134 ± 30^{a}	143 ± 27^{a}
		Udder butts	35 ± 13^{a}	25 ± 7^{a}	17 ± 4^{a}	20 ± 7^{a}	24 ± 2^{a}

m = minutes

^{a,b,c} Means with different superscripts differ ($P < 0.05$)

Total diurnal suckling activity

The total udder stimulation (stripping phase teat changes and udder butts) experienced by cows over the period from 0500 hours to 1900 hours increased significantly ($P < 0.01$) with an increase in the number of times the calves suckled during the observation period (Fig. 2). The mean total teat changes ranged from 330 (suckled twice daily) to 615 in cows suckled four times a day, while udder butts increased from 59 to 125. For cows suckled more than five times per observation period, the time spent suckling and total udder stimulation rose linearly, yielding figures in excess of 1100 teat changes over a suckling period of 100 minutes.

Fig. 2. Total suckling stimulation over all sucklings between 0500 and 1900hrs. as a % of highest frequency. Total teat changes differed significantly ($P < 0.05$) between suckling frequencies.

Experiment 2

Suckling activity in relation to milk yield

Suckling activity was classified into four categories on the basis of estimated milk intake during suckling. These four groups covered the range from 0 to 4.2 kg per suckling (Table 4). The duration of a suckling event increased significantly ($P < 0.01$) only when the milk production from below 1kg to more than 1kg per at the suckling after overnight starvation (Table 4).

With increasing milk intake at each suckling bout, the length of time between teat changes, during the extraction phase, increased significantly ($P < 0.01$) by 2.8 seconds. At the same time the total duration of this extraction phase increased significantly ($P < 0.01$). During this phase the number of udder butts did not differ between milk-intake groups. With the longer extraction phase in the higher intake groups, the time between udder butts was significantly ($P < 0.01$) longer than amongst cows yielding less than 1kg at the first suckling after overnight separation from cows. suckling.

In contrast to the extraction phase, milk intake did not significantly influence the factors studied.

TABLE 4

Patterns of suckling behaviour associated with four levels of milk intake during a single suckling bout in Mashona cows

Suckling component	Estimated milk production in kg			
	0 - 0.9	1.0 - 1.9	2.0 - 2.9	3.0 - 4.2
Extraction phase:				
Teat changes	55 ± 5.6^{ab}	64 ± 3.1^{a}	54 ± 2.7^{ab}	44 ± 3.9^{b}
Udder butts	4.5 ± 0.7	3.1 ± 0.3	3.1 ± 0.5	2.9 ± 0.8
Phase duration (minutes)	3.2 ± 0.2^{a}	3.4 ± 0.1^{a}	3.8 ± 0.1^{ab}	4.3 ± 0.2^{b}
Seconds between teat changes	4.0 ± 0.5^{a}	3.3 ± 0.1^{a}	4.8 ± 0.3^{b}	6.8 ± 0.3^{c}
Seconds between udder butts	46.8 ± 6.7^{a}	90.0 ± 9.0^{b}	105.0 ± 10.1^{b}	102.6 ± 15.9^{b}
Stripping phase:				
Teat changes	131 ± 11.5	162 ± 7.7	161 ± 8.7	141 ± 20.5
Udder butts	9.3 ± 1.5	13.8 ± 1.3	13.8 ± 1.4	14.7 ± 2.1
Phase duration (minutes)	5.6 ± 0.4	6.52 ± 0.19	6.31 ± 0.3	6.08 ± 0.5
Seconds between teat changes	2.8 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	3.3 ± 0.4
Seconds between udder butts	73.8 ± 18.4	41.4 ± 4.2	46.2 ± 6.8	32.4 ± 4.7
Duration of one suckling (mins)	8.7 ± 0.3^{a}	9.8 ± 0.2^{b}	10.1 ± 0.2^{b}	10.4 ± 0.4^{b}

[a,b,c] Means within the same row with different superscripts differ ($P < 0.01$)

DISCUSSION

Patterns within, duration of, and daily incidence of suckling bouts

The two distinct phases (extraction and stripping) of a suckling bout described in this study have apparently not been identified before. It was seen that during the extraction phase, while milk (presumably) was being easily removed from the udder, calves changed teats infrequently (24 ± 2 times) and slowly. When the rate of milk removal appeared to decrease markedly, the behaviour reflected a stripping action with numerous (135 ± 4) and frequent teat changes. Thus, the process can be likened to handmilking.

The mean duration of a suckling bout of 14.4 minutes in the present study is longer than the 9 to 11 minutes reported by others (Drewry *et al.*, 1959; Wagnon, 1963; Nicol and Sharafeldin, 1975; Reinhardt and Reinhardt, 1981; Odde *et al.*, 1985).

The limited data available from this study indicate that a large variation exists in the number of times cows will suckle their calves during daylight hours and that this is not materially affected by breed. With only a few animals available, the frequency of suckling could not be related to calf growth as done by Drewry *et al.* (1959).

Effect of milk yield and stage post-partum

The question arises as to why patterns of suckling activity differ between cows. Milk production, and to a lesser extent, cow mothering instincts, are obvious independent variates on the dam's side. On the other hand, calf appetite (a function of growth potential) and suckling persistence could be viewed as driving forces peculiar to the calf that will contribute to differences in suckling behaviour. With the majority (83%) of sucklings being initiated by the calf (Wagnon, 1963) and milk production being limited largely by calf appetite (Gifford, 1949; 1953), suckling behaviour appears to be more a function of calf genotype than milking potential of the dam.

As a general rule it would appear that a calf with good appetite, suckling a high yielding dam will tend to change teats and butt the udder less often and with a slower change rate during the extraction phase than a calf suckling a lower producing cow (Table 4). For this reason the extraction phase is of longer duration in the high producing cow. In sharp contrast to this relaxed extraction phase, the higher yielding cow will be subjected to a greater number of udder butts, administered at a higher rate than her lower yielding contemporary (Table 4). The net effect appears to be a similar amount of total udder stimulation in high and low yielding cows.

An evaluation of the effect of age of the calf (or of stage post partum) on suckling behaviour is confounded by changes in milk yield as lactation progresses. Thus, while Cole and Johannson (1933), Heyns (1960a) and Kress and Anderson (1974) noted that milk production peaked within three to four weeks after calving, others have recorded maximum yields towards the end of the second month after parturition (Dawson *et al.*, 1960; Gleddie and Berg, 1968; Louw, 1984). The duration of a suckling event, in turn, reached a maximum 40 to 60 days post-partum and remained constant to 120 days after calving (Nicol and Sharafeldin, 1975).

The Afrikaner cows in this study showed a peak in the number of stripping-phase teat changes (181) 53 days after calving. This level was significantly higher ($P < 0.05$) than either Mashona (134) or Hereford (142) cows (Table 2). After this time, suckling activity within a suckling event, declined in all three breeds. No such peak was recorded by Reinhardt and Reinhardt (1981) in the East African zebu.

The major objective of this study was to determine whether differences in the duration of lactation anoestrus between Afrikaner and Mashona cows (Wells *et al.*, 1985) could be accounted for in terms of peculiarities of the suckling behaviour of these breeds. Unfortunately, the limited numbers that could be observed (five cow-calf pairs of each breed) left this objective unsatisfied.

From the suckling patterns observed, Afrikaner and Mashona cows appeared to differ very little in their patterns of suckling behaviour, yet the reproductive performance of these two breeds is very different (Wells *et al.*,1985; Wells, 1986). The only notable difference was the higher incidence of udder butts in Afrikaner cows at about the time they would be expected to initiate ovarian activity (Wells *et al.*, 1985;1986)

It has been suggested that high milk production in beef cows may have a detrimental effect on post partum reproduction (Deutscher and Whiteman, 1971; McGinty and Frerichs, 1971; Holloway *et al.*, 1975; Boggs *et al.*,1980; Montano-Bermudez and Nielsen, 1990). Therefore, if the total number of udder butts were positively correlated with milk production then an association between udder butting and reproduction could be expected. However, no such relationship was evident (Table 4) except perhaps at very low milk yields.

Drewry *et al.* (1959) established that total suckling time and frequency were positively correlated with calf weight up to 60 days post partum. Furthermore, Nicol and Sharafeldin (1975) reported that each additional minute of total suckling time per 24 hours increased daily gain by 0.05 kg. Since calf gain and milk yield during early lactation are highly correlated (Heyns, 1960b; Neville, 1962; Melton *et al.*, 1967; Boggs *et al.*, 1980; Holloway *et al.*, 1983; Louw, 1984; Clutter and Nielsen, 1987; Beal *et al.*, 1990) it could be expected that rapid calf growth would inhibit resumption of breeding post-partum and thereby reduced subsequent calving rates. However, Wells (1986) could demonstrate no relationship between weight at 75 days post-partum of Afrikaner and Mashona calves and the interval to the first ovulation of their dams. Thus, the cows that yielded high levels of milk and reared heavy calves did not breed late. This agrees with the results obtained by Beal *et al.* (1990).

The intensity of a suckling stimulus appears to be more a function of total suckling stimulus per 24 hours than the rate of either teat changing or udder butting during a suckling event. Consequently, the trends observed across different levels of milk production (Table 4) within a suckling event may be of little significance in reproduction function *per se*. The only point of interest would be the correlation between these patterns of behaviour and either the total suckling time or frequency of suckling bouts per 24 hours. Obviously, a study, incorporating sufficient animals, should be conducted to relate suckling behaviour to milk yield and duration of lactation anoestrus. Of particular interest would be the relationship between number of sucklings per day and post-partum breeding. This is especially relevant in view of the marked increase in conception rates in cows allowed to suckle only once daily (Wells *et al.*, 1986).

Since Afrikaner and Mashona cows appear to differ little in their patterns of suckling activity, the inhibitory effect of suckling on post-partum reproduction appears to operate at different thresholds of sensitivity in these two breeds. To investigate this possibility the calves would need to be of similar genotype and independent of the dam's genotype. Embryo transfer would be useful in meeting this objective.

REFERENCES

Beal, W.E., Notter, D.R. and Akers, R.M., 1990. Techniques for estimation of milk yield in beef cows and relationships of milk yield to calf weight and postpartum reproduction. J. Anim. Sci., 68: 937-943.

Boggs, D.L., Smith, E.F., Schalles, R.R., Brent, B.E., Corah, R. and Pruit, R.J., 1980. Effects of milk and forage intake on calf performance. J. Anim. Sci., 51: 550-553.

Cole L.J. and Johansson, I., 1933. The yield and composition of milk from Aberdeen-Angus cows. J. Dairy Sci., 16: 565-572.

Drewry, K.J., Brown, C.J. and Honea, R.S., 1959. Relationship amongst factors associated with mothering ability in beef cattle. J. Anim. Sci., 18: 938-953.

Clutter, A.C. and Nielsen, M.K., 1987. Effect of level of beef cow milk production on pre- and postweaning calf growth. J. Anim. Sci., 64: 1313-1322.

Dawson, W.M. Cook, A.C. and Knapp, B., 1960. Milk production of beef Shorthorn cows. J. Anim. Sci., 19: 502-508.

Deutscher, G.H. and Whiteman, J.V., 1971. Productivity as two-year-olds of Angus-Holstein crossbreds compared to Angus heifers under range conditions. J. Anim. Sci., 33: 337-342

Ewbank.R., 1969. The frequency and duration of the nursing periods in single suckled Hereford beef cows. Br. Vet. J., 2: IX.

Geddie, V.M. and Berg, R.T., 1968. Milk production in range beef cows and its relationship to calf gains. Canad. J Anim. Sci., 48: 323-333.

Gifford, W., 1949. Importance of high milk production in beef cows overestimated. J. Anim. Sci., 8: 605 (Abstr.).

Gifford, W., 1953. Records of performance for beef cattle in breeding herds, milk production: milk production of dams and growth of calves. Ark. Agr. Exp. Sta. Bul. No. 531.

Heyns, H., 1960a. The growth of the Afrikaner calf in relation to the production and composition of the milk. I. The production and composition of the milk. S. Afr. J. Agric. Sci., 3: 349- 366.

Heyns, H., 1960b. The growth of the Afrikaner calf in relation to the production and composition of the milk of its dam. II. The milk production of the dam and growth of the calf. S. Afr. J. Agric. Sci., 3: 517-530.

Holloway, J.W., Stephens, D.F., Whiteman, J.V. and Totusek, R., 1975. Efficiency of production of 2- and 3-year-old Hereford, Hereford x Holstein and Holstein cows. J. Anim. Sci., 41: 855-867.

Hutchinson, H.G. Woof, R., Mabon, R.M., Salehe, I. and Robb, J.M., 1962. A study of the habits of zebu cattle in Tanganyika. J. Agric. Sci., 58: 301-317.

Kress, D.D. and Anderson, D.C., 1974. Milk production in Hereford cattle. J. Anim. Sci., 38: 1320 (Abstr.).

Louw, B.P., 1984. Intensive beef production on cultivated pastures in a sub-tropical environment. Ph.D. Thesis, Dept. of Anim. Sci. University of Natal, Pietermaritzburg.

McGinty, D.D. and Frerichs, W.G., 1971. Milk yields and calf performance from crossbred and Hereford cows. J. Anim. Sci., 33: 210 (Abstr.).

Montano-Bermudez, M. and Nielsen, M.K., 1990. Reproductive performance and variation in body weight during annual cycles for crossbred beef cows with different genetic potential for milk. J. Anim. Sci., 68: 2289-2296.

Melton, A.A., Riggs, J.K., Nelson, L.A. and Cartwright, T.C., 1967. Milk production, composition and calf gains of Angus, Charolais and Hereford cows. J. Anim. Sci., 26: 804-809.

Neville, W.E., 1962. Influence of dam's milk production and other factors on 120- and 240-day weight of Hereford calves. J. Anim. Sci., 21: 315-320.

Nicol, A.M. and Sharafeldin, M.A., 1975. Observations on the behaviour of single-suckled calves from birth to 120 days. N. Z. Soc. Anim. Prod., 35: 221-230.

Odde, K.G., Kiracofe, G.H. and Schalles, R.R., 1985. Suckling behaviour in range beef cows. J. Anim. Sci., 61: 307-309.

Rayner. A.A., 1967. A first course in Biometry for agriculture students. University of Natal Press. Pietermaritzburg. South Africa.

Reinhardt, R.R. and Reinhardt, A., 1981. Natural suckling performance and age of weaning in zebu cattle (Bos indicus). J. Agric. Sci., 96: 309-312.

Somerville, S.H. and Lowman, B.G., 1979. Observations on the nursing behaviour of beef calves suckling Charolais cross calves. N. Z. J. Agric. Res., 5: 331-337.

Wagnon, K.A., 1963. Behaviour of beef cows on a California range. Calif. Agric. Exp. Sta. Bull. No. 799.

Ward, H.K. and Dlodlo, S., 1985. Post-weaning growth and carcase characteristics of crossbred cattle. Ministry of Agriculture. Zimbabwe. Matopos Res. Sta. Res. Rep.

Wells, P.L., 1986. The influence of suckling during early lactation on the resumption of ovarian activity and oestrous behaviour in the Afrikaner cow. Ph.D. Thesis, Dept. of Anim. Sci. University of Natal, Pietermaritzburg.

Wells, P.L., Holness, D.H., Freymark, P.J., McCabe, C.T. and Lishman, A.W., 1985. Fertility in the Afrikaner cow. 2. Ovarian recovery and conception in suckled and non-suckled cows post partum. Anim. Reprod. Sci., 8: 315-326.

Wells, P.L., Holness, D.L., McCabe, C.T. and Lishman, A.W., 1986. Fertility in the Afrikaner cow. 3. Once a day suckling and its effect on the pattern of resumption of ovarian activity and conception rate in early lactation. Anim. Reprod. Sci., 12: 1-12.

Suckling behaviour and fertility in beef cows on pasture
1. Suckling behaviour

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The suckling behaviour of one- to three-month-old calves, suckled by 66 Hereford, Simmentaler and Hereford × Simmentaler cows, was studied. The most common frequency of suckling in 24 h was 4, and the mean duration of each suckling event was 9.6 min. Suckling events were not evenly distributed throughout the 24-h period. Regardless of suckling frequency or days *post partum*, the most common suckling period was between 04:00 and 06:00. The lowest frequency of suckling recorded was in the period from midnight to dawn. The longest interval between two suckling events in all cows over the 24-h period always occurred before dawn and became longer as the calf grew older ($P < 0.01$). Once the mating season commenced, the onset of oestrus was positively correlated ($P < 0.01$) with the length of the longest inter-suckling period.

Die soginggedrag van een tot drie maande oue kalwers van 66 Hereford, Simmentaler en Hereford × Simmentaler koeie is bestudeer. Die mees algemene frekwensie van soging was 4 maal in 24 h, en die gemiddelde lengte van elke sogingperiode was 9.59 min. Sogings was nie eweredig oor 'n 24 h-periode versprei nie. Ongeag die frekwensie van soging en dae na kalwing, was die mees algemene sogingperiode tussen 04:00 en 06:00. Die minste sogingsperiodes is waargeneem tussen middernag en ongeveer 04:00. Die langste interval tussen twee sogingsperiodes binne 'n 24 h-periode het altyd voor 4:00 voorgekom en hierdie interval het langer geword met 'n toename in die ouderdom van die kalf ($P < 0.01$). Na aanvang van die dekseisoen was die aanvang van estrus positief gekorreleerd ($P < 0.01$) met die lengte van die langste interval tussen twee sogingsperiodes.

Keywords: Beef calves, beef cows, oestrus, suckling behaviour.

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Introduction

The calving percentage of non-dairy cattle in South Africa has been reported to be about 50% (Boulle, 1986; Lishman, 1988; RSA Livestock and Meat Statistics, 1990). One of the major reasons for the low calving rate in beef cows is the extended *post partum* anoestrous period exhibited by suckling cows (Williams, 1989).

Suckling is an external stimulus which exerts a major role in governing the reproductive cycles in female mammals (Edgerton, 1980). Suckling induces a delay in ovulation (Carruthers & Hafs, 1980). When cows suckle their calves, the release of gonadotrophin is suppressed, thus inhibiting ovarian activity (Convey *et al.*, 1983). The removal of the suckling stimulus increases the release of pulsatile luteinizing hormone (LH) (Walters *et al.*, 1982). In the cow suckling a calf, low levels of basal LH prevail for longer than in the non-suckling cow (Radford *et al.*, 1978).

The aim of this study was to observe suckling behaviour patterns in cows and their calves during the first 100 days *post partum* under intensive grazing conditions, in an effort to understand the effect of suckling behaviour on the onset of oestrus. Differences in suckling behaviour patterns have been consistently reported by investigators. These differences could possibly provide the reason why some cows exhibit longer suckling-induced, *post partum* anoestrous periods than others; a possibility which was examined in this study.

Materials and Methods

Suckling behaviour

All observations were made at the Cedara Agricultural Experiment Station, 29°32′ latitude and 30°17′ longitude, where relatively warm, wet summers and cool, dry winters prevail. Sixty-six cows, consisting of Herefords or Simmentalers or Hereford/Simmentaler crosses, calved down between mid-August and mid-October. Observations were taken during the spring of three consecutive years. The suckling events were recorded when the calves were between one and three months old.

Each calf observation period commenced at about 10:00 and lasted for 24 h. A suckling event constituted the period between the time that a calf first mouthed a teat to the time it stopped suckling. The times of these events were recorded to the nearest second.

Because primiparous cows show longer *post partum* intervals to oestrus than do multiparous cows, the data were categorized into first lactation cows and others. The stages *post partum* were chosen on the basis of the:

(i) possibility of cows being anoestrus (<45 days *post partum*),
(ii) likely onset of first oestrus (46 to 90 days *post partum*),
(iii) possibility of cows having been bred (>90 days *post partum*).

Oestrus

The herd was rebred by artificial insemination (AI) between November 1 and January 1 each year. Oestrus was detected by heat-spotting in the early morning (06:00–07:00) and late afternoon (17:00–18:00), which constituted the only record of ovarian activity.

Statistical analyses

The frequency with which a cow suckled her calf was defined as the number of times that suckling occurred within a 24-h period. The duration of each suckling event was recorded as the time, to the nearest minute, that a calf suckled. Since the time at which suckling occurred was recorded, the distribution of the suckling events over 24 h and the length of the intervals between these events could be determined. The standard error of the mean (±) for both the frequency and duration of suckling for the various cow/calf categories were calculated.

The Genstat 5 statistical programme was used to fit a log-linear model to the suckling frequency data and test for the goodness of fit of the Poisson distribution. The difference in means of the age classes was tested using this model. Frequency of suckling showed a significant Poisson fit with a mean of 5.03 over all calves ($P > 0.95$). However, there are indications that this fit, although still Poisson, is different for the age groups <45 days and >45 days.

Results

Frequency of suckling

Of the 332 suckling events observed, the modal suckling frequency was 4 per observation period (Figure 1). Mean suckling frequency for calves younger than 45 days was 5.8 ± 2.1, and for calves 45 days and older, it was 4.6 ± 1.5, which is significant at the 5% level (Table 1).

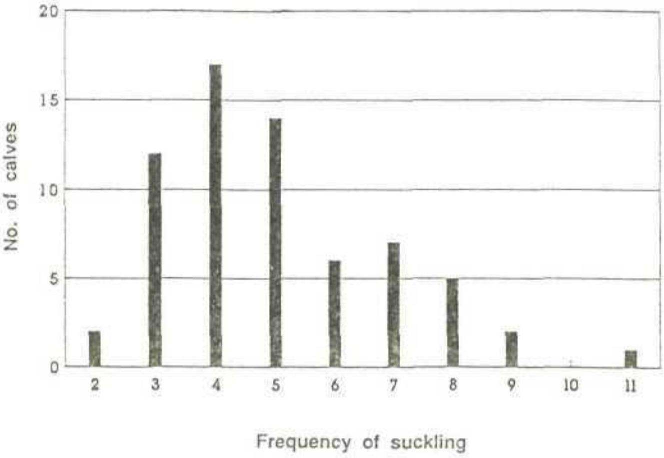

Figure 1 The frequency at which calves suckled over a 24-h period.

Table 1 Frequency and the duration of suckling events categorized according to age of cow and age of calf, data expressed as mean ± *SE*

	Frequency per 24 h		Duration of suckling (min)		*n*
Class of cow					
All cows	4.95	± 1.9	9.6	± 2.2	66
1 st lactation	5.2	± 2.1	8.7	± 2.8	6
> 1 st lactation	4.9	± 1.6	9.6	± 2.1	60
Calf age					
<45 days	5.8	± 2.1	9.88	± 2.4	28
45–90 days	4.4	± 1.3	8.72	± 2.0	17
>90 days	4.6	± 1.5	9.92	± 1.9	21

Duration of suckling events

Over all stages *post partum*, the mean duration of the suckling events for individual cow/calf pairs varied from 5.6 to 14.8 min. The mean duration of suckling events was 9.6 min (Table 1). In certain cows that had been observed on three occasions in the one season and again the following season, the duration of suckling events varied from 7.6 to 10.1 min.

The period of suckling was not significanty different for first-lactation and multiparous cows (Table 1). Similarly, there was no significant difference between the duration of suckling in the younger and older calves.

The variation in duration of suckling events for all animals observed over 24-h periods is shown in Figure 2. A negative correlation ($r = -0.2068$; $P < 0.01$) between the duration and frequency of suckling was found.

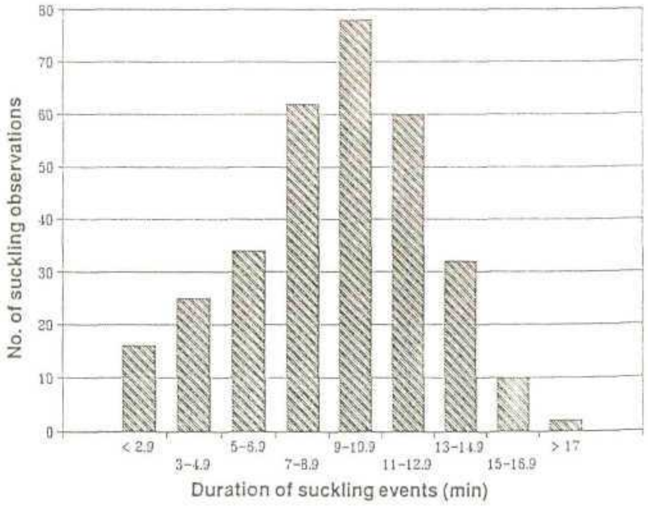

Figure 2 Variation in the duration of suckling events in beef cows.

Total suckling time

The frequency with which a calf suckled in a 24-h period, multiplied by the mean duration of each suckling event, yielded the total suckling time. The mean total time spent suckling in a 24-h period was 47.4 min, covering a wide range, i.e. from 28 to 80 min.

Distribution of suckling events

The most common suckling time (17% of all events) was between 04:00 and 06:00 (Figure 3). The second most common (16%) suckling time was from 17:00 to 19:00. The distribution of suckling events throughout the 24-h period for calves less than 45 days old and over 90 days of age is illustrated in Figure 4. Younger calves (<45 days old) which fed slightly more frequently (5.8 times a day) than older calves, had a more even distribution of suckling events throughout the 24-h period, although early morning remained the most favoured time. With the exception of two calves out of 95, no suckling occurred between midnight and dawn (Figure 4).

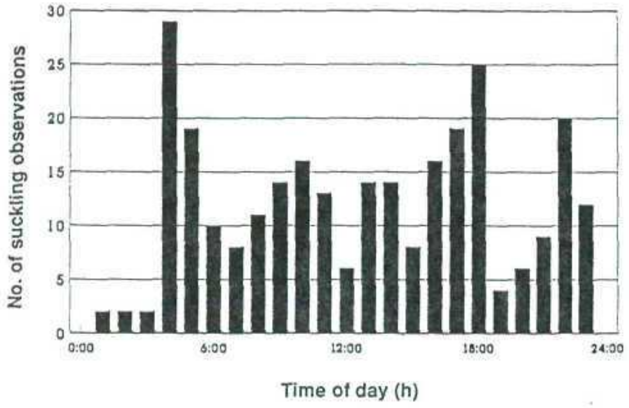

Figure 3 Daily distribution of suckling bouts in beef cows on cultivated pastures.

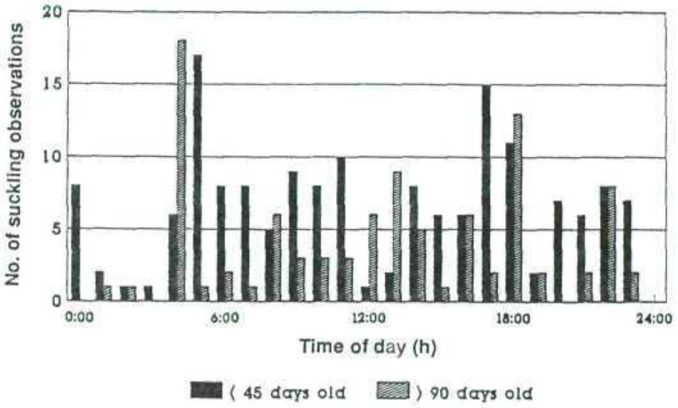

Figure 4 Daily distribution of suckling bouts among beef cows prior to 45 days and subsequent to 90 days *post partum*.

Intervals between suckling events

It was evident (Figure 4) that the suckling events were not evenly distributed throughout a 24-h period. As the age of the calf increased, the longest interval between suckling events was extended ($P < 0.01$; Table 2). There was also a significant ($r = 0.2167$; $P < 0.01$) correlation between calf mass and length of the longest interval between two suckling events. With the exception of the two aforementioned cow/calf pairs, all the longest intervals occurred in the early hours of the morning. The younger calves, nursed by cows less than 45 days into lactation, averaged longest intervals of 345 min. This time-span increased until at three months and over, the average length of the longest interval was 458 min. Cows which exhibited oestrus as early as 55 days *post partum* had longest inter-suckling periods of 422 min (Table 3). During the longest interval between suckling events, the calves rested and their dams were also mostly inactive. Occasionally a cow lay chewing the cud, but for four or five hours, late at night, there was marked inactivity, not seen at any other time during the 24-h period.

Table 2 The longest interval between suckling events. Data expressed as the mean ± *SE*

Calf age (days)	*n*	Longest interval (min)
All ages	57	380 ± 105.2
<45	25	345 ± 69.8
46–90	13	363 ± 32.2
>90	19	458 ± 140.5

Table 3 A comparison of suckling patterns and *post partum* intervals to oestrus

	Suckling patterns			
Interval: calving – oestrus (days)	Av. frequency of suckling per 24 h	Av. duration of suckling events (min)	Av. long interval between events (min)	*n*
55–80	4.8 ± 1.9	8.5 ± 2.4	422 ± 130.7	12
81–100	4.2 ± 1.1	9.3 ± 2.6	433 ± 140.8	12

The mean period between parturition and first AI was 72.5 days ($n = 24$). The relationship between *post partum* interval to first oestrus during the mating period and suckling behaviour variables is described in Table 3.

Discussion

The results show that it is possible to devise a management strategy whereby cows and their calves are separated to stimulate ovarian activity with little disruption to the animals.

Wells (1987) expressed the opinion that the frequency of suckling per 24-h period was the only component likely to be correlated with *post partum* reproduction. In this study, relatively young calves suckled more frequently than did those nearly two months of age. The negative correlation between frequency and duration of suckling events was expected. The total time spent suckling over a 24-hour period varied considerably (Figure 3). This characteristic is not considered to be of importance because of its variability and because it bore no relation to the onset of oestrus.

The negative correlation between the frequency of suckling events and the longest interval between successive events (Table 3) is logical, since the less frequent the suckling events, the greater the time interval between these.

The daily pattern, or distribution of suckling events throughout a 24-h period, warrants careful evaluation. Firstly, as shown in Figure 4, the most marked pattern was the concentration of suckling events between 04:00 and 05:00 and then between 17:00 and 18:00, followed by late night (20:00) and then more variably, late morning (10:00). These results differ considerably from those of Odde *et al.* (1985) and others who reported a concentrated nursing time at dawn, and then at 20:00. Their results were obtained in the northern hemisphere, with longer day lengths than encountered in Natal, which may account for this discrepancy. Nevertheless, in the present study, the intensity of the early morning suckling activity was very marked (17.3% of all observations), followed by 15.8% in the late afternoon. The period of least activity was between midnight and 04:00 (2.2% of sucking events observed). The *post partum* interval to oestrus in suckling cows has been reported to vary from 52 to 88 days (Short *et al.*, 1972; Dobson & Kamonpatana, 1986). Of all the suckling behaviour characteristics examined, the longest interval between suckling events was the most significant when comparing or relating suckling behaviour patterns to the anticipated onset of oestrus, at about 50 days *post partum* (Table 3).

It has been established that once-a-day suckling can promote ovarian activity (Odde *et al.*, 1986; Wells, 1987). This raises a number of questions, viz.

(i) What is the minimum interval between sucklings required to trigger ovarian activity, in other words, what is the minimum time needed for daily cow-calf separation at any particular time in the 24-h suckling cycle?

(ii) Is the longest natural interval between sucklings a natural clue or cue to the answer?

(iii) Would ovarian activity be stimulated by artificially manipulating the longest interval just before the breeding season?

(iv) Was it significant that the longest interval was always before the early morning feed, and that little physical activity occurred during this interval?

In relating suckling behaviour patterns to oestrus or ovarian activity, it appears from this study that an as yet, unknown minimum interval, when no suckling occurs, is required to trigger oestrus. This minimum interval between suckling events probably occurs in normally-suckled calves when the cow is about 70 days *post partum*.

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References

BOULLE, J.P., 1986. A survey of management factors affecting production in Natal beef breeding herds. M.Sc.(Agric.) thesis, Univ. Natal, Pietermaritzburg.

CARRUTHERS, T.D. & HAFS, H.D., 1980. Suckling and four times daily milking: Influence on ovulation, oestrus and serum luteinising hormone, glucocorticoids and prolactin in postpartum Holsteins. *J. Anim. Sci.* 50, 919.

CONVEY, E.M., TUCKER, H.A. & SHORT, R.E., 1983. Acute effect of suckling on gonadotropin, prolactin and glucocorticoid concentrations in serum of intact and overiectomised beef cows. *Theriogenology* 20, 661.

DOBSON, H. & KAMONPATANA, M., 1986. A review of female cattle reproduction with special reference to a comparison between buffaloes, cows and zebus. *J. Reprod. Fert.* 77, 1.

EDGERTON, L.A., 1980. Effect of lactation on the post partum interval. *J. Anim. Sci.* 51 (Suppl. 11), 40.

LISHMAN, A.W., 1988. Reproduction of beef cattle under ranching conditions. Paper presented at 27th Congr., S. Afr. Soc. Anim. Prod., March, 1988.

RSA LIVESTOCK AND MEAT STATISTICS, 1990. Meat Board, *Q. Rep.* May, 1990.

ODDE, K.G., KIRACOFE, G.H. & SCHALLES, R.R., 1985. Suckling behaviour in range beef cows. *J. Anim. Sci.* 61, 307.

ODDE, K.G., KIRACOFE, G.H. & SCHALLES, R.R., 1986. Effect of forty eight hour calf removal, once or twice daily suckling and norgestamet on beef cow and calf performance. *Theriogenology* 26, 3.

RADFORD, H.M., NANCARROW, C.D. & MATTNER, P.E., 1978. Ovarian function in suckling and non-suckling beef cows post partum. *J. Reprod. Fert.* 54, 49.

SHORT, R.E., BELLOWS, R.A., MOODY, E.L. & HOWLAND, B.E., 1972. Effects of suckling and mastectomy on bovine post partum reproduction. *J. Anim. Sci.* 34, 70.

WALTERS, D.L., KALTENBACH, C.C., DUNN T.G. & SHORT, R.E., 1982. Pituitary and ovarian function in beef cows. 1. Effect of suckling on serum and follicular fluid hormones and follicular gonadotropin receptors. *Biol. Reprod.* 26, 640.

WELLS, P.L., 1987. The influence of suckling during early lactation on the resumption of ovarian activity and oestrous behaviour in the Afrikaner cow. Ph.D. thesis, University of Natal, Pietermaritzburg.

WILLIAMS, G.L., 1989. Suckling as a regulator of postpartum rebreeding in cattle: A review. *J. Anim. Sci.* 68, 831.

Suckling behaviour and fertility in beef cows on pasture
2. Influence of twelve-hour calf separation on interval to first oestrus after onset of mating period

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For two weeks prior to the start of the breeding season, the suckling behaviour of beef calves, separated from their dams for 12 h out of 24, was investigated. Fifty-six per cent of the 44 cows which were separated from their calves from 18:00 to 06:00 (day-sucklers) exhibited oestrus within 30 days of the start of the breeding season, compared to 22% of the group separated from 06:00 to 18:00 ($P < 0.01$). It was concluded that not only the suppression of suckling may be involved in the onset of ovarian activity. The specific time period during the 24 h when suckling is prohibited may also play a role.

Vir twee weke voor die aanvang van die jaarlikse teelseisoen, is die sogingsgedrag van vleisbeeskalwers wat vir 12 h uit elke 24 h van hul moeders geskei was, bestudeer. Ses-en-vyftig persent van die 44 koeie wat tussen 18:00 en 06:00 (dag-sogend) van hul kalwers geskei was, het binne 30 dae na aanvang van die teelseisoen tekens van hitte getoon, in vergelyking met slegs 22% van die groep wat tussen 06:00 en 18:00 (nag-sogend) geskei was. Die aanvang van eierstokaktiwiteit word nie slegs deur die beperking van soging beïnvloed nie. Die spesifieke periode gedurende 'n 24 h-tydperk, waarin die beperking plaasvind, mag ook 'n rol speel.

Keywords: Oestrus, ovarian activity, separation, suckling.

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Introduction

It is widely recognized that a cow which suckles a calf has a delayed *post partum* oestrus. Accordingly, attempts have been made to improve the fertility of cows by restricting the calf's access to its dam (Eduvie & Dawuda, 1986; Wells, 1987). By removing calves from anoestrous cows at three weeks of age or older, an immediate increase in tonic luteinizing hormone (LH) secretion has been observed (Walters *et al.*, 1982).

Some researchers have attempted to remove calves temporarily before the breeding season, with variable results. For example, Alberio *et al.* (1985) temporarily removed Aberdeen Angus calves, age 40 days, for 48 or 72 h, and achieved conception rates of 67% and 72%, respectively, as opposed to 50% in cows which kept their calves.

Another approach has been to limit calf suckling each day. When calves were restricted to nursing only once or twice daily, commencing 11 days before the breeding season, 75 and 78.6% of the cows exhibited ovarian activity, respectively, compared to 51% in control cows (Odde *et al.*, 1986). Wells (1987) found that conception rates in Afrikaners increased by 40% when suckling was reduced to twice a day, starting on day 28 *post partum* and then once a day from day 42 *post partum*.

When the inhibitory effect on ovarian cycles, exerted by the suckling calf, is artificially removed, an increased pulse frequency triggers the first ovulation (Carruthers *et al.*, 1980; Walters *et al.*, 1982). However, even in the cow which suckles her calf normally, ovulation will occur eventually. Thus, it appears reasonable to assume that cycling will recommence spontaneously when the calf withdrawal period has increased beyond a certain threshold limit.

From previous observations (Odde *et al.*, 1985; Stewart, 1991) it was evident that as the calf grew older, the interval between suckling events lengthened, particularly in the period from midnight to the early feed. A natural form of temporary calf removal thus appears to facilitate ovarian activity.

A trial was therefore conducted to investigate the effect of a 12-h separation period on the onset of oestrus, and on the normal suckling behaviour patterns. In other words, would the calves compensate for the enforced separation by suckling more often during the 12 hours when they are together? Furthermore, the significance of the nocturnal separation was also investigated.

Procedure

Over three seasons a total of 88 suckling cows were allocated to one of two treatments according to stage *post partum*. The one group was separated from their calves during the night (day-suckled), and the other during the day (night-suckled). Two weeks before the start of the breeding season, the day-sucklers were separated from their dams from 18:00 to 06:00, whereas the night-suckling group were separated from 06:00

to 18:00. Cows and calves were separated by removing the cows from the pasture camps and leaving their calves behind. Cows were placed in a paddock adjoining the calves. There was little change to the forage or herbage component of their diets.

Suckling behaviour patterns were monitored. Observations were taken over 12 h, commencing at 06:00 when the day-sucklers were reunited with their mothers, and for another 12 h at 18:00 for the night-sucklers.

At the end of the two-week period, all the cows and calves remained together as one herd, and breeding by artificial insemination commenced. During the subsequent 60-day mating period, heat-spotting was used to check for signs of oestrus on a twice-daily basis. The heat-spotting period lasted for about 30 min and was done at 06:00 and at 18:00.

Results

The suckling behaviour patterns observed after the cows and their calves were reunited after 12 h of separation, are summarized in Table 1.

Table 1 Frequency and duration of suckling in day- and night-sucklers. Data expressed as the mean ± *SE*

	Day-suckled	Night-suckled
No. of cows	44	44
No. of suckling bouts	3.3 ± 0.96	2.4 ± 0.49
Range in no. of bouts	2 – 5	2 – 3
Duration (min)	12.6 ± 2.19	11.4 ± 1.7
Range	10.1 – 15.1	8.7 – 14.0
Longest interval (h)	2.4 (+ 12)	1.2 (+ 12)
Range	5.3 – 0.0	1.8 – 0.1
Total suckling time (min)	40.2	27.2
% Cycling in 30 days	56.8	22.0

The overall length of the longest interval between two suckling events for the night-sucklers was the imposed 12 h plus the relatively short time between the 4:00 suckling event and 06:00 (1.16 h), when separation took place. The inter-suckling interval for the day-sucklers was as long as 12 plus 5.3 h (those which suckled at about 12:30 and were then separated at 18:00) and as short as 12 h.

There appeared to be no attempt to 'make up for lost time' by calves which fed at night and were nursed less often than those during the day, and their total sucking time (27.2 min) was much shorter than that of the calves with their dams during the day (40.2 min).

Of the 44 cows in the day-suckler group, 25 were inseminated within the first month of the breeding season whereas 13 of the 44 in the night-suckler group were inseminated during the same period. Of these groups, 81 and 66%, respectively, subsequently calved. The mean period *post partum* to first insemination in cows which exhibited oestrus, in the day-suckler and night-suckler groups was 72.4 and 71.1 days, respectively.

Discussion and Conclusions

Results obtained by Stewart (1991) showed that cows and calves do not change their suckling behaviour patterns as a result of a 12-h separation. The day-sucklers nursed more frequently during the 12 h with their dams than did the night-sucklers. This was consistent with the normal daytime suckling behaviour of calves (Stewart, 1991) which were with their dams continuously. Similarly, the night-sucklers reflected suckling behaviour characteristics consistent with normally-suckled calves, i.e. less frequent suckling during the night than during the day. It thus appears that calves which are with their dams for only 12 out of 24 hours, do not compensate for the period during which they were separated from their mothers.

The two groups of cows were separated from their calves for 12 h each day. If the length of time of no suckling was the only trigger to stimulate the onset of oestrous cycles, a similar response would have occurred in the two groups. However, a higher percentage of day-sucklers exhibited oestrus within the first month of the breeding season than the night-sucklers ($P < 0.01$), even though the total suckling time in the day-sucklers was nearly twice as long as in the night-sucklers (Table 1).

It is possible that the day-suckler cows altered their pattern of oestrus, coming into season during the day when they were more readily heat-spotted. This possible shifting of oestrous display could have been evoked by elimination of the suckling bout around midnight, which occurs in cows with access to their calves throughout a 24-h period (Stewart, 1991).

In a study of Afrikaner cattle, normally-suckled cows displayed 50% of their oestrous behaviour between 19:00 and 05:00 (Wells, 1987). However, when the calves were partially weaned, the occurrence of oestrus shifted and only 17% occurred between 19:00 and 05:00.

Another possible explanation for the difference in response to day suckling and night suckling, observed in this study, is the episodic release in LH that is necessary to restore ovarian cyclicity in *post partum* cows and that can be triggered by removing the suckling stimulus (Walters *et al.*, 1982; Williams *et al.*, 1982). Furthermore, there is evidence that a control pathway involves endogenous opioid peptides which suppress LH secretion in suckling cows (Whisnant *et al.*, 1986; Gregg *et al.*, 1986). It is known that endogenous opioids suppress LH secretion in suckling cows, since an administration of an antagonist will increase the LH concentrations (Whisnant *et al.*, 1986).

Research using ovariectomized heifers has indicated that photoperiod alters the circulatory concentrations of LH, and the pulsatile release of LH and melatonin. LH levels and pulse amplitude were higher in blood samples taken nocturnally than in those taken diurnally. Melatonin concentrations showed similar patterns, though melatonin pulse amplitude was higher during periods of light stimulation (Crister *et al.*, 1987b; Short *et al.*, 1972). Melatonin injections given at 16:00 tended to inhibit a decrease in LH concentrations (Crister *et al.*, 1987a).

In fact, Short *et al.* (1988) were excited about recent additions to available options for further study of ovulation control using central nervous system(CNS)-related hormones, e.g. endogenous opioids, oxytocin, melatonin, or their antagonists. Not only do calves suckle less frequently at night than during the day, but from midnight to dawn is a very quiet and ıactive period when cows and calves alike rest and sleep, hether they are together for 12 or 24 h.

It would appear from the present study that there could be ı association between the onset of oestrus and the longest ıterval between suckling events and the regular occurrence of ıis interval before about 04:00.

Factors such as suckling, endogenous opioids and melatonin ıodify the pulsatile pattern of LH release. As the calf grows lder, in other words the *post partum* period increases, the ıtensity of suckling is reduced by a lengthening of the longest ıterval between two suckling bouts. This takes place during ıe hours of darkness. The inhibitory effect of the endogenous pioids is reduced and melatonin, with higher nocturnal concentrations, influence the LH concentrations in the anoestrous ow until a point is reached where an LH surge is sufficient to ıitiate the onset of oestrus.

It is suggested from the results of this study that it is the ıng, predawn interval between two suckling bouts that holds ıe clue to the suppressive effects of suckling on *post partum* estrus.

leferences

LBERIO, R.H., BUTLER, H.M., PALMA, C.R. & TORQUATT, O., 1985. Effect of temporary calf removal and/or parenteral copper on post partum sexual activity in multiparous cows. Abstract. Asociaco n Latinamericano de Prodducio n Animal, 1983.

ARRUTHERS, T.D., CONVEY, E.M., KESNER, J.S., HAFS, H.D. & CHENG, K.W., 1980. The hypothalamo-pituitary gonadotropic axis of suckled and non-suckled dairy cows. *J. Anim. Sci.* 51, 949.

RISTER, J.K., HINSHELWOOD, M.M. & HAUSER, E.R., 1987a. The effect of exogenous melatonin administration on gonadotropin and prolactin patterns in ovariectomised estradiol treated heifers exposed to increasing photoperiod. *Theriogenology* 28, 257.

CRISTER, J.K., BLOCK, T.M., FOLKMAN, S. & HAUSER, E.R., 1987b. Effect of photoperiod on LH, FSH, prolactin and melatonin patterns in ovariectomised prepubertal heifers. *J. Reprod. Fert.* 81, 29.

EDUVIE, L.O. & DAWUDA, P.M., 1986. Effect of suckling on reproductive activities of Bunaji cows during the post partum period. *J. agric. Sci., Camb.* 107, 235.

GREGG, D.W., MOSS, G.E., HUDGENS, R.E. & MALVEN, P.V., 1986. Endogenous opioid modulation of luteinizing hormone and prolactin secretion in post partum ewes and cows. *J. Anim. Sci.* 63, 838.

ODDE, K.G., KIRACOFE, G.H. & SCHALLES, R.R., 1985. Suckling behaviour in range beef cows. *J. Anim. Sci.* 61, 307.

ODDE, K.G., KIRACOFE, G.H. & SCHALLES, R.R., 1986. Effect of forty eight hour calf removal, once or twice daily suckling and norgestamet on beef cow and calf performance. *Theriogenology* 26, 3.

SHORT, R.E., BELLOWS, R.A., MOODY, E.L. & HOWLAND, B.E., 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. *J. Anim. Sci.* 34, 70.

SHORT, R.E., STAIGMILLER, R.B. & BELLOWS, R.A., 1988. Hormonal treatments to induce ovulation. 11th International Congress on Animal Reproduction and Artificial Insemination, Dublin. Vol. 5.

STEWART, I.B., 1991. Suckling behaviour and fertility in beef cows on pasture. M.Sc.(Agric.) thesis, Univ. Natal, Pietermaritzburg.

WALTERS, D.L., KALTENBACH, C.C., DUNN, T.G. & SHORT, R.E., 1982. Pituitary and ovarian function in beef cows. 1. Effect of suckling on serum and follicular fluid hormones and follicular gonadotropin receptors. *Biol. Reprod.* 26, 640.

WELLS, P.L., 1987. The influence of suckling during early lactation on the resumption of ovarian activity and oestrous behaviour in the Afrikaner cow. PhD. thesis, University of Natal, Pietermaritzburg.

WHISNANT, C.S., KISER, T.E., THOMSON, F.N. & BARB., C.R., 1986. Naloxone infusion increases pulsatile luteinizing hormone release in post partum beef cows. *Dom. Anim. Endocrin.* 3, 49.

WILLIAMS, G.L., KOTWICA, J., SLANGER, W.D., OLSEN, D.K., TILTON, J.E. & JOHNSON, L.J., 1982. Effect of suckling on pituitary responsiveness to gonadotropin-releasing hormones throughout the early post partum period of beef cows. *J. Anim. Sci.* 54, 3.

Late-night suckling inhibits onset of postpartum oestrous activity in beef cows

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To determine whether suckling of calves late at night would prolong lactation anoestrus, 51 Hereford-type cows (21 – 29 days postpartum) were divided into three treatment groups. In treatments 1 (night suckling) and 2 (day suckling) cows suckled their calves five times every 24 hours; the calves in treatment 1 did not suckle between 09:00 and 17:00 and those in treatment 2 between 21:00 and 05:00 . Cows in treatment 3 suckled only once daily at 17:00. By 80 days after calving a significantly greater proportion ($P < 0.001$) of the night-suckled cows had not returned to oestrus than those from the other treatments. The mean interval to first oestrus was significantly ($P < 0.001$) longer in treatment 1 (67.0 ± 1.5 days) than in treatments 2 (42.2 ± 2.5) and 3 (45.7 ± 3.2). Lactation anoestrus does not appear to be dependent on the number of sucklings per 24 hours, but rather on the time of night when suckling occurs.

Om te bepaal of suiping van kalwers laat in die nag, laktasie anestrus sal verleng, is 51 Hereford-tipe koeie, 21 – 29 dae na kalwing, in drie groepe verdeel. In behandelings 1 en 2 het die koeie hul kalwers vyf keer elke 24 uur laat suip. Die kalwers is in behandeling 1 (nag suiping) nie tussen 09:00 en 17:00, en dié in behandeling 2 (dag suiping) nie tussen 21:00 en 05:00 toegelaat om te suip nie. Die koeie in behandeling 3 het net eenkeer per dag om 17:00 laat suip. 'n Betekenisvolle groter verhouding ($P < 0.001$) van die koeie wat gedurende die nag laat suip het (behandeling 1), het nie estrus teen 80 dae na kalwing, as by die ander behandelings groepe getoon nie. Die gemiddelde interval tot eerste estrus was betekenisvol ($P < 0.001$) langer by behandeling 1 (67.0 ± 1.5 dae) as by behandelings 2 (42.2 ± 2.5) en 3 (45.7 ± 3.2). Klaarblyklik is laktasie anestrus van die tyd van die nag wanneer suiping plaasvind afhanklik en nie van die aantal suipings per 24 uur nie.

Keywords: Beef cow, night suckling, postpartum anoestrus.

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introduction

When Williams (1990) reviewed suckling-induced anoestrus in cattle, he stated that either the frequency, intensity or duration of suckling had been considered to be the major controlling factor. Although species differences are undoubtedly important, a similar conclusion has been reached for nursing women (McNeilly *et al.*, 1985), with the evidence favouring frequency as opposed to duration (Robyn *et al.*, 1985). To date, no relationship between suckling frequency and onset of oestrus, postpartum, has been detected in bovines (Williams *et al.*, 1984; Day *et al.*, 1987). However, it appeared that two or three sucklings per day would delay breeding after parturition in cows (Williams, 1990), while in women, ovarian activity was suppressed by suckling frequencies exceeding five times, and more than 65 minutes in duration (McNeilly *et al.*, 1982; 1983). Thus, an early resumption of oestrous cycles, by limiting suckling to once daily, has been achieved in beef cows (Randel, 1981; Wells *et al.*, 1986; Browning *et al.*, 1993).

Studies have been undertaken (Short *et al.*, 1972; McVey & Williams, 1988; Viker *et al.*, 1989; 1993) in an attempt to elucidate which aspect of suckling behaviour is responsible for the inhibition of reproductive cycles. When attention was focused on the hours of darkness, it appeared that regardless of the natural suckling frequency, cows initiated breeding only if a consistently long interval (more than 7 hours) between sucklings occurred (Stewart *et al.*, 1993a). Furthermore, it appeared that this long interval tended to occur late at night. Based on research with human females, indirect support for this hypothesis has been provided by Howie & McNeilly (1982), who proposed that the absence of breast-feeding between midnight and 08:00 might favour the recovery of the hypothalamic-pituitary-ovarian axis to permit ovulation. When cows were separated from their calves, either during the night or day, the former treatment resulted in more cows exhibiting oestrus during the 90-day breeding period (Stewart *et al.*, 1993b). This study was therefore conducted to establish whether cows that continued to suckle their calves late at night would exhibit a prolongation of postpartum anoestrus.

Materials and Methods

This study was conducted at the Cedara Research Centre (longitude 30°17′, latitude 29°32′). Between 21 and 29 days postpartum, spring-calving, Hereford-type cows (aged 3 – 7 years) and their calves were randomly allocated to 1 of 3 treatment groups (Table 1). Cows were allocated in batches (ranging in calving date by not more than 7 days) to the treatment groups. The first cow calved on 27 July and the last cow on 25 September. The cows in treatments 1 and 2 suckled their calves every 4 hours five times every day. When not suckling, the calves were kept separate from their dams by

Table 1 Time of the day when cows were allowed (+) or not (0) to suckle their calves

	Suckling times					
Treatment	09:00	13:00	17:00	21:00	01:00	05:00
1 – Night suckling	+	0	+	+	+	+
2 – Day suckling	+	+	+	+	0	+
3 – Once daily	0	0	+	0	0	0

confining them to a small pen within the cow-holding area (1 600-m² feedlot). At the appointed suckling times the cows were allowed to enter the calf pen and the calves suckled for only 5 minutes whereafter the cows were again removed. Free contact between cows and calves was possible through the sides of the pen, except that suckling was prevented.

In treatment 1, the calves (n = 15) did not suckle for 8 hours from 09:00 to 17:00 (night suckling), while in treatment 2, this long non-suckling period was from 21:00 to 15:00 (day suckling; n = 17). On 25 August, when the first cows entered the experiment, sunrise and sunset were at 06:25 and 17:37, respectively. The experiment ended on 4 December, at which stage sunrise and sunset occurred at 04:56 and 18:43, respectively. Cows in treatment 3 suckled their calves for a total time (25 minutes) equivalent to that of treatments 1 and 2, but only at 17:00 each day (once daily suckling; n = 18). In this study emphasis was placed on the postpartum stage at which the cow could have become pregnant, i.e. the day on which serve first became possible. Accordingly, at each suckling time (Table 1), regardless of whether the cows had been suckled, observations for oestrus were conducted for 20 minutes after their separation from the calves. Oestrus was defined as standing while being mounted by other cows. Additional signs of behavioural oestrus, such as nasal exploration of the perineal area by other cows, not in oestrus, and of mucous discharge from the vagina, were also carefully noted. In order to minimize stress due to the experimental procedures, no rectal palpations to determine the presence of corpora lutea were conducted. Similarly, to avoid stimulating reproductive activity, the examination of the ovarian status per rectum was excluded. However, in order to eliminate cows that may have ovulated prior to the commencement of treatments, jugular blood samples were collected into heparinized tubes for progesterone assay of the plasma, one day before the treatment was initiated. The method of Butcher *et al.* (1974) was used for progesterone assay.

The calves had access to drinking water and hay while the cows were fed maize silage. *Eragrostis curvula* hay and a mineral lick (equal parts salt, maize meal, urea and di-calcium phosphate) so as to maintain a condition score of 2.5 (0 = thin, 5 = fat). Cows that had not shown oestrus by 80 days after calving (once daily suckling, n = 2; night suckling, n = 13) were removed from the experiment to facilitate re-breeding for other experiments. First-calvers which could not compete effectively for feed and which thus lost condition severely, were also omitted from the analysis (treatment 2, n = 3).

Statistical analysis

The proportions of cows which had not exhibited oestrus by 80 days postpartum were compared by the Chi-square test. The number of days to first oestrus for the three treatment groups was compared using the log rank test (Armitage & Berry, 1987) which is appropriate for survival data. The days for cows that had not shown oestrus by 80 days postpartum were treated as censored observations.

Results

Night suckling (treatment 1) increased ($P < 0.001$) the proportion (13/15) of cows that did not cycle (Figure 1) compared with cows suckled only once daily (treatment 3) or suckled during the day (treatment 2).

The difference in mean days to first oestrus between cows suckled once daily (treatment 3, $\bar{x} = 45.7 \pm 3.2$) and those that missed the suckling at 01:00 (treatment 2, $\bar{x} = 42.2 \pm 2.5$ days) was not statistically significant at the 5% level, although there was some evidence (Figure 1) that treatment 2

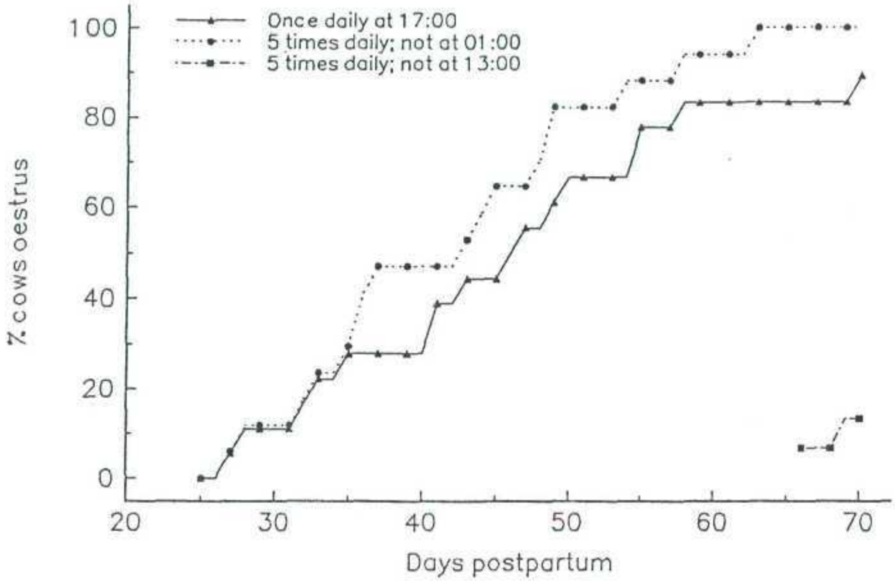

Figure 1 The cumulative % of cows exhibiting first oestrus as influenced by frequency and time of suckling.

had a slightly shorter mean interval to first oestrus ($P < 0.07$). Cows that suckled at night, but not at 13:00 (treatment 1, $\bar{x} = 67.0 \pm 1.5$ days), showed oestrus later ($P < 0.0001$) than treatments 1 and 3 (Figure 1).

Discussion

Just as in the beef cow, nursing of the infant is believed to be the primary cause of lactational acyclicity in women (Short, 1984)). As the infant grows older, the frequency and duration of nursing decrease and this facilitates the resumption of ovarian cycles in the lactating mother (Short, 1984). However, among !Kung women, where the period between births frequently exceeds 3 years (Howell, 1979), the interval between nursings was strongly correlated with the age of the infant, but not the duration of each nursing (Konner & Worthman, 1988). Similarly, no effect of age of the calf on duration of suckling bouts has been recorded (Drewry *et al.*, 1959; Odde *et al.*, 1985; Wells, 1986; Stewart *et al.*, 1993a).

Konner & Worthmann (1988) maintained that the critical change that occurred as the infant aged was a lengthening of the period between nursings. Obviously, the number of nursings per 24 hours must then decrease accordingly. A similar response has been seen in beef calves where the suckling frequency decreased with the age of the calf (Drewry *et al.*, 1959; Hutchinson *et al.*, 1962; Walker, 1962; Reinhardt & Reinhardt, 1981; Wells, 1986; Day *et al.*, 1987). In contrast, Odde *et al.* (1985) have reported no effect of calf age on suckling frequency.

The association between frequency of suckling and interval to first ovulation, postpartum, may not be as clear-cut as presumed. Thus, Stewart *et al.* (1993) observed that at about the time cows could be expected to end postpartum anoestrus, i.e. when their calves were about 45 days or older, the calves were less likely to suckle from 22:00 h to dawn than younger calves. Attention has been drawn to the important ovulation-delaying effect of night-time breast feeding (Howie & McNeilly, 1982; Short, 1984) and, in the discussion following Frisch's (1985) paper, McNeilly proposed that breast feeding before 23:00 was as effective in disrupting onset of normal ovarian activity as suckling between 23:00 and 06:00. McNeilly (pers. comm., 1994) now maintains that the time of day or night is unimportant.

The results obtained here clearly demonstrate (Figure 1) that night suckling prolonged lactation anoestrus. In contrast, when suckling was prevented between 21:00 and 05:00, this was as effective as once daily suckling in stimulating the onset of first oestrus. Thus, it would appear that there may be a time of the night when beef cows are particularly sensitive to the inhibitory influence of suckling. The possibility that the hours after onset of darkness or before first light may be of greater importance than the actual time needs to be considered.

The mechanism by which suckling delays the time of first ovulation after parturition has been the subject of speculation and research. It has been proposed that the release of prolactin in response to nipple stimulation (Short, 1984) may not be the cause of the hypothalamic inhibition observed. A more likely mechanism appears to be the suppressive effects of beta-endorphins on the release of gonadotrophin-releasing hormone (Short, 1984). Williams (1990) has expanded on this thesis as it pertains to the beef cow and Viker *et al.* (1993) have shown that the teat may not be the only sensitive area of the udder. Recent results have shown the mother-offspring bond to be a vital component of the mechanism by which suckling delays ovulation (Silveira *et al.*, 1993) and somatosensory cues appear to play no role in suckling-induced anoestrus (Williams *et al.*, 1993).

A further possibility involves the mechanism by which melatonin mediates the effect of variations in day length on seasonal breeding (Yellon *et al.*, 1992). This hormone is released in a characteristic pattern during darkness in many mammalian species (Deveson *et al.*, 1992; Lincoln, 1992) and the period of exposure to elevated levels of melatonin may be important in modifying the inhibitory influence of night-time suckling. While seeking an explanation, it should not be forgotten that there is also a negative association between calving date and delay to first oestrus (Peters & Riley, 1982; Hauser, 1984). Whatever the final explanation, our results provide strong supportive evidence for Short's (1984) contention that the abandonment of night-time feeding might remove the suckling inhibition of GnRH release from the hypothalamus. This explanation is also supported by the finding that the return of ovarian function in breast-feeding women is associated with night-time pulsatile secretion of LH (Tay *et al.*, 1989). In order to obtain further support for the conclusions regarding late-night suckling, cows in treatment 1 that had reached 80 days postpartum without having exhibited oestrus were then prevented from suckling between 21:00 and 05:00, but continued to suckle their calves five times per 24 hours, as for treatment 2. Of the 7 cows treated in this way 57% were in oestrus within 18 days.

The failure to observe a positive association between milk yield and duration of postpartum anoestrus in beef cows (Day *et al.*, 1987; Beal *et al.*, 1990) also needs to be accounted for. This is especially important for selection in the beef industry where there has been much attention on improving weaning weights. The associated increased pre-weaning growth rates are at least partly dependent on higher milk production of the dams (Beal *et al.*, 1990), with attendant greater nutritional demands. If such demands are not satisfied, the cows could fail to re-breed in time. To date, experimental results have not supported such a trend (Deutscher & Whiteman, 1971; Holloway *et al.*, 1975; Montaño-Bermudez & Nielsen, 1990). At the other end of the scale, cows which fail to produce sufficient milk to satisfy the appetite of the calf are likely to be suckled frequently (Drewry *et al.*, 1959; Odde *et al.*, 1985). Consequently, the chances of suckling occurring during the 'critical' hours of darkness are increased. Such cows may thus be anoestrus for longer than cows producing more milk.

The above conclusions support the proposal (Short, 1984) that supplementation of energy to nursing mothers may decrease the frequency of suckling as a consequence of the improved milk yield. These additional calories might result in night-time nursing being curtailed. The lack of an association between milk yield and duration of lactation anoestrus in beef cows can then be explained in terms of suckling occurring outside the sensitive period of the night.

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References

ARMITAGE, P. & BERRY, G., 1987. Statistical methods in medical research. Blackwell Scientific Publications, Oxford.

BEAL, W.E., NOTTER, D.R. & AKERS, R.M., 1990. Techniques for estimation of milk yield in beef cows and relationships of milk yield to calf weight gain and postpartum reproduction. *J. Anim. Sci.* 68, 937.

BROWNING, R., NEUENDORFF, D.A. & RANDEL, R.D., 1993. Effects of postpartum nutrient intake and once daily suckling on return to estrus and endocrine profiles in fall-calving Brahman cows. *J. Anim. Sci.* 71 (suppl. 1), p. 447 (Abstr).

BUTCHER R.L., COLLINS, W. & FUGO, N., 1974. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. *Endocrinology,* 94, 1704.

DAY, M.L., IMAKAWA, K., CLUTTER, A.C., WOLFE, P.L., ZALESKY, D.D., NIELSEN, M.K. & KINDER, J.E., 1987. Suckling behavior of calves with dams varying in milk production. *J. Anim. Sci.* 65, 1207.

DEUTSCHER, G.H. & WHITEMAN, J.V., 1971. Productivity as two-year-olds of Angus-Holstein crossbreds compared to Angus heifers under range conditions. *J. Anim. Sci.* 33, 337.

DEVESON, S.L., ARENDT, J. & FORSYTH, I.A., 1992. The influence of the pineal gland and melatonin on the reproductive performance of domesticated ungulates. *Anim. Reprod. Sci.* 30, 113.

DREWRY, K.J., BROWN, C.J. & HONEA, R.S., 1959. Relationships among factors associated with mothering ability in beef cattle. *J. Anim. Sci.* 18, 938.

FRISCH, R.E., 1985. Maternal Nutrition and Lactational Amenorrhoea: Perceiving the Metabolic Costs. In: Maternal Nutrition and Lactational Infertility. Ed. Dobbing, J., Raven Press, New York.

HAUSER, E.R., 1984. Seasonal effects on female reproduction in the bovine (*Bos taurus*) (European Breeds). Theriogenology 21, 150.

HOLLOWAY, J.W., STEPHENS, D.F., WHITEMAN, J.V. & TOTUSEK, R.,1975. Performance of 3-year-old Hereford, Hereford × Holstein and Holstein cows on range and in drylot. *J. Anim. Sci.* 40, 114

HOWELL, N., 1979. Demography of the Dobe !Kung. Academic Press, New York.

HOWIE, P.W. & MCNEILLY, A.S., 1982. Effect of breast-feeding patterns on human birth intervals. *J. Reprod. Fert.* 65, 545.

HUTCHINSON, H.G., WOOF, R., MABON, R.M., SALEHE, I. & ROBB, J.M., 1962. A study of the habits of zebu cattle in Tanganyika. *J. Agric. Sci.* 59, 301.

KONNER, M. & WORTHMAN, C.,1988. Nursing frequency, Gonadal function, and birth spacing among !Kung hunter-gatherers. *Science* 207, 788.

LINCOLN, G.A., 1992. Photoperiod-pineal-hypothalamic relay in sheep. *Anim. Reprod. Sci.* 28, 203.

McNEILLY, A.S., GLASIER, A. & HOWIE, P.W., 1985. Endocrine Control of Fertility. I. In: Maternal Nutrition and Lactational infertility. Ed. Dobbing, J., Raven Press, New York.

McNEILLY, A.S., HOWIE, P.W., HOUSTON, M.J., COOK, A. & BOYLE, H., 1982. Fertility after child-birth: adequacy of postpartum luteal phases. *Clin. Endocrinol.* 17, 609.

McNEILLY, A.S., GLASIER, A.F., HOWIE, P.W., HOUSTON, M.J., COOK, A. & BOYLE, H., 1983 Fertility after childbirth: pregnancy associated with breast feeding. *Clin. Endocrinol.* 19, 167.

MONTAÑO-BERMUDEZ, M. & NIELSEN, M.K., 1990. Reproductive performance and variation in body weight during annual cycles for crossbred beef cows with different genetic potential for milk. *J. Anim. Sci.* 68, 2289.

McVEY, W.R &WILLIAMS, G.L., 1988. Mammary somato sensory masking in suckled anestrous cows: Endocrine, reproductive and lactational characteristics. Biol. Reprod. 38 (Suppl. 1), 109(Abstr).

ODDE, K.G., KIRACOFE, G.H. & SCHALLES, R.R., 1985. Suckling behavior in range beef calves. *J. Anim. Sci.* 61, 307.

PETERS, A.R. & RILEY, G.M., 1982. Is the cow a seasonal breeder? *Br. Vet. J.* 138, 535.

RANDEL, R.D., 1981. Effect of once-daily suckling on postpartum interval and cow-calf performance of first-calf Brahman × Hereford heifers. *J. Anim. Sci.* 53, 755.

REINHARDT, V. & REINHARDT, A., 1981. Natural suckling performance and age of weaning in zebu cattle (*Bos indicus*). *J. Agric. Sci.* 96, 309.

ROBYN, C., MEURIS, S. & HENNART, P., 1985. Endocrine Control of Lactational Infertility. In: Maternal Nutrition and Infertility. Ed. Dobbing, J., Raven Press, New York.

SHORT, R.V., 1984. Breast feeding. *Sci. Am.* 250, 23.

SHORT, R.E., BELLOWS, R.A., MOODY, E.L. & HOWLAND, B.E., 1972. Effects of suckling and masectomy on bovine postpartum reproduction. *J. Anim. Sci.* 34, 70.

SILVEIRA, P.A., SPOON, R.A., RYAN, D.P., WILLIAMS, G.L., 1993. Evidence for maternal behaviour as a requisite link in suckling-mediated anovulation in cows. *Biol. Reprod.* 49, 1338.

STEWART, I.B., LISHMAN, A.W. & LOUW, B.P., 1993a. Suckling behaviour and fertility in beef cows on pasture. 1. Suckling behaviour. *S. Afr J. Anim. Sci.* 23, 176.

STEWART, I.B., LOUW, B.P. & LISHMAN, A.W., 1993b. Suckling behaviour and fertility in beef cows on pasture. 2. The influence of twelve-hour calf separation on interval to first oestrus after onset of mating period. *S. Afr. J. Anim. Sci.* 23, 180.

TAY, C.C.K, GLASIER, A. & McNEILLY, A.S., 1989. Twenty-four hour secretory profiles of gonadotrophins and prolactin in breastfeeding women. *J. Reprod. Fert. Abstr. Series* No.3, p. 10 (Abstr).

VIKER, S.D., McGUIRE, W.J., WRIGHT, J.M., BEEMAN, K.B. & KIRACOFE, G.H., 1989. Cow-calf association delays postpartum ovulation in mastectomised cows. *Theriogenology* 32, 467.

VIKER, S.D., LARSON, R.L., KIRACOFE, G.H., STEWART, R.E. & STEVENSON, J.S., 1993. Prolonged postpartum anovulation in mastectomised cows requires tactile stimulation by the calf. *J. Anim. Sci.* 71, 999.

WALKER, D.E., 1962. Suckling and grazing behavior of beef heifers and calves. *N.Z. J. Agric. Res.* 5,331.

WELLS, P.L., 1986. The influence of suckling during early lactation on the resumption of ovarian activity and oestrous behaviour in the Afrikaner cow. PhD. thesis, University of Natal, Pietermaritzburg.

WELLS, P.L., HOLNESS, D.H., McCABE, C.T. & LISHMAN, A.W., 1986. Fertility in the Afrikaner cow.3. Once-a-day suckling and its effect on the pattern of resumption of ovarian activity and conception rate in early lactation. *Anim. Reprod. Sci.* 12, 1.

WILLIAMS, G.L., 1990. Suckling as a regulator of postpartum rebreeding in cattle: A review. *J. Anim. Sci.* 68,831.

WILLIAMS, G.L., McVEY, W.R. & HUNTER, J.F., 1993. Mammary somatosensory pathways are not required for suckling-mediated inhibition of luteinizing hormone-secretion and delay of ovulation in cows. *Biol. Reprod.* 49,1328.

WILLIAMS, G.L., OSBORN, R.G., KIRSCH, J.D. & TILTON, J.E., 1984. Suckling, milking and calf presence as regulators of tonic gonadotropin release and postpartum interval. 10th Int. Congr. Anim. Reprod. Artif. Insem. Univ. Illinois, Urbana-Champaign, p. 410.

YELLON, S.M., FOSTER, D.L., LONGO, L.D. & SUTTIE, J.M., 1992. Ontogeny of the pineal melatonin rhythm and implications for reproductive development in domestic ruminants. *Anim. Reprod. Sci.* 30, 91.

Interaction between melatonin and suckling in prolonging post partum oestrous activity in suckled cows

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The effect on suckling-induced postpartum anoestrus of artificially raising melatonin levels during the day (two 30mg injections) and of abolishing high blood melatonin levels at night (provision of lights) was investigated in this study. As in a previous study, suckling five times in 24 hours, but not at 01:00h (day suckled) resulted in the same delay to first oestrus as suckling only once daily. In contrast, suckling five times in a 24-hour period, but not at 13:00h (night suckled) significantly delayed the time of first oestrus, whereas provision of lights at night (night suckled + lights) abolished this effect. Permitting suckling when melatonin levels were artificially raised during the day (day suckled + melatonin) prolonged anoestrus similar to night suckling. Plasma progesterone levels after first oestrus were significantly higher ($P < 0.001$) in cows suckled once daily than for any of the other treatments.

Suckling while plasma melatonin levels are high prolongs postpartum anoestrus and reduced suckling intensity improves luteal function after first oestrus.

Keywords: melatonin, suckling, anoestrus, beef cows

Introduction

Prolongation of lactation anoestrus in beef cows, due to suckling by the calf, continues to have enormous economic implications for the beef producer. Restriction of suckling to once daily has provided an important management tool for the producers to enable them to negate the inhibitory effects of suckling on resumption of breeding activity (Wells *et al.*, 1986; Browning *et al.*, 1994). However, the mechanisms involved remain unclear, in spite of numerous studies in this field (Williams, 1990)

Stewart *et al.* (1995) provided new insight by demonstrating that frequent suckling was no more suppressive than suckling once daily, provide that suckling did not occur late at night. In attempting to account for their findings, Stewart *et al.* (1995) proposed that night-time elevated secretion of melatonin might be involved. They based this conclusion on the now well established role of melatonin in mediating seasonal control of breeding in domestic ruminants (Yellon *et al.*, 1992) and on the night-time rise in circulating melatonin levels in cows (Martin et al., 1983; Kennaway, 1984) and in ewes (Rollag & Niswender, 1982).

The purpose of this trial was to investigate further the interaction between elevated levels of melatonin and suckling by a calf in postponing the onset of oestrus after parturition.

Procedure

Forty multiparous Hereford-type cows and their spring-born (31 July - 10 September) calves were used in this trial. The body condition score of the cows ranged between 2.75 and 3.5 (0 = emaciated, 5 = obese). The cows were fed an *ad lib* diet of maize silage, *Eragrostis curvula* hay, soyabean and a mineral supplement, to maintain condition during lactation.

The treatments (Table 1) began when the cows were 21 to 26 days post partum and five treatments were applied. For each group, the cows and calves were separated, with the calves being kept in a small enclosure within the cow-pen. Contact between cows and calves was possible through the sides of the enclosure, but suckling was prevented during separation.

The basic procedure (Table 1) was to allow suckling to occur on five separate occasions during a 24-hour period in four treatment groups, while the control group suckled only once-a-day (05:00h). Thus, in two groups an eight-hour rest from suckling occurred at night (21:00h to 05:00h), while in the remaining two groups this eight-hour rest from suckling was during the day (09:00h to 17:00h). The suckling omitted was thus either at 01:00h or at 13:00h. One group of cows that suckled their calves at 01:00h was exposed to normal daylight-darkness (night suckled), while in the other group additional light was provided during the hours of darkness (night suckled + light). The latter treatment was applied in order to prevent the night-time rise in melatonin. Artificial light was provided via two 500 Watt halogen lamps at either end of the 15 metre-wide pen. The lamps were placed at a height of two metres and the light beams just overlapped in the centre of the pen such that the light at this point was 12 Lux. At one meter from the light source the reading was 210 Lux. In both cases the light meter faced the lights. Lights were switched on at 17:00 and switched-off at 7:00.

Table 1 Suckling times and treatments applied

Suckling treatment	Treatment group	No. in group	Suckling times:					
			01:00	05:00	09:00	13:00	17:00	21:00
Once daily	1	8	rest	rest	+	rest	rest	rest
Day suckled	2	9	rest	+	+	+	+	+
Night suckled	3	7	+	+	+	rest	+	+
Day suckled + melatonin	4	8	rest	+	+	+	+	+
Night suckled + lights	5	8	+	+	+	rest	+	+

Where suckling occurred at 13:00, one group of cows received intramuscular injections of melatonin (30mg in 2ml arachis oil) at 08:55 and again at 12:55 (day suckle + melatonin) in an effort to mimic the night-time elevation in circulating melatonin levels. The dose was based on that used in sheep (Nett & Niswender, 1982; Turner & Halford, 1993). The other group received no additional melatonin (day suckle) and did not receive injections of vehicle. This practice was adopted for two reasons. Firstly, this treatment was to be compared to cows treated in exactly the same manner except for the time at which suckling did not occur (night suckled). Secondly, if the procedure involved with injection of the cows twice daily was to influence their response then it should be limited to this group only. The obvious solution was to duplicate the day suckling treatment in a separate group of cows that were sham treated, but numbers of experimental animals were limiting.

At each appointed suckling time the cows were allowed into the calf-pen to suckle their calves, for five minutes, and were then removed. In the control group the cows remained with their calves for 25 minutes to equate the total suckling time allowed the other groups.

All groups were intensively heat spotted after each suckling session to determine the onset of oestrus. A young, active bull ran with each group of cows to assist in heat detection. Bulls were interchanged between treatment groups every three or four days.

To verify the observations of oestrus jugular blood samples were taken approximately five days after observed oestrus or after termination of the experiment and assayed for progesterone according to Butcher *et al.* (1974). The inter- and intra -assay coefficients of variation were 10.8 and 5.4%, respectively. Ovarian palpation was also performed at the time of sampling for progesterone content time to check for the presence of corpora lutea.

For the assay of melatonin, blood samples were taken from three of the cows (randomly selected) that were kept in constant light (NSL). These samples were drawn at 21:00 and 01:00. In order to characterise plasma melatonin levels in the cows that were injected at 09:00 and again at 13:00h a series of hourly blood samples was obtained on a single day. Sampling commenced immediately after the eight cows had been injected at 08:50h and sampling ceased at 16:00h. The time-lapse between injection and sample collection was not more than five minutes for the first and 15 minutes for the last cow to be sampled. On one occasion, cows exposed to normal daylight-darkness were sampled at hourly intervals for 24 hours. Melatonin concentrations were assayed according to the method of Rollag & Niswender (1976) as modified by Visser (1991). The intra- and inter-coefficients of variation were 8 and 13.5%, respectively and the sensitivity of the assay was 8pg/ml.

The number of days to first observed oestrus for the five treatment groups was compared using the logrank test (Armitage & Berry, 1987) which is appropriate for the analysis of survival data. Days for cows that did not exhibit oestrus by the end of the experiment were treated as censored observations. The test was carried out using Proc Lifetest in the statistical package SAS, which provides summary quantiles for each treatment group. Treatment effects on progesterone levels were tested by analysis of variance.

Results

One calf (night suckled) regularly refused to suckle at 01:00h. Where melatoninwas administered, one cow would not permit suckling shortly after having been injected, while another cow was reluctant to suckle on numerous occasions after injection. The data for these three cows was omitted from the results.

The mean number of days to first observed oestrus for each treatment group is presented in Table 2 and the rate at which the cows emerged from anoestrus is depicted in Figure 1.

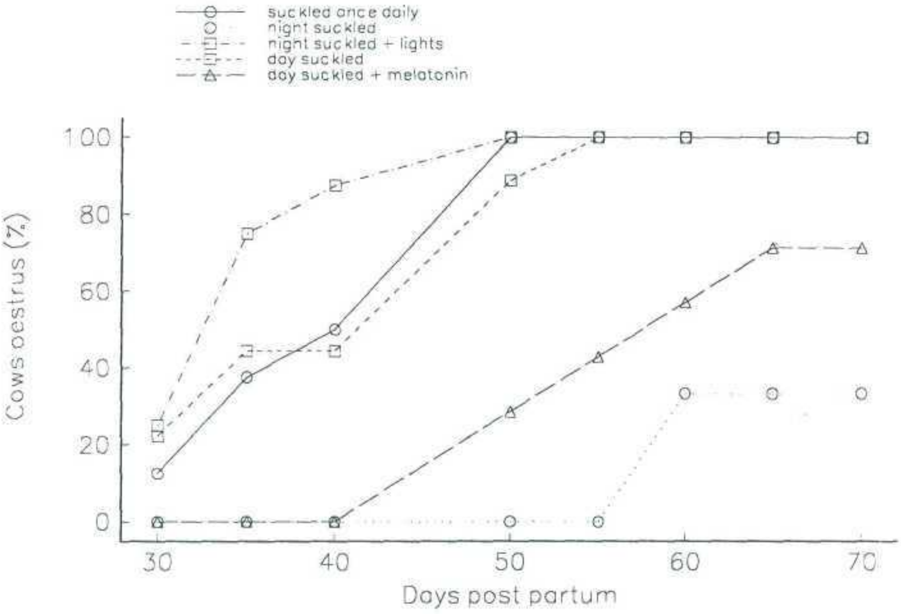

Figure 1 Cumulative percentage of cows showing first oestrus at different stages post partum after being subjected to various calf-suckling treatments

Comparison of the treatment groups using the Proc Lifetest showed that the distribution of number of days to oestrus differed significantly between groups (Table 2). The rate at which cows showed first oestrus was obviously different (Figure 1, Table 2) for the cows suckled at 01:00h and also for the cows injected with melatonin. Therefore, the statistical tests were repeated omitting the data for each of these treatments, in turn. The resultant overall logrank chi-square statistics of 13.35 (3 d.f.) and 0.53 (2 d.f.) indicated that cows receiving melatonin did not exhibit oestrus in the same pattern as the remaining groups, while there was little difference between the other three groups (once daily,, night rest and lights at night).

Table 2 Mean (± SEM) number of days post partum to oestrus and the distribution of days to first oestrus for cows suckled differently and receiving melatonin or additional light

Suckling treatment	Treatment group	Number of cows:-		Days to first observed	Days required for various % to show oestrus			Logrank chi-square
		Oestrus	No oestrus	oestrus	25	50	75	
Once daily	1	8	0	37.7 ± 2.5	34	40	43	4.30
Day suckled	2	9	0	39.9 ± 3.3	31	43	49	3.92
Night suckle	3	2	4	58.5 ± 2.5	0	0	0	-8.47
Day suckled + Melatonin	4	5	2	54.2 ± 2.7	50	54	61	-3.46
Night suckled + lights	5	8	0	35.2 ± 2.1	30	37	41	3.70

Overall logrank chi-square = 26.43 with 4 d.f. (P < 0.0001)

The melatonin content of the plasma samples taken over the period just after administration of the first injection until 16:00h (Figure 2) showed that the level exceeded 200pg/ml for most of this period with the the first peaks occurring one hour after the 09:00h injection and the second within 15 minutes of the 13:00h injection. In contrast, the provision of lights at night resulted in mean levels of 162.5 ± 0.05 and 131.1 ± 0.02 at 21:00 and 01:00h, respectively. The results for the cows exposed to normal light are not yet available.

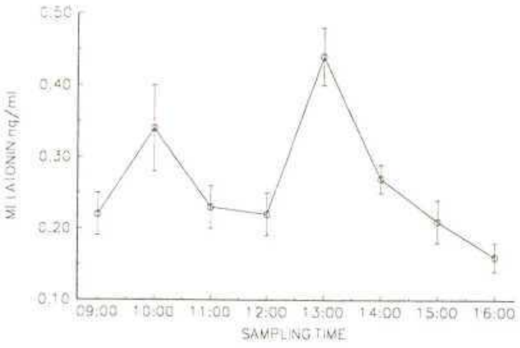

Figure 2 Plasma melatonin in cows injected with 30 melatonin at 08:50 and 12:50h

Assay of the blood samples taken on average 5.6 ± 0.4 days after first observed oestrus indicated that cows suckled only once daily had significantly higher ($P < 0.001$) plasma progesterone concentrations than any of the other treatment groups (Table 3). If a progesterone level of 1 ng/ml plasma is assumed to indicate normal luteal activity then only the cows suckled once daily showed evidence of an active corpus luteum post oestrus. None of the cows that did not exhibit oestrus were shown to have a level of progesterone suggesting that oestrus had been missed in such cows. However, three of these six cows had a palpable corpus luteum when examined prior to termination of oestrous observations.

Table 3 Plasma progesterone concentrations after the first observed oestrus in cows subjected to various suckling treatments

Suckling treatment	Mean plasma progesterone (ng/ml)	% cows oestrus with progesterone > 1pg/ml
Once daily	3.11 ± 0.78	87.5
Day suckled	0.45 ± 0.12	11.1
Night suckled	0.42 ± 0.23	50.0
Day suckled + melatonin	0.32 ± 0.10	0.0
Night suckled + lights	0.28 ± 0.03	0.0

Discussion

The results obtained in this investigation confirm previous findings showing that suckling late at night (after 21:00 and before 05:00h) prolongs lactational anoestrus in beef cows (Stewart *et al.*, 1995). In addition, it has now been demonstrated that abolishment of the night-time rise in melatonin by the provision of additional lights negates the effect of suckling at night. Furthermore, when suckling occurred during the time that daylight melatonin levels were artificially raised this also delayed the time of first oestrus (Figure 1). Sharpe *et al.* (1986) also lengthened the ppi in beef cows by longterm treatment with melatonin, but the response seen may have been the result of suckling occurring during the time of high melatonin levels.

From the results presented in Figure 1 it is evident that day-time melatonin levels in lactating cows injected with melatonin at 08:50 and 12:50h had declined to slightly more than 100pg/ml by 16:00h. Furthermore, cows exposed to light at night exhibited a mean level below 170pg/ml. These levels can be compared to the normal daylight-night concentrations once these are available. The present results are somewhat higher than the day-time levels of 6-60pg/ml in cloprostenol treated heifers (Martin et al., 1983), of 40 - 60pg/ml in ovariectomized, prepubertal heifers (Critser *et al.*, 1987) or 80pg/ml seen in pregnant heifers (Newbold *et al.*, 1991). Assay differences confuse direct comparisons between studies and species.

In this study intramuscular injection of 30 mg of melatonin resulted in a rapid increase in circulating levels and the peak level within 15mins of injection at 12:50h exceeded the level at 16:00h by over 250%, while it was of the same magnitude as the night-time levels seen in calves (Kennaway *et al.*, 1977; Hedlund *et al.*, 1977). By comparison Arendt *et al.*(1983) increased melatonin levels approximately 600% with a single daily injection of 3 mg in ewes and 2.5 mg elevated plasma levels by over 500% within an hour of injection (Turner & Hallford, 1993). Kennaway & Seamark (1980) were mindful of administering unphysiological levels of melatonin and showed that a single s.c.injection of as little as 100ug in oil achieved the normal high levels seen during the night in untreated ewes. They also noted that circulating levels of melatonin were markedly increased within 15 minutes of administration.

When melatonin was administered by the oral route, 2mg in the food mimicked the high night-time levels in ewes (Kennaway & Seamark, 1980), and 4 mg/kg BW in the protein supplement of cows trebled plasma levels within half an hour (Wetteman *et al.*, 1994). Although, oral administration would appear to be preferable to injection in attempting to duplicate the night-time pattern of melatonin secretion (Kennaway & Seamark, 1980), this method does not mimic the marked episodic secretion seen at night in ewes (Bittman *et al.*, 1983).

The slightly accelerated return to oestrus seen in the cows that were suckled while melatonin was artificially raised, as compared to cows suckled late at night (Figure 1), might be accounted for by the failure to duplicate the night-time pattern of secretion reported by Martin et al. (1983). Severe limitation of the number of samples that could be assayed hampers conclusions form the present investigation. An additional consideration is the negative behaviour of three cows toward suckling shortly after having been injected. One cow had to be excluded on this basis. This response to injection partly justifies the decision not to inject cows suckled during daylight (Group 2) with vehicle as a positive control.

Importance of time of night

Most wild animals time their breeding season so that the young are born in spring when their chances of survival are maximised (Malpaux *et al.*, 1996). This applies particularly in non-equatorial regions where the seasonal fluctuations in temperature and food supplies have considerable impact on survival of young (Deveson *et al.*, (1992; Lincoln, 1992).

Photoperiod is the main environmental cue that determines the timing of the breeding period (see Lincoln & Short, 1980; Follet, 1982; Karsch *et al.*, 1984, for reviews). In both long and short day domesticated ungulates the inhibitory and stimulatory photoperiodic stimuli to the reproductive axes are integrated through a complex pathway (Deveson *et al.*, 1992) with alterations in the rhythm of night-time melatonin secretion via the pineal gland being of cardinal importance (Karsch *et al.*, 1988; Deveson *et al.*, 1992). Depending on the species the same signal (day length) thus has different consequences (Deveson *et al.*, 1992) so that parturition can occur at the chosen time.

Although it is generally believed that domestication has led to an almost complete loss of seasonal breeding in cattle and pigs (Malpaux *et al.*, 1996) there is evidence that seasonality persists, at least in beef cattle. Thus, cows are responsive to seasonal changes in the environment immediately post calving (Hauser, 1984). This is based on the negative correlation between calender day of calving and duration of anoestrus (Peters & Riley, 1982; King & MacLeod, 1983; Hauser, 1984; Sharpe *et al.*, 1986, Paterson, 1994; personal communication).

With melatonin being secreted only at night (Karsch *et al.*, 1988), two hypotheses have been proposed to explain how melatonin codes for daylength. These depend on, (1) the duration of the nocturnal phase of high melatonin levels signalling the length of the night (Carter & Goldman, 1983) and (2) the phase of melatonin release, with respect to the light-dark cycle, being important (see Arendt, 1986). Most of the experimental evidence in sheep supports the duration hypothesis (Karsch *et al.*, 1988). However, the proposal of a melatonin sensitive phase of the day, entrained by the light-dark cycle (Deveson et al., 1992) seems to be supported by the suggestion of a rhythm linked to sunset in mares (Palmer & Guillaume, 1992) and a photo-sensitive phase set close to dusk in ewes (Guerin *et al.*, 1994). On the basis of these proposals it seems reasonable to conclude that the inhibitory effect of late-night suckling depends more on the time when it occurs after onset of darkness, and the consequent prevalence of melatonin, than on the time of sunrise. It is also perhaps significant to note that under conditions of natural summer-time daylength, melatonin reached a peak around 24:00h in ovariectomized ewes (Bottman *et al.*, 1983).

Linking melatonin and suckling

The basic reason why anoestrus persists after calving in suckled beef cows is because of suppression of the pulsatile release of LH (see Willliams *et al.*, 1990 for review). It appears that suckling inhibits LH pulsatility through endogenous opioids (EOP) in neural tissue that are related to GnRH synthesis (Malven *et al.*, 1986; Gordon *et al.*, 1987; Barb *et al.*, 1991) and ultimately to LH release.

Similarly, it has been proposed that melatonin could control seasonal anoestrus through its effect on the catecholamine and/or opioid secreting neuronal networks in the hypothalamus (Rasmussen 1991; Lincoln & Maeda, 1992a). Through this mechanism the pulsatile secretion of GnRH, and ultimately that of LH, could be altered (Lincoln, 1992).

If both suckling and melatonin control pulsatile LH secretion via endogenous EOPs, then this may be the link between the prolongation of anoestrus by suckling and time of day. Whether the effects of suckling and night-time melatonin are interactive or additive remains to be determined.

Suckling and luteal function

The finding that once daily suckling results in improved luteal function subsequent to the first observed oestrus after calving (Table) confirms earlier findings where ovulation was induced in anoestrous beef cows by means of GnRH (Lishman *et al.*, 1988). Although a subsequent investigation failed to duplicate the earlier findings there are now two separate pieces of evidence which show that reduced suckling frequency improves luteal function. Whether this result is achieved through improved luteotropic support (see Lishman & Inskeep, 1991, for review) consequent to the greater frequency of episodic LH release

Table 17.1. Variations Among Breeds of Beef Cattle in Intervals from Parturition to First Estrus or First Ovulation[a]

Breed of Cattle	Interval from Parturition to: First Estrus Number Observed	First Estrus Days	First Ovulation Number Observed	First Ovulation Days
Angus	161	72	61	19
Hereford	857	76	76	51
Shorthorn	318	100	52	54

[a] Data are weighted means of values presented by Casida et al. (*5*).

longer postpartum intervals to conception. Certainly this possibility should receive critical study.

The quicker return to functional reproductive status with increasing age observed in this study is of considerable interest. It is widely accepted that two-year-olds suckling their first calves have long postpartum intervals; these data reveal the same problem in three-year-olds, most of which were suckling their second calves. Such findings have led Wiltbank (*52*) to propose breeding all replacement heifers at synchronized estrus in advance of the cow herd. Both two- and three-year-old cows can be expected to perform poorly when they compete with older cows, as appears to have been the case in several experiments reported (*10, 24*) and is the usual situation in the small herds that contain the majority of beef cattle in this country. Higher energy intake either pre- or post-calving (*12*) or weaning of calves eight days before the start of a 42-day breeding season hastened estrus in young cows (*24*).

EFFECTS OF LACTATION OR SUCKLING AND OF PLANE OF NUTRITION

Casida et al. (*5*) concluded that beef cows have longer intervals to first estrus and first ovulation than dairy cows and that the interval to first estrus is increased by suckling and by more frequent milking. These conclusions were confirmed by more recent work (*2, 40*). Weaning calves at a mean age of 55 days shortened the interval from calving to first estrus only in two-year-old cows (*23*), which is not surprising because the mean interval to first estrus in mature cows in that study was 55 days.

Level of energy intake, both before and after calving, influences the length of the postpartum anovulatory and/or anestrous period (*12, 50, 56, 57*). Although Topps (*44*) has indicated that, under certain conditions of natural grazing, protein intake may influence calving percentage of lactating beef cows, work in the United States does not support his conclusion (*10, 23, 54*). Both Wiltbank (*53*) and Topps (*44*) have concluded independently that condition of the cow and whether she is gaining or losing

(Williams *et al.*, 1996) or due to changes prior to ovulation remains open to speculation.

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REFERENCES

ARENDT, J., 1986. Role of the pineal gland and melatonin in seasonal reproductive function in mammals. *Oxf. Rev. Reprod. Biol.* 8, 266.

ARENDT, J., SYMONS, A.M., LAUD, C.A. & PRYDE,S.J., 1983.Melatonin can induce early onset of the breeding season in ewes. *J. Endocrin.* 97, 395.

ARMITAGE, P. & BERRY, G., 1987. Statistical Methods in Medical Research. Blackwell Scientific Publications, Oxford. pp 429 - 433.

BARB,C.R., KRAELING, R.R. & RAMPACEK, G.B., 1991. Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. *Dom. Anim. Endocrinol.* 8,15.

BITTMAN, E.L., DEMPSEY, R.J. & KARSCH, F.J., 1983. Pineal melatonin secretion drives the reproductive response to daylength in the ewe. Endocrinology, 113, 2276.

BROWNING, R., ROBERT, B.S., LEWIS, A.W., NEUENDORFF, D.A. & RANDEL, R.D., 1994. Effects of post partum nutrition and once-daily suckling on reproductive efficiency and preweaning calf performance in fall-calving Brahman (*Bos indicus)* cows. J.Anim.Sci. 72, 984.

BUTCHER, R.L., COLLINS, W. & FUGO, N., 1974. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17ß throughout the 4 day estrous cycle of the rat. *Endocrinology,* 94, 1704.

CARTER, D.S. & GOLDMAN, B.D., 1983. Antigonadal effects of timed melatonin infusion in pinealectomized male Djungarian hamsters (*Phodopus sungorus sungorus*): Duration is the critical parameter. *Endocrinology*, 113: 1261-1267.

CRITSER, J.K., HINSHELWOOD, M.M. & HAUSER, E.R., 1987. The effect of exogenous melatonin administration on gonadotropin and prolactin patterns in ovariectomized estradiol-treated heifers exposed to increasing photoperiod. *Theriogenology*, 28, 257.

DEVESON SHARON L., FORSYTH, ISABEL A. & ARENDT, JOSEPHINE.,1992. Induced out-of-season breeding in British Saanen dairy goats: use of artificial photoperiods and/or melatonin administration. *Anim.Reprod.Sci.* 29, 1.

FOLLET, B.K., 1982. Physiology and photoperiodic time-measurement. In: Vertebrate Circadian Systems. Eds. J. Aschoff, S. Daan & G. Groos. Springer- Verlag. Berlin., pp. 268-275.

GORDON, K., RENFREE, M.B., SHORT, R.V. & CLARKE, I.J., 1987. Hypothalamo-pituitary portal blood concentrations of ß-endorphin during suckling in the ewe. *J.Reprod.Fert.* 79, 397.

GUERIN, M.V., NAPIER, A.J. & MATTHEWS, C.D., 1994. Effect of exogenous melatonin and extending the dark period at dusk before the summer solstice on the onset of oestrus in Romney Marsh ewes. *J. Reprod. Fert.* 101, 145.

HANSEN, P.J. & HAUSER, E.R., 1984. Photoperiodic alteration of postpartum reproductive function in suckled cows. *Theriogenology,* 22,1.

HAUSER, E.R., 1984. Seasonal effects on female reproduction in the bovine (*Bos taurus*)(European breeds). *Theriogenology*, 21, 151.

HEDLUND, L., LISCHKO, M.M., ROLLAG, M.D. & NISWENDER, G.D., 1977. Melatonin daily cycle in plasma and cerebrospinal fluid of calves. Science, N.Y. 195, 686.

KARSCH, F.J., BITTMAN, E.L., FOSTER, D.L., GOODMAN, R.L., LEGAN, S.J. & ROBINSON, J.E., 1984. Neuroendocrine basis of seasonal reproduction. *Recent. Prog. Horm. Res.* 40, 185.

KARSCH, F.J., MALPAUX, B., WAYNE, L.E. & ROBINSON, J.E., 1988. Characteristics of the melatonin signal that provide the photoperiodic code for timing seasonal reproduction in the ewe. *Reprod. Nutr. Dev.*, 28, 459.

KENNAWAY, D.J. & GILMORE, TA., 1984. Effects of melatonin implants in ewe lambs. *J.Reprod. Fert.* 70, 39.

KENNAWAY, D.J. & SEAMARK, R.F., 1980. Circulating levels of melatonin following its oral administration or subcutaneous injection in sheep and goats. *J. Endocr.* 33, 349.

KENNAWAY, D.G., FRITH,R.C., PHILLIPOU, G., MATTHEWS, C.D. & SEAMARK, R.F., 1977. A specific radioimmunoassy for melatonin in biological tissue and fluids and its validation by gas chromatography-mass spectrometry. *Endocrinology*, 101, 119.

KING, G.J. & MACLEOD, G.K., 1983. Reproductive function in beef cows calving in the spring or fall. *Anim. Reprod. Sci.* 25, 255.

LINCOLN, G.A., 1992. Photoperiod-pineal-hypothalamic relay in sheep. *Anim. Reprod. Sci.* 28, 203.

LINCOLN, G.A. & MAEDA, K.I., 1992. Reproductive effects of placing micro-implants of melatonin in the mediobasal hypothalamus and preoptic area in rams. *J. Endocrin*. 132, 201.

LINCOLN, G.A. & SHORT, R.V., 1980. Seasonal breeding: Nature's contraceptive. *Recent. Progr. Horm. Res.* 36, 1.

LISHMAN, A.W. & INSKEEP, E.K., 1991. Deficiencies in luteal function during re-initiation of cyclic breeding activity in beef cows and in ewes. *S. Afr. J. Anim. Sci.* 21,59.

LISHMAN, A.W., LOUW, B.P., VAN NIEKERK, A. & RACIBORSKA, C., 1988. Once daily suckling after GnRH to improve luteal function in beef cows. *29th Congr. S. Afr. Soc. Anim. Prod.*

MALPAUX, B., VIGUIE, C. SKINNER,D.C., THIERY, J.C., PELLETIER, J. & CHEMINEAU, P.,1996. Seasonal breeding in sheep: Mechanism of action of melatonin. *Anim. Reprod. Sci.* 42, 109.

MALVEN, P.V., PARFET, J.R., GREGG, D.W. ALLRICH, R.D. & MOSS,G.E., 1986. Relationships among concentrations of four opioid neuropeptides and luteinizing hormone-releasing hormone in neural tissues of beef cows following early weaning. *J.Anim.Sci.* 62, 723.

MARTIN, T.C. CUNNINGHAM, N.F. & SABA, N., 1983. Estimation of melatonin in bovine plasma: patterns of Jersy heifers treated with cloprostenol at two times of the day. *J. Endocrin.* 98, 189.

NETT, T.M. & NISWENDER, G.D., 1982. Influence of exogenous melatonin on seasonality of reproduction in sheep. *Theriogenology*, 17, 645.

NEWBOLD, J.A., CHAPIN, L.T., ZINN, S.A. & TUCKER, H.A., 1991. Effects of photoperiod on mammary development and concentration of hormones in serum of pregnant dairy heifers. *J. Dairy Sci.* 74, 100.

PALMER, E. & GUILLAUME, D., 1992. Photoperiodism in the equine species - what is a long night? *Anim. Reprod. Sci.* 28, 21.

PETERS, A.R. & RILEY, G.M., 1982. Is the cow a seasonal breeder? *Br. Vet. J.* 138, 533.

RASMUSSEN, D.D., 1991. The interaction between mediobasalhypothalamic dopaminergic and endorphinergic systems as a key regulator of reproduction: a hypothesis. *J. Endocrinol. Invest.* 14, 323.

ROLLAG, M.D. & NISWENDER, G.D., 1982. Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. *Endocrinology*, 98, 482.

SHARPE, P.H., GIFFORD, D.R., FLAVEL, P.F., NOTTLE, M.B. & ARMSTRONG, D.T., 1986. Effect of melatonin on postpartum anestrus in beef cows. *Theriogenology,* 26, 621.

STEWART, IONA B., LOUW, B.P. & LISHMAN, A.W., 1995. Late night suckling inhibits onset of post partum oestrous activity in beef cows. *S.Afr.J.Anim.Sci.* 25, 26.

TURNER, M.L. & HALLFORD, D.M., 1993. Return to estrus and endocrine patterns in early postpartum, spring-lambing ewes treated with melatonin. *Theriogenology,* 39,1245.

VISSER, J.H., 1991. 'N Geslagsendokrinologiese ondersoek na die seisoenaliteit van ramme van vyf verskillende skaap- en bokrasse. M.Sc. Thesis. University of Stellenbosch.

WELLS, P.L., HOLNESS, D.H., MCCABE, C.T. & LISHMAN, A.W., 1986. Fertility in the Africaner cow. 3. Once a day suckling and its effect on the pattern of resumption of ovarian activity and conception rate in early lactation. *Anim. Reprod. Sci.* 12, 1.

WETTEMANN, R.P., TUCKER, H.A., LUSBY, K.S., BISHOP, D.K., YELICH, J.V. & VIZCARRA, J.A., 1994. Treatment with melatonin increases pregnancy rate of beef cattle. *Anim.Sci.Res.Report.*

WILLIAMS, G.L. 1990. Suckling as a regulator of postpartum rebreeding in cattle: A review. J. Anim. Sci. 68, 831.

WIILIAMS, G.L., GAZAL, O.S., GUZMAN VEGA, G.A. & STANKO, R.L. ,1996. Mechanisms regulating suckling-mediated anovulation in the cow. *Anim. Reprod. Sci.* 42, 289.

YELLON, S.M., BITTMAN,E.L., LEHMAN, M.N., OLSTER, D.H., ROBINSON, J.E. & KARSCH, F.J., 1985. Importance of duration of nocturnal melatonin secretion in determining the reproductive response to inductive photoperiod in the ewe. *Biol. Reprod.* 32, 523.

weight are major determinants of postpartum interval to estrus. Each has proposed that a system of condition scoring, or weights at 28-day intervals (*53*), be used to plan the nutritional regimen during late pregnancy and early lactation so that cows are in moderate condition at calving and weight losses during early lactation are limited.

Holstein cows suckling calves produced more milk and required more roughage or winter supplement than Herefords for successful rebreeding performance; Hereford × Holstein crosses were intermediate (*18, 22*). The effects of nutritional requirements for lactation have been difficult to separate from the effects of suckling per se. When suckled cows were fed so that they gained 12 kg during the first two weeks postpartum and nonsuckled cows were fed so that they lost 20 kg, the nonsuckled cows still showed estrus 40 days earlier (*38*). In an Oklahoma study (*49, 59*), Hereford × Holstein cows rearing either single calves or their own and a foster calf were fed so that their winter weight losses were the same despite higher milk production by the cows nursing two calves. Similar weight losses were obtained by feeding 72% more supplement to the cows nursing two calves. The first postpartum estrus occurred an average of 27 days later in the more intensively suckled cows. Similarly, feeding 125% of the NRC-recommended energy level beginning at 220 days of gestation was less effective than once-daily suckling in shortening postpartum interval to estrus in primiparous Brahman × Hereford cows (*34*). However, mastectomy shortened the postpartum interval in cows only if they were on a high level of TDN for 90 days before calving (*39*).

CHANGES OCCURRING DURING THE POSTPARTUM ANESTROUS PERIOD

Wagner and Oxenreider (*45*) reviewed the literature on patterns of follicular growth, estrus, ovulation and fertility after calving and endocrine changes associated with these patterns. Since that time, the use of more sensitive assays for hormones has provided some additional information. Most work has been in dairy cows, and not all observations will apply to lactating beef cows. For example, concentrations of LH in plasma increased with time after calving in dairy cows (*14, 21*), in agreement with changes in pituitary content of LH reported earlier (*5*). In suckled beef cows, plasma concentrations of LH did not vary significantly with time after calving (*20*), even though pituitary LH activity increased with time in the same manner as in dairy cows (*37*). This discrepancy may be real or an artifact due to the small numbers of beef cows sampled in each study (*20, 37*). The ability of the pituitary to release LH in response to GnRH is regained by day seven to ten postpartum (*14, 21*) in dairy cows and by day 15 to 16 in beef cows (*20, 47*).

When plasma concentrations of hormones were followed throughout postpartum anestrus, one of the most significant findings was an increase in progesterone for a few days before first estrus (*1, 9*). Corpora lutea or luteinized follicles have been reported to be the source of the progesterone

(*6*). Indeed, exogenous progestogens have been used successfully to initiate cyclic reproductive activity in anestrous beef cows (*15*). In studies by Wiltbank and his colleagues, the response to progestogen has been enhanced by removal of calves for 48 hours (*55*). This treatment was not effective in thin cows, however (*46*). The reasons for estrus and ovulation after withdrawal of progestogens in cows in moderate condition but not in thin cows have not been determined.

CHANGES IN HORMONAL PATTERNS WITH FACTORS THAT ALTER LENGTH OF ANESTRUS

Only recently have experiments been designed to compare hormonal patterns in cows receiving treatments known to create differences in the postpartum interval to estrus. In most of these studies, comparisons have been made of LH, estrogen, and progesterone levels in plasma. Some workers have measured the response to steroid treatment and others have examined the response to gonadotropins or gonadotropin releasing hormones.

Effects on release of LH. Lishman et al. (*29*) conducted an experiment to determine whether plane of nutrition or prior treatment with FSH would alter the pattern of release of LH in response to 300 μg GnRH. Forty-three two-year-old, pregnant Hereford heifers were subjected to either adequate feeding (100% of NRC-1970-recommended energy requirements) or restricted feeding (60% of requirements) beginning approximately 30 days prior to parturition. None of the cows had palpable CL by 29 days after calving. Animals on each level of nutrition were divided randomly into three groups. Commencing on day 29, the cows received twice-daily intramuscular injections of 0, .125, or .25 mg of FSH for three days. All cows were injected with 300 μg of GnRH one day after the last injection of FSH, and LH was quantified in samples of jugular venous plasma collected every 30 minutes during the ensuing seven hours. The pattern of release of LH, as reflected by concentrations in jugular plasma, was examined by analysis of variance; time was used as an independent, continuous variable and tests were made for interactions of treatment with the partitioned linear, quadratic, and cubic regressions of hormonal concentration on time.

The profile of LH was shown to rise to a peak more rapidly (30 minutes earlier) in the cows on the high plane of nutrition. This effect was not seen by workers who used very few animals and looked only at the peak of LH (*13*). In Lishman's study, release of LH was greater in cows that had palpable follicles in the ovaries when treatment with FSH was initiated. Treatment with FSH did not alter the pattern of release of LH significantly. The proportion of cows with palpable CL seven days after GnRH was similar regardless of plane of nutrition or dose of FSH.

Weaning of calves at 12 hours postpartum shortened the interval to estrus from 50 to 16 days in mature Hereford and Hereford × Angus cows (*35*). In the nonsuckled cows, concentration of LH in peripheral serum was higher during the first seven days postpartum.

In mature Charolais × Hereford cows at an average of 24 days postpartum calves were removed for 0, 24, or 32 hours and 300 μg GnRH was injected at 24 hours after calf removal. The profile of LH in plasma collected at 30-minute intervals over seven hours tended to be higher in the group in which calves were removed for 32 hours than in the other two groups (*19*). Similar results were obtained by Smith and Wiltbank (*41*). In their study, cows with calves removed for 48 hours and receiving 150 μg of GnRH 24 hours after calf removal showed a more rapid rise to the peak of LH than suckled cows. These results agree with those obtained earlier in anestrous ewes, where lactation depressed or delayed release of LH after treatment with progestogen, putresine, and estrogen (*28*) or with progestogen, pregnant mare serum gonadotropin (PMSG), and estrogen (*32*). In mature cows receiving GnRH (*19*), the profile of LH in plasma was positively correlated with size of follicle at palpation one day prior to the injection of GnRH.

Effects relative to estrogen. It has been more difficult to clarify the role of estrogen in determination of the postpartum interval than was the case for progesterone. Changes in estrogen may be more subtle (*1*).

In dairy cows, the peak of LH in response to GnRH was correlated (.67; $P<.01$) with pretreatment concentrations of estradiol in plasma (*14*). In contrast, the pattern of GnRH-induced release of LH in beef cows was unrelated to pretreatment concentrations of estradiol (*19, 29*). It does not appear from examination of the data that this difference can be accounted for by the fact that estradiol was measured in a greater number of pretreatment samples per dairy cow studied (*14*).

In the study by Lishman et al. (*29*) discussed earlier, neither plane of nutrition nor dose of FSH up to .25 mg daily altered concentrations of estradiol-17β in plasma during the period from four days before through eight hours after GnRH. In contrast, treatment for three days with 4 mg of FSH per day did increase estradiol-17β in plasma and follicular development in anestrous cows that were 56 days postpartum (*29*). In cows given GnRH 24 hours after calves were removed (*19*), concentrations of estradiol-17β in plasma collected eight hours after GnRH were greater (2.9 pg/ml) than in control animals (1.8 pg/ml).

Progesterone and luteal function. When CL formed after treatment with GnRH (*19, 29*), their life span, as judged by palpation and concentrations of progesterone in peripheral plasma, was invariably shorter than normal (Fig. 17.2). In contrast to dairy cows, in which estrous cycles were initiated by treatment with GnRH on day 14 postpartum (*3*), beef cows returned to anestrus after these induced CL regressed (*19, 29*). Luteal life span or function did not vary with plane of nutrition (*29*), dose of FSH (*29*), or intensity of suckling during treatment with GnRH (*19*). A number of workers have reported that CL induced by GnRH in anestrous beef cows (*4, 47*) and ewes

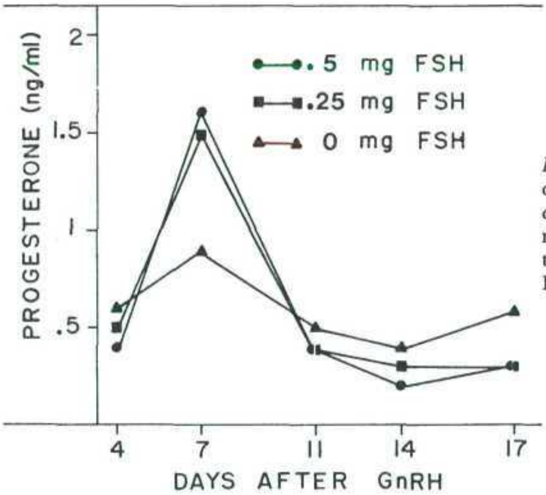

Figure 17.2. Pattern of progesterone in jugular venous plasma of cows that formed corpora lutea in response to 300 μg GnRH after three days of pretreatment with FSH, by daily dose of FSH.

(*8*, *16*) have a short life span, as do many CL occurring spontaneously in the early postpartum period (*39*).

Fall-lambing ewes have been studied as a possible model for the anestrous cow (*26*). One-half of a group of mature ewes was treated with progestogen for four days (17 to 21 days postpartum), and one-half was maintained as controls. Lambs were weaned from one-half of each group on day 20, 24 hours prior to withdrawal of progestogen. All ewes received 150 μg of GnRH intramuscularly on the morning of day 22 postpartum. All ewes formed CL in response to GnRH. Luteal life span was estimated from daily measurements of progesterone in jugular venous plasma, condition of CL at laparotomy on day 7 after GnRH (day 29 postpartum), and the interval from injection of GnRH to estrus. Luteal life span averaged 12 days overall, but was quite variable; an average of three ewes per group had luteal phases of normal length (14 days or greater) while five had luteal phases of short duration. In ewes with short luteal phases, the lengths of those short luteal phases averaged 6.6 ± .9 days in ewes pretreated with progestogen, compared to 9.8 ± 1.0 days in untreated ewes. In the ewes with luteal phases of normal length, pretreatment with progestogen reduced concentrations of progesterone in plasma throughout the sampling period. Weaning had no effect on either luteal life span or profiles or progesterone in either control or progestogen-treated ewes.

Multiple injections or infusions of GnRH were reported to reduce the incidence of short-lived CL in anestrous ewes (*11*, *36*). However, Crighton et al. (*8*) found no difference in plasma progesterone profiles between ewes receiving single and multiple injections of GnRH despite a significantly greater release of LH in response to the multiple injections. In mature beef cows in which CL were induced by a single injection of 300 μg of GnRH at 25 to 30 days postpartum, daily injections of 5 mg of LH in sesame oil:beeswax (95:5) did not extend luteal life span or increase concentrations of progesterone in jugular plasma on days 4, 7, 10, or 14 after GnRH (*27*).

Corpora lutea induced by injection of HCG also had a short life span (*27, 31*).

If stimulation of follicles by FSH or treatment with LH are not able to produce normal life span and function of CL induced during postpartum anestrus, other alternatives must be considered. Because $PGF_2\alpha$ is elevated at parturition, Lewis (*26*) considered the possibility that $PGF_2\alpha$ may remain high in the early postpartum period. He collected uterine venous plasma seven days after an injection of 150 μg of GnRH on day 22 postpartum in fall-lambing ewes and assayed it for $PGF_2\alpha$. Mean concentrations of $PGF_2\alpha$ were lower in uterine venous plasma of ewes receiving prior treatment with progestogen (1.7±.5 ng/ml of plasma vs. 4.2±1.0 in controls), but luteal life span was not associated with concentrations of $PGF_2\alpha$.

The one situation in which CL induced during early lactation in beef cows have had a normal life span was in animals treated with PMSG. Oxenreider (*31*) induced formation of two to eight CL 7 to 9 days after calving by injection of 2500 IU of PMSG on day 2 postpartum and 2000 IU of HCG on day 6 in four cows. Spontaneous estrus and ovulation occurred 20 to 23 days after the induced ovulation. More recently (*30*), treatment with PMSG increased the calving rate in progestogen-treated lactating beef cows. Response to progestogen followed by PMSG varied with breed of cows (*33*). It must be presumed that luteal life span was relatively normal in those animals that became pregnant, but anestrous and reproductively active animals were not considered separately, and concentrations of progesterone were not determined.

PMSG has long been used as an aid to inducing fertile ovulation in the seasonally anestrous ewe. The fact that some fertility is obtained by this technique in lactating ewes implies that luteal life span is relatively normal in at least some ewes. Recently Haresign and Lamming (*17*) reported a relatively normal luteal life span in four anestrous ewes induced to ovulate by GnRH after pretreatment with PMSG. They stated that concentrations of progesterone approached normal when corrected for a presumed higher metabolic clearance rate in anestrous ewes. Luteal life was short in four ewes induced to ovulate by GnRH after pretreatment with estradiol benzoate (*17*). Because PMSG stimulates and estrogen inhibits follicular development (*43*), the degree of follicular development at the time ovulation is induced might be an important determinant of luteal life span. Yet Lishman et al. (*29*) found no relation of luteal life span to size of follicle before treatment with GnRH and no effect of pretreatment with FSH on luteal life span in lactating anestrous beef cows (Fig. 17.2). It is clear that induction of formation of CL is not followed spontaneously by a normal luteal phase during lactational anestrus in beef cows.

CONCLUSIONS

Several factors have been identified that affect the duration of postpartum anestrus in the beef cow. Notable among these are breed, age, intensity of suckling, and plane of nutrition. Recent studies have delineated some changes that occur during this period, but variations in hormonal patterns and in ovarian function in association with the factors known to affect the interval from calving to first estrus are only beginning to be studied. These studies have focused attention upon three points: (a) formation of a corpus luteum does not always occur in response to GnRH or HCG; (b) hormonal patterns may vary with factors known to affect the length of postpartum anestrus; and (c) corpora lutea induced by injection of GnRH have a short life span. Future work should be designed to compare changes in structure and function of follicles and corpora lutea in relation to treatments known to affect postpartum interval to estrus and ovulation. Attention should be directed especially toward factors that affect follicular development and to the hypothalamic control of pituitary hormones involved in this process.

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LITERATURE CITED

1. Arije, F. R., J. N. Wiltbank, and M. L. Hopwood. 1974. *Hormone levels in pre- and postparturient beef cows.* J. Anim. Sci. 39: 338-347.

2. Bellows, R. A., R. E. Short, J. J. Urick, and O. F. Pahnish. 1974. *Effects of early weaning on postpartum reproduction of the dam and growth of calves born as multiples or singles.* J. Anim. Sci. 39: 589-600.

3. Britt, J. H., R. J. Kittok, and D. S. Harrison. 1974. *Ovulation, estrus and endocrine response after GnRH in early postpartum cows.* J. Anim. Sci. 39: 915-919.

4. Britt, J. H., T. E. Kiser, B. E. Seguin, H. D. Hafs, W. D. Oxender, and H. D. Ritchie. 1975. *Fertility after GnRH and* $PGF_{2}\alpha$ *in suckling cows.* J. Anim. Sci. 41: 345. (Abstr.)

5. Casida, L. E., W. E. Graves, J. W. Lauderdale, J. W. Riesen, S. Saiduddin, E. R. Hauser, and W. J. Tyler. 1968. *Studies on the postpartum cow.* Univ. of Wisc. Res. Bull. 270. 54 pp.

6. Castenson, P. E., A. M. Sorenson, Jr., C. R. Cobos, and J. L. Fleeger. 1976. *Source of postpartum P and 20β-OHP preceding estrus in heifers.* J. Anim. Sci. 43: 277. (Abstr.)

7. Chapman, A. G., and L. E. Casida. 1935. *Length of service period in relation to productive and reproductive efficiency in dairy cows.* Proc. Amer. Soc. Anim. Production 28: 66-70.

8. Crighton, D. D., J. P. Foster, W. Haresign, and S. A. Scott. 1975. *Plasma LH and progesterone levels after single or multiple injections of synthetic LH-RH in anoestrous ewes and comparison with levels during the oestrous cycle.* J. Reprod. Fert. 44: 121-124.

9. Corah, L. R., A. P. Quealy, T. G. Dunn, and C. C. Kaltenbach. 1974. *Prepartum and postpartum levels of progesterone and estradiol in beef heifers fed two levels of energy.* J. Anim. Sci. 39: 380-385.

10. Davis, D., R. R. Schalles, G. H. Kiracofe, and D. L. Good. 1977. *Influence of winter nutrition on beef cow reproduction.* J. Anim. Sci. 45: 430-437.

11. Domanski, E., F. Przekop, B. Skubiszewski, B. Wroblewska, and E. Stupnicka. 1977. *Effect of prolonged infusion of small doses of LH-RH on the release of LH and ovulation in ewes during mid-anoestrus.* J. Reprod. Fert. 51: 457-460.

12. Dunn, T. G., J. E. Ingalls, D. R. Zimmerman, and J. N. Wiltbank. 1969. *Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake.* J. Anim. Sci. 29: 719–726.

13. Entwistle, K. W., and L. A. Oga. 1977. *Effect of plane of nutrition on luteinizing hormone (LH) response to luteinizing hormone releasing hormone (LH-RH) in anoestrus post partum beef cows.* Theriogenology 8: p. 190.

14. Fernandes, L. C., W. W. Thatcher, C. J. Wilcox, and E. P. Call. 1978. *LH release in response to GnRH during the postpartum period of dairy cows.* J. Anim. Sci. 46: 443–448.

15. Foote, W. D., and J. E. Hunter. 1964. *Post-partum intervals of beef cows treated with progesterone and estrogen.* J. Anim. Sci. 23: 517–520.

16. Haresign, W. 1975. *Ovarian response to synthetic LH-RH in anoestrous ewes.* J. Reprod. Fert. 44: 127–129.

17. Haresign, W., and G. E. Lamming. 1978. *Comparison of LH release and luteal function in cyclic and LH-RH treated anoestrous ewes pretreated with PMSG or oestrogen.* J. Reprod. Fert. 52: 349–353.

18. Holloway, J. W., D. F. Stephens, J. V. Whiteman, and R. Totusek. 1975. *Performance of 2-year-old Hereford, Hereford × Holstein and Holstein cows on range and in drylot.* J. Anim. Sci. 40: 114–125.

19. Inskeep, E. K., A. W. Lishman, R. L. Butcher, and S. M. J. Allison. 1977. *Effect of short-term removal of the calf on release of LH, ovulation and CL function following GnRH in beef cows.* Abstracts, Amer. Soc. Anim. Sci. 69th Ann. Mtg., pp. 171–172. (Abstr.)

20. Irvin, H. J., H. A. Garverick, A. A. Zaied, D. J. Kesler, B. N. Day, and R. S. Youngquist. 1977. *LH response to GnRH in postpartum suckled beef cows.* Abstracts, Amer. Soc. Anim. Sci. 69th Ann. Mtg., p. 172. (Abstr.)

21. Kesler, D. J., H. A. Garverick, R. S. Youngquist, R. G. Elmore, and C. J. Bierschwal. 1977. *Effect of days postpartum and endogenous reproductive hormones on GnRH-induced LH release in dairy cows.* J. Anim. Sci. 45: 797–803.

22. Kropp, J. R., D. F. Stephens, J. W. Holloway, J. V. Whiteman, L. Knori, and R. Totusek. 1973. *Performance on range and in drylot of two-year-old Hereford, Hereford × Holstein and Holstein females as influenced by level of winter supplementation.* J. Anim. Sci. 37: 1222–1232.

23. Lamm, W. D., J. K. Ward, and G. C. White. 1977. *Effects of nitrogen supplementation on performance of gestating beef cows and heifers grazing corn crop residues.* J. Anim. Sci. 45: 1231–1238.

24. Laster, D. B., H. A. Glimp, and K. E. Gregory. 1973. *Effects of early weaning on postpartum reproduction of cows.* J. Anim. Sci. 36: 734–740.

25. Laster, D. B., H. A. Glimp, L. V. Cundiff, and K. E. Gregory. 1973. *Factors affecting dystocia and the effects of dystocia on subsequent reproduction in beef cattle.* J. Anim. Sci. 36: 695–705.

26. Lewis, G. S. 1977. *Factors affecting concentrations of prostaglandins* $F_2\alpha$ *and* E_2 *in uterine venous blood of ewes.* Ph.D. Dissertation. West Virginia University, Morgantown. 118 p.

27. Lewis, G. S., and E. K. Inskeep. 1977. Unpublished data. West Virginia University, Morgantown.

28. Lewis, P. E., D. J. Bolt, and E. K. Inskeep. 1974. *Luteinizing hormone release and ovulation in anestrous ewes.* J. Anim. Sci. 38: 1197–1203.

29. Lishman, A. W., S. M. J. Allison, R. L. Butcher, and E. K. Inskeep. 1977. *Release of LH, ovulation and CL function after GnRH in underfed lactating beef cows primed with FSH.* Abstracts, Soc. Anim. Sci., 69th Ann. Mtg., p. 184.

30. Mulvehill, P., and J. M. Sreenan. 1977. *Improvement of fertility in post-partum beef cows by treatment with PMSG and progestogen.* J. Reprod. Fert. 50: 323–325.

31. Oxenreider, S. L. 1968. *Effects of suckling and ovarian function on postpartum reproductive activity in beef cows.* Amer. J. Vet. Res. 29: 2099–2102.

32. Pelletier, J., and J. Thimonier. 1973. *Comparison of the induced preovulatory LH discharge in lactating and dry sheep during anestrus.* J. Reprod. Fert. 33: 310–314.

33. Pelot, J., D. Chupin, D. Auger, F. LeProvost, and P. Mauleon. 1976. *Fertility of nursing cows of Aubrac, Charolais and Salers breeds after oestrus induction and synchronization by*

SC21009 implants during postpartum anoestrus. 8th Int. Congr. Anim. Reprod. Art. Insem. 3: 499-502.

34. Randel, R. D., and G. A. Welker. 1977. *Effect of energy intake and once daily suckling on postpartum interval in Brahman × Hereford heifers.* Abstracts, Amer. Soc. Anim. Sci., 69th Ann. Mtg., p. 198.

35. Randel, R. D., R. E. Short, and R. A. Bellows. 1976. *Suckling effect on LH and progesterone in beef cows.* J. Anim. Sci. 42: 267. (Abstr.)

36. Restall, B. J., R. D. Kearins, and B. G. Starr. 1977. *Studies of pituitary function in lactating ewes.* J. Reprod. Fert. 49: 291-296.

37. Saiduddin, S., and W. D. Foote. 1964. *Pituitary luteinizing hormone activity of postpartum bovine.* J. Anim. Sci. 23: 502.

38. Short, R. E., R. A. Bellows, E. L. Moody, and B. E. Howland. 1972. *Effects of suckling and mastectomy on bovine postpartum reproduction.* J. Anim. Sci. 34: 70-74.

39. Short, R. E., R. D. Randel, and R. A. Bellows. 1974. *Factors affecting reproduction in the postpartum cow.* J. Anim. Sci. 39: 226. (Abstr.)

40. Smith, L. E., Jr., and C. K. Vincent. 1972. *Effects of early weaning and exogenous hormone treatment on bovine postpartum reproduction.* J. Anim. Sci. 35: 1228-1232.

41. Smith, M. F., and J. N. Wiltbank. 1977. Unpublished data. Texas A & M University Agricultural Research Station, Beeville.

42. Spelbring, M. C., T. G. Martin, and K. J. Drewry. 1977. *Maternal productivity of crossbred Angus × Milking Shorthorn cows. II. Cow reproduction and longevity.* J. Anim. Sci. 45: 976-982.

43. Squires, E. L., R. J. Scaramuzzi, B. V. Caldwell, and E. K. Inskeep. 1972. *LH release and ovulation in the prepuberal lamb.* J. Anim. Sci. 34: 614-619.

44. Topps, J. H. 1977. *The relationship between reproduction and undernutrition in beef cattle.* World Rev. Anim. Prod. 13: 43-49.

45. Wagner, W. C., and S. L. Oxenreider. 1971. *Endocrine physiology following parturition.* J. Anim. Sci. 32, Suppl. 1: 1-16.

46. Walters, D. L., B. Songster, M. Valencia, W. C. Burrell, and J. N. Wiltbank. 1977. *Steroids in conjunction with 48 hr calf removal or early weaning in thin anestrous cows.* Abstracts, Amer. Soc. Anim. Sci. 69th Ann. Mtg., pp. 215-216.

47. Webb, R., G. E. Lamming, N. B. Haynes, H. D. Hafs, and J. G. Manns. 1977. *Response of cyclic and post-partum suckled cows to injections of synthetic LH-RH.* J. Reprod. Fert. 50: 203-210.

48. Welch, J. A., A. J. Hackett, C. J. Cunningham, J. O. Heishman, S. P. Ford, R. N. Nadaraja, W. Hansel, and E. K. Inskeep. 1975. *Control of estrus in lactating beef cows with prostaglandin* $F_{2}\alpha$ *and estradiol benzoate.* J. Anim. Sci. 41: 1686-1692.

49. Wetteman, R. P., E. J. Turman, R. D. Wyatt, and R. Totusek. 1976. *Suckling intensity and reproduction in range cows.* J. Anim. Sci. 42: 267. (Abstr.)

50. Whitman, R. W., E. E. Remmanga, and J. N. Wiltbank. 1975. *Weight change condition and beef cow reproduction.* J. Anim. Sci. 41: 387. (Abstr.)

51. Whitmore, H. L., W. J. Tyler, and L. E. Casida. 1974. *Effects of early postpartum breeding in dairy cattle.* J. Anim. Sci. 38: 339-346.

52. Wiltbank, J. N. 1974. *Management programs to increase reproductive efficiency of beef herds.* J. Anim. Sci. 38, Suppl. 1: 58-67.

53. Wiltbank, J. N. 1978. *Management of heifer replacements and the brood cow herd through the calving and breeding periods.* Pages 156-208 *in* C. C. O'Mary and I. A. Dyer, eds. *Commercial Beef Cattle Production,* Lea & Febiger, Philadelphia.

54. Wiltbank, J. N., J. Bond, E. J. Warwich, R. E. Davis, A. C. Cook, W. L. Reynolds, and M. W. Hazen. 1965. *Influence of total feed and protein intake on reproductive performance in the beef female through second calving.* U.S. Dept. Agric. Tech. Bull. 1314.

55. Wiltbank, J. N., and S. E. Mares. 1977. *Breeding at a predetermined time following Synchro-Mate-B treatment.* Proc. 11th Conf. on A.I. of Beef Cattle, Denver, pp. 57-65.

56. Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Gregory, and R. M. Koch. 1962. *Effect of energy level on reproductive phenomena of mature Hereford cows.* J. Anim. Sci. 21: 219-225.

57. Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, and D. R. Zimmerman. 1964. *Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving.* J. Anim. Sci. 23: 1049–1053.

58. Woloshuk, J. M. 1977. *Application of* $PGF_{2\alpha}$ *and estradiol benzoate to synchronize estrus in beef cattle.* M. Agric. Thesis, West Virginia University, Morgantown, 22 pp.

59. Wyatt, R. D., M. B. Gould, and R. Totusek. 1977. *Effects of single vs. simulated twin rearing on cow and calf performance.* J. Anim. Sci. 45: 1409–1414.

Failure to induce ovulation by short-term calf removal in lactating beef cows on dry-lot

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Beef heifers were fed during late pregnancy to produce cows in poor, average, and good condition at calving late in winter. The animals were fed on dry-lot for the major part of the experiment. At 50 days of age half the calves were removed from their dams for 7 days, but no cows were induced to ovulate. The remaining calves were temporarily weaned for one week at 80 days of age. Only two cows exhibited oestrus shortly after calf removal.

Vleisbeesverse is gedurende laatdragtigheid gevoer om koeie in maer, gemiddelde, en goeie kondisie te kry ten tye van kalwing laat in die winter. Die diere is grotendeels op kraal gevoer. Die helfte van die kalwers is op 'n ouderdom van 50 dae tydelik gespeen vir 7 dae, maar geen van die koeie het geovuleer nie. Die oorblywende kalwers is ook vir 'n week tydelik gespeen toe hulle 80 dae oud was. Slegs twee koeie was kort hierna bronstig.

Keywords: Temporary weaning, beef cows, ovulation, dry-lot

The low calving rate of many beef herds is of national importance when considering increased production from a relatively fixed cattle population. The greatest wastage of potential weaners can be traced to cows failing to be mated during the limited annual breeding period (Reynolds, 1967; Inskeep & Lishman, 1978). This, in turn, is a consequence of a delay in the return to reproductive cyclicity after parturition and is influenced by both nutrition and suckling of the calf (Chenoweth, 1984).

Considerable interest is being shown world-wide in methods whereby the inhibitory influence of suckling can be overcome. The findings have been reviewed by Chenoweth (1984), who noted that weaning stratagems are likely to have their greatest beneficial effect on first-calf heifers. However, inadequate feeding of such breeding females and weaning too soon after calving will reduce the response.

A trial was conducted using 45 yearling *Bos taurus* (Aberdeen Angus, Sussex, and Hereford crossbreds) type heifers which had been inseminated with Brahman semen so as to begin calving during the second week in August. During late pregnancy (May) the pregnant heifers were randomly divided, after blocking for livemass and date of service, into three groups (15/group) and fed for approximately three months on high, medium, and low planes of nutrition. These levels of feeding were intended to provide for a wide range in the body condition of the heifers at calving. The diets consisted of varying amounts of maize or kikuyu silage, veld hay, and NPN lick. After calving all the cows received 4 kg per day of a maize silage-chicken litter-molasses mixture with NPN lick and veld hay supplied *ad lib*.

At 50 days post-partum the calves were removed from half the cows while suckling was not interrupted for the remaining cows (controls). For the 7 days that the calves did not suckle they were held approximately 1 km from the cows where they received concentrate and hay. Throughout the winter – early summer period the experimental animals were maintained on dry-lot except that the calves had access to an adjacent kikuyu pasture. All animals were turned out to grazing in mid-December.

Regular determinations of bodymass and body condition (five-point scale with 0 = thin and 5 = fat) were made, the latter being judged by two independent observers. The ovaries of all the cows were palpated per rectum at 50 days post-partum and again 7 days later to check for ovulations or changes in size distribution of ovarian follicles. Observations for oestrus (standing to be mounted by other cows) occurred throughout the daylight hours.

The nutritional levels that were applied succeeded in achieving differences in body condition at 50 days after calving (Table 1). However, the change in bodymass was not according to expectation with the medium and low levels resulting in similar gains during pregnancy.

Table 1 Bodymass and body condition characteristics of cows prior to and during lactation

	Nutritional level		
Measurement	High	Medium	Low
Change in bodymass (kg) during last 3 months of gestation	47,0 ± 3,8	2,5 ± 3,1	−3,0 ± 3,6
Bodymass (kg) : $n = 47$			
7 days before calving commenced		392,6 ± 5,5	
50 days post-partum		324,6 ± 6,4	
80 days post-partum		328,0 ± 5,9	
Number of cows with body condition[a]			
Good		9	
Average		18	
Poor		18	

[a]Assessed 50 days post-partum on a five-point scale: Good = >2,5; Average = 2 – 2,5; Poor = <2.

Not a single cow ovulated or showed oestrus following temporary removal of the calf, and no marked growth in follicles was evident. This result was unexpected in view of the success obtained by Symington & Hale (1967), Holness, Hopley & Hale (1978) and Holness, Hale & Hopley (1980) who interrupted suckling for similar periods.

Since there was no measurable response at 50 days post-partum the treatment groups were switched so that when the calves were 80 days old, those cows which earlier served as controls had their calves removed for 7 days. Checks for ovulation and oestrus were conducted as before. Only two cows exhibited oestrus and ovulated 5 days after removal of their calves. A further two cows showed oestrus 29 and 33 days after suckling was interrupted. None of the cows which continued to suckle their calves ovulated or exhibited oestrus. Possible reasons for the failure of the cows to respond to calf removal include the confinement to dry-lot for an extended period (Warnick, Koger & Dixon, 1977), the absence of stimulation by bulls (Chenoweth, 1984) or inadequate bodymass (Meaker, Coetsee, Smith & Lishman, 1980).

Based on the average bodymass of the lactating cows at 80 days post-partum (Table 1) at least 50% of them should have ovulated and exhibited oestrus by this time (Meaker, *et al.*, 1980). A similar conclusion is reached when body condition is used as measure of the physiological status of the cows (Van Niekerk, 1982). However, this occurred only 30 days after the cows had been removed from dry-lot when 26 of the 45 cows had a corpus luteum within the ovary. A further possible explanation of the lack of positive response is that the total removal of the calves may have imposed undue stress on their dams (Symington, Gregor & Hale, 1967). The use of nose-plates to reduce this stress was not shown to be beneficial (unpublished data) although Holness, *et al.* (1978; 1980) had obtained positive results with this method of weaning. Clearly, the circumstances under which suckling can be manipulated to promote early onset of reproductive activity in the lactating beef cow need careful examination. Furthermore, the limiting factors require detailed elucidation before temporary weaning can become a useful tool for on-the-farm application.

References

CHENOWETH, P.J., 1984. Reproductive management procedures in control of breeding. *Proc. Aust. Soc. Anim. Prod.* 15, 28.

HOLNESS, D.H., HALE, D.H. & HOPLEY, J.D.H., 1980. Ovarian activity and conception during the post-partum period in Afrikaner and Mashona cows. *Zim. J. Agric. Res.* 18, 3.

HOLNESS, D.H., HOPLEY, J.D.H. & HALE, D.H., 1978. The effects of plane of nutrition, liveweight, temporary weaning and breed on the occurrence of oestrus in beef cows during the post-partum period. *Anim. Prod.* 26, 47.

INSKEEP, E.K. & LISHMAN, A.W., 1978. Factors affecting postpartum anoestrus in beef cattle. *Beltsville Symp. Agric. Res. [3] Anim. Reprod.*, 277.

MEAKER, H.J., COETSEE, T.P.N., SMITH, M. & LISHMAN, A.W., 1980. The relationship between bodymass and fertility of beef cows of different ages. *S. Afr. J. Anim. Sci.* 10, 83.

REYNOLDS, W.L., 1967. Breeds and reproduction. In: Factors affecting calf crop. Eds T.J. Cunha, A.C. Warnick & M. Koger. University Florida Press, Gainesville. pp. 244 – 259.

SYMINGTON, R.B., GREGOR, A. & HALE, D.H., 1967. Sexual activity in lactating ranch cows. *Rhod. Zamb. Mal. J. Agric. Res.* 5, 233.

SYMINGTON, R.B. & HALE, D.H., 1967. Methods to stimulate sexual activity in lactating Zebu cows. *Rhod. Zamb. Mal. J. Agric. Res.* 5, 13.

VAN NIEKERK, A., 1982. The effect of body condition as influenced by winter nutrition, on the reproductive performance of the beef cow. *S. Afr. J. Anim. Sci.* 12, 383.

WARNICK, A.C., KOGER, M. & DIXON, W.P., 1977. Effect of breed and management on reproduction in beef cows. *Abstracts 69th Ann. Mgt Amer. Soc. Anim. Sci.* University of Wisconsin, 217.

PAPER 42

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FERTILITY IN THE AFRIKANER COW. 2. OVARIAN RECOVERY AND CONCEPTION IN SUCKLED AND NON-SUCKLED COWS POST PARTUM

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ABSTRACT

Wells, P.L., Holness, D.H., Freymark, P.J., McCabe, C.T. and Lishman, A.W., 1985. Fertility in the Afrikaner cow. 2. Ovarian recovery and conception in suckled and non-suckled cows post partum. *Anim. Reprod. Sci.*, 8: 315—326.

The post-partum reproductive performance of suckled and non-suckled Afrikaner cows was compared in order to assess the importance of suckling in the resumption of post-partum ovarian function in this breed. Non-suckled cows were weaned at 3 days post partum whilst calves in the control group had continuous access to their dams. Commencement of ovarian activity was significantly ($P < 0.01$) earlier in non-suckled cows at 18.1 ± 5.2 (mean ± SD) days post partum than in suckled cows 53.2 ± 19.7 days. Conception rates were significantly higher ($P = 0.01$) in non-suckled cows (80%) than in suckled cows (50%). Mean intervals from parturition to conception were 45.8 ± 15.8 (mean ± SD) days in non-suckled cows as compared with 70.9 ± 17.9 days in suckled cows. The conception rate associated with each ovulation appears to be highest between 50 and 70 days post partum regardless of the degree of suckling stimulus. Four classes of ovarian activity were recorded from the progesterone profiles compiled. The incidence of short cycles was unaffected by the degree of suckling stimulus. Weaning may ($P = 0.05$) reduce the proportion of cows which exhibit regular cyclic ovarian activity without conceiving. Coitus during oestrus may ($0.01 < P > 0.05$) be more easily observed in non-suckled cows than in their suckled contemporaries. Suckling altered the diurnal distribution of coitus, with non-suckled cows showing the highest frequency for this component of oestrus before 09.00 h and after 15.00 h, whilst in suckled cows coitus appeared to be more evenly distributed throughout the day.

INTRODUCTION

The Afrikaner cow is used extensively in Zimbabwe and yet has a record of sub-fertility characterized by prolonged post-partum anoestrus and poor conception rates (55—65%) within a 90-day bulling period (Trail et al., 1977; APRU Report, 1980). The breed is known to possess strong mothering instincts as indicated by considerable aggression soon after calving and the absence of any tendency to allow calves apart from her own to suckle.

Much research effort has been directed towards the physiological aspects of reproduction in relation to the cow-calf association during lactation. It has been shown that the resumption of ovarian activity in early lactation, conception rate and post-partum interval (PPI) to conception are influenced to varying degrees by suckling stimulus and suckling intensity (Wiltbank and Cook, 1958; Randel and Welker, 1976; Wetteman et al., 1978; Gonzalez-Stagnaro, 1980; Inskeep and Lishman, 1981).

A reduction in the intensity of the suckling stimulus is achieved by either a complete interruption in suckling for a period or by limited access of the calf to its dam each day. The former, involving short term (< 96 h) complete removal of the calf from its dam (temporary weaning), has shown variable results in terms of stimulation of oestrus and improvement of conception rates (Rodriguez et al., 1980). Temporary weaning has been used in conjunction with the synchronization of oestrus and artificial insemination programmes to improve conception rates (Smith et al., 1979; Kiser et al., 1980; Pace and Sullivan, 1980). The alternative is a long-term reduction in suckling intensity (partial weaning) where daily access of the calf to its dam is restricted (Randel and Welker, 1976; Reeves et al., 1980).

Studies on the manipulation of suckling have mainly been conducted with *Bos taurus* breeds. The possibility exists that the endocrine systems of *B. taurus* and *B. indicus* animals, in regard to their sensitivity to environmental stimuli and endogenous hormone output, are different. The Brahman breed appears to differ from the Hereford with regard to timing of peak oestrogen concentration prior to oestrus and timing of ovulation in relation to the onset of oestrus (Randel, 1980). *Bos indicus* and *B. taurus* breeds may differ in progesterone concentrations and susceptibility to fertility problems (Adeyemo and Heath, 1980). Moreover, timing of ovulation in relation to the onset of behavioural oestrus may differ between Afrikaner and other breeds (McCabe et al., 1980).

This study was undertaken in the Afrikaner cow to ascertain the extent to which suckling affects ovarian activity, behavioural components of oestrus and conception rates during lactation.

MATERIALS AND METHODS

Experimental cows

Sixty mature (range from 3 to 9 years), multiparous (range 1 to 5 calves) Afrikaner cows were used in the study. They were considered to have fat reserves at the start of the experimental period in excess of those found in similar animals in commercial herds. Conditions scores ranged from 2.5 to 3 on a scale of 0.5 (very thin) to 5 (excessively fat).

Method

Cows in the control group had continuous access to their calves. Cows in the weaned group had their calves removed at 3 days of age. All weaned calves were artificially reared. The two treatment groups were grazed in separate paddocks to avoid contact between weaned cows and calves of the control group. All cows remained on the trial until 100 days post partum. Duplicate blood samples were collected on days 7, 14 and 21. From day 23 to 100 post partum blood samples were collected three times each week by jugular venipuncture at 08.00 h. Samples were centrifuged and the plasma stored at −20°C within 120 min of collection.

Progesterone assay

The assay was performed according to the method of Holness et al. (1980). Sensitivity of the assay was 200 pg/ml of plasma with a 200 μl sample of plasma. For each assay, 8 control plasma samples were included with the 70 plasma progesterone determinations per standard curve. These control plasma samples were derived from a single plasma pool which was the same for the duration of progesterone analyses for this trial. From these control plasma inclusions, inter- and intra-assay variation was estimated. Intra-assay variation was ± 0.35 ng/ml (standard error of the mean, SEM) or 7% and inter-assay variation was ± 0.44 ng/ml (SEM) or 9% based on a control plasma value estimated to be 4.95 ng/ml. Standard error of a single determination was 0.96 ng/ml.

Bulls

All bulls used in the study were semen-tested and physically examined to ensure the absence of any structural abnormalities of testes, sheath and penis. Bulls were rotated each week between treatment groups to minimise any effects of sire fertility on conception rates. Bulls had access to cows continuously from calving until the end of the trial. Within this period cows were removed from the trial as they reached 100 days post partum.

Behaviour

Behaviour records were compiled for each cow whilst under observation from 04.30 h until 18.30 h each day for the duration of the trial. Only activities associated with oestrus were recorded and included association of cows and bull in a sexually active group, homosexual mounts, homosexual stands, heterosexual mounts by cows and coitus.

RESULTS

Ovarian activity

Ovulation was considered to have occurred where plasma progesterone levels rose above 1 ng/ml for a minimum of 72 h. Based on these criteria, Table I provides the post-partum intervals to first ovulation. In the absence of suckling, resumption of ovarian activity is advanced significantly ($P < 0.01$) by 35 days to 18.1 ± 5.2 in non-suckled cows. Following the resumption of ovarian activity short oestrous cycles (< 13 days) were ob-

TABLE I

Mean post-partum intervals in days to first ovulation, first observed oestrus, first observed service and conception

	Suckled (mean ± SD)	Non-suckled (mean ± SD)	Significance
First ovulation	53.2 ± 19.7	18.1 ± 5.2	$P < 0.01$
Length of shortened cycle	10.8 ± 1.9	9.5 ± 1.6	NS
Incidence of short luteal phase at first ovulation	6/26	8/29	NS
First observed oestrus	53.3 ± 21.0	18.6 ± 10.2	NS
Incidence of observed oestrus	24/30	28/30	NS
First observed service	58.0 ± 23.5	24.5 ± 15.6	NS
Incidence of observed service	12/30	26/30	$P < 0.05$
Conception	70.9 ± 17.9	45.8 ± 15.8	NS

TABLE II

Post-partum intervals to each ovulation at which conception did or did not occur in suckled and non-suckled cows

Number of of ovulation		Non-suckled		Suckled	
		n	(days)	*n*	(days)
First	no conception	27	17.9 ± 5.3	24	51.5 ± 19.5
	conception	2	21.5 ± 0.7	2	74.5 ± 2.1
Second	no conception	18	36.1 ± 7.9	13	66.2 ± 18.1
	conception	9	34.1 ± 8.2	10	69.9 ± 19.9
Third	no conception	7	64.1 ± 18.9	6	79.5 ± 10.9
	conception	11	54.0 ± 6.8	2	83.5 ± 17.7
Fourth	no conception	3	97.0 ± 18.0	0	—
	conception	2	72.5	1	94.0

served in a similar proportion of each treatment group 6/26 (suckled) and 8/29 (non-suckled). Weekly blood sampling over the first 3 weeks is likely to have failed to detect shortened luteal phases within this period. The length of the shortened oestrous cycles observed was 10.8 ± 1.9 days (suckled cows) and 9.5 ± 1.6 days (non-suckled cows).

Post-partum intervals to each ovulation, up to and including conception, are summarized in Table II. Ovulations which were accompanied by conception have been distinguished from those where cows did not conceive. All ovulations in non-suckled cows were earlier than comparable ovulations in suckled cows. There was no difference in post-partum intervals between fertile and non-fertile ovulations.

Oestrus and coitus

The minimum requirement for a cow in oestrus was a period of not less than 1.5 h in association with the bull. This criterion was set following the inspection of behaviour data in conjunction with timing of ovulation, estimated from progesterone profiles. The first evidence of oestrus and the first incidence of coitus closely followed the early onset of ovarian activity. A reduction of 35 days occurred in post-partum interval to first oestrus (18.6 ± 10.2 days) and first observed coitus (24.5 ± 15.6 days) in non-suckled cows compared to their suckled contemporaries.

The diurnal distribution of oestrus appeared to differ between suckled and non-suckled cows ($P < 0.05$), with non-suckled cows being observed in coitus more frequently before 09.00 h and after 15.00 h each day than their suckled contemporaries (Fig. 1).

Oestrous behaviour

Examination of behaviour records in conjunction with ovulation dates indicated that active association of cow and bull without any interaction such as chin resting by the bull or attempted mounting by either cow or bull was the only evidence of behavioural oestrus in a proportion of animals in each group. Thus oestrus varied, from a short period of less than 3 h of cow-bull association without any interactive behaviour, to oestrus lasting 13 h and being accompanied by homosexual and heterosexual activity culminating in coitus. The four components of post-partum ovarian activity by which the two treatment groups were assessed were behavioural oestrus, coitus, ovulation and conception. In some instances one or two of these components occurred in the absence of the remainder (Table III). In less than 2% of cows in each group oestrus was not accompanied by ovulation. The incidence of ovulation in the absence of apparent oestrus was lower in non-suckled cows than in the suckled group ($P < 0.1$) (Table III). A similar trend was observed in the incidence of oestrus and ovulation together in suckled cows and in non-suckled cows. The incidence of coitus

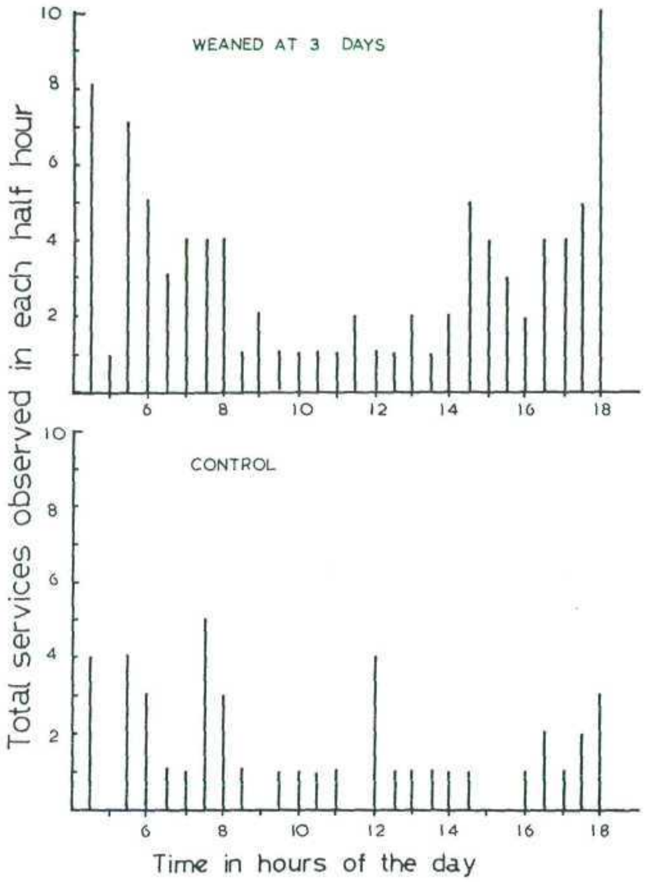

Fig. 1. Diurnal distribution of oestrus, based on a 30 min observation interval. Observations carried out daily from 20 September until 12 March.

TABLE III

A summary of oestrous behaviour and ovarian activity in suckled and non-suckled postpartum Afrikaner cows

Event	Suckled	Non-suckled	Significance
Oestrus only	3	2	NS
Coitus only	—	1	NS
Ovulation only	10	4	$P < 0.10$
Oestrus + ovulation	22	14	$P < 0.05$
Oestrus + coitus + ovulation	12	26	$P < 0.05$
Oestrus + ovulation + conception	2	5	NS
Oestrus + coitus + ovulation + conception	7	17	NS
Ovulation + conception	2	1	NS

in each group indicated that fewer suckled cows were observed being served by the bull compared to their non-suckled contemporaries. From the recorded services it would appear that the diurnal distribution of coitus was affected by suckling.

Conception

The post-partum interval to conception in non-suckled cows (45.8 ± 15.8) was 25 days shorter than in suckled cows (70.9 ± 17.9), Table I. Conception rates were significantly ($P < 0.01$) improved from 50% in suckled cows to 80% in non-suckled cows (Table IV). Amongst suckled cows the highest conception rate (10/15) was associated with the second ovulation at 69.9 ± 19.9 days, whilst in non-suckled cows conception rates were highest (11/24) at the third ovulation at 54.0 ± 6.8 days (Table II).

TABLE IV

Classification of ovarian activity within 100 days of calving

Class	Characteristics of ovarian activity	Probability (P)	Suckled	Non-suckled
1	Conception 100 days	0.01	50% (15/30)	80% (24/30)
2	Conception failure Regular ovarian activity Partial oestrus	0.05	27% (8/30)	7% (2/30)
3	Conception failure Irregular ovarian activity Largely anoestrous	0.33	10% (3/30)	10% (3/30)
4	Anovulatory, anoestrous	0.15	13% (4/30)	3% (1/30)

Classification of ovarian activity

From progesterone profiles compiled for each cow four classes of ovarian activity were recognized and described as follows (Wells et al., 1981):

Class 1. Resumption of cyclic ovarian activity and conception within 100 days of calving. Conception may occur at any ovulation within this period.

Class 2. Resumption of cyclic ovarian activity characterized by at least one oestrous cycle of 19—23 days with luteal phase progesterone levels attaining a minimum of 6 ng/ml plasma. Conception fails to occur within 100 days of calving.

Class 3. Within 100 days of calving intermittent surges of progesterone (1 ng/ml) lasting less than 7 days occur. These do not follow a cyclic pattern. Conception fails to occur.

Class 4. No evidence of any ovarian activity within 100 days of calving, as indicated by the absence of any plasma progesterone concentrations in excess of 1 ng/ml.

Removal of the suckling stimulus failed to stimulate detectable ovarian activity in all cows within 100 days of calving, since 13% of suckled cows and 3% of non-suckled cows were anovulatory for this period ($P = 0.15$) (Class 4, Table IV). Weaning at 3 days after calving appears to have reduced the number of cows which failed to conceive whilst exhibiting regular cyclic ovarian activity from 27% to 7% ($P = 0.05$) (Class 2, Table IV).

DISCUSSION

The results of this study indicate that suckling is a major contributing factor in firstly, the prolonged anoestrous periods observed in some Afrikaner cows, secondly, the low conception rates often recorded for this breed and thirdly, the diurnal distribution of coitus during oestrus.

The observation that reduction in suckling is accompanied by a shortening of the interval to first ovulation is in agreement with the work of Oxenreider (1968), Short et al. (1972), Wetteman et al. (1978), Radford et al. (1978), Suzuki and Sato (1979), Carter et al. (1980) and LaVoie et al. (1981). It appears that the early resumption of ovarian activity is a result of an earlier increase in frequency and amplitude of pulsatile LH release in weaned cows (Randel et al., 1976; Carruthers and Hafs, 1980) and in partially suckled cows (Walters et al., 1977; Echternkamp, 1978). The behavioural component (cow-calf interaction) of suckling appears to contribute to the delayed resumption of ovarian activity, as Peters et al. (1981) observed that milking does not suppress the resumption of pulsatile LH release to the same extent as suckling does.

After the onset of ovarian activity as indicated by plasma progesterone levels in excess of 1 ng/ml for at least 72 h, non-suckled cows ovulated more times (2.5 ovulations per conception) before they conceived than did normally suckled cows (2.1 ovulations per conception). Plasma progesterone determinations were too infrequent (once each week) prior to 21 days post partum to assess short luteal cycle incidence in non-suckled cows. From the data available not all cows exhibited a shortened luteal phase prior to the first apparently normal oestrous cycle. This would appear to be a common finding (Edgerton and Hafs, 1973; Arije et al., 1974; Corah et al., 1974). Hinshelwood et al. (1982) also noted that the absence of suckling in post-partum cows did not alter the incidence of short cycles.

Although suckled cows ovulated for the first time later than non-suckled cows, both groups exhibited a peak in conception at 50—70 days post partum. Reasons for failure of conception at these early ovulations in non-suckled cows cannot be determined from the available data. Two explanations exist: either these ovulations were not accompanied by satisfactory coitus or, after natural insemination, ova were not fertilized; or the embryos died as a result of inadequate luteal support. Nevertheless, conception rates at similar intervals post partum in each group were always higher in non-suckled cows.

Oestrous behaviour at the time of these early ovulations was more readily detected in the absence of suckling. The incidence of observed coitus was higher in non-suckled cows ($P < 0.05$) which supports the finding of Laster et al. (1973), Odde et al. (1980) and Da Fonseca et al. (1981). Mawhinney and Roche (1980) proposed that suckling shortened the length of oestrus and reduced its intensity. LaVoie et al. (1981) noted a decrease in non-standing oestrous behaviour associated with an increase in oestrous intensity in suckled cows compared with non-suckled cows. Suckled cows also exhibited their first oestrus later than non-suckled cows in the latter study.

In the present study cow-calf activity would have to be assessed in conjunction with cow-bull activity in order to determine whether the oestrus and coitus observations in suckled cows were dependent on calf-cow-bull opportunity interactions or whether they were due entirely to physiological factors. Larger post-partum intervals appear to alter the nature of oestrus-related behaviour by increasing standing oestrous activity (Saiduddin et al., 1968). In the present study intervals to first observed service indicate that the absence of conception to initial ovulations in non-suckled cows is unlikely to be due entirely to the absence of coitus. Therefore conception failure at this stage would not seem to be due to lack of opportunity.

The diurnal distribution of coitus in this study differed significantly between suckled and non-suckled cows. In non-suckled cows, coitus was seen more frequently before 09.00 h and after 15.00 h than in cows with calves at foot. The diurnal distribution of suckling may have influenced the overt expression of oestrus or the endocrine events which culminate in oestrus. During daylight hours fewer ($P < 0.10$) ovulations occurred in non-suckled cows without oestrus being observed than in suckled cows. Although oestrus was in evidence for 85% of ovulations in suckled cows and 94% of ovulations in non-suckled cows, coitus associated with these oestrous periods was observed less frequently ($P < 0.05$) in suckled cows than in non-suckled cows. There were either less coital events per oestrus in suckled cows or oestrus in these suckled cows was prolonged until coitus occurred at night, outside the surveillance period (04.30—18.30 h). These two situations cannot be distinguished. The hypothesis that suckled cows will stand for the bull when their calves are least active is tempting.

The earlier resumption in ovarian activity (35 days) and the substantial improvement in conception rates (30%) achieved with the removal of the suckling stimulus in Afrikaner cows was unexpected. These results may indicate that "maternal instinct" could be more suppressive in terms of ovarian activity in the *Bos indicus* breeds than in the *Bos taurus* breeds. This effect may in part be due to an endocrine component that delays the onset of ovarian activity post partum and a behavioural component that alters the opportunity of service and conception.

CONCLUSION

The Afrikaner, a *Bos indicus* breed, has responded well to the removal of the suckling stimulus, with an advance of 35 days in post-partum interval to resumption of ovarian activity and improvement of 30% in conception rate. In fact, the results are higher than expected, indicating that the calf-dam interaction in *B. indicus* may be more suppressive in terms of ovarian activity than in *B. taurus* breeds. By contrast, Gehlon and Sekhon (1969) concluded that as a result of the strong maternal instinct, Hariana dams (*B. indicus*) did not respond well reproductively to having their calves removed at birth.

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REFERENCES

Adeyemo, O. and Heath, E., 1980. Plasma progesterone concentration in *Bos taurus* and *Bos indicus* heifers. Theriogenology, 14: 411–420.

APRU Report. Animal Production Research Unit, 1980. Ten Years of Animal Production and Range Research in Botswana. Ministry of Agriculture, P.B. 0033, Gaborone, Botswana, pp. 123–140.

Arije, G.R., Wiltbank, J.N. and Hopwood, M.L., 1974. Hormone levels in pre- and post-parturient beef cows. J. Anim. Sci., 39: 338–342.

Carruthers, T.D. and Hafs, H.D., 1980. Suckling and four times daily milking: influence on ovulation, oestrus and serum luteinising hormone, glucocorticoids and prolactin in post-partum beef cows. J. Anim. Sci., 50: 919–925.

Carter, M.L., Dierschke, D.J., Turledge, J.J. and Hauser, E.R., 1980. Effect of gonadotropin-releasing hormone and calf removal on pituitary-ovarian function and reproductive performance in post-partum beef cows. J. Anim. Sci., 51: 903–910.

Corah, L.R., Quealy, A.P., Dunn, T.G. and Kaltenbach, C.C., 1974. Prepartum and postpartum levels of progesterone and estradiol in beef heifers fed two levels of energy. J. Anim. Sci., 39: 380–385.

Da Fonseca, V.O., Chow, L.A., Do Norte, A.L. and Lima, O.P., 1981. Effect of suckling on the reproductive efficiency of zebu (*Bos indicus*) cows. Anim. Breed. Abstr., 59: 239.

Echternkamp, S.E., 1978. Stimulation of oestrogen and luteinising hormone secretion in post-partum beef cows. J. Anim. Sci., 47: 521–531.

Edgerton, L.A. and Hafs, H.D., 1973. Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. J. Dairy Sci., 56: 451–458.

Gehlon, M.S. and Sekhon, G.S., 1969. Practicability of weaning calves in Hariana cattle. Indian Vet. J., 46: 1062–1065.

Gonzalez-Stagnaro, C., 1980. Effect of milk production and suckling on ovarian activity and post-partum reproductive performance in tropical cattle. Anim. Breed. Abstr., 48 (12): 870.

Hinshelwood, M.M., Dierschke, D.J. and Hauser, E.R., 1982. The effect of suckling and time post-partum on GnRH-induced LH release in ovariectomized cows. J. Anim. Sci., 55 (Suppl. 1): 359 (abstr.).

Holness, D.H., Hale, D.H. and Hopley, J.D.H., 1980. Ovarian activity and conception during the post-partum period in Afrikaner and Mashona cows. Zim. J. Agric. Res., 18: 3—11.

Inskeep, E.K. and Lishman, A.W., 1981. Factors affecting post-partum anoestrus in beef cattle. Anim. Reprod. (Barcelona Symposium 3): 277—289.

Kiser, T.E., Dunlap, S.E., Benyshek, L.L. and Mares, S.E., 1980. The effect of calf removal on oestrus response and pregnancy rate of beef cows after SyncroMate-B treatment. Theriogenology, 13: 381—389.

Laster, D.B., Glimp, H.A. and Gregory, K.E., 1973. Effects of early weaning on post-partum reproduction of cows. J. Anim. Sci., 36: 734—739.

LaVoie, V., Han, D.K., Foster, D.B. and Moody, E.L., 1981. Suckling effect on estrus and blood plasma progesterone in post-partum beef cows. J. Anim. Sci., 52: 802—812.

Mawhinney, S. and Roche, J.F., 1980. Effect of suckling and environmental change of oestrous behaviour in beef cows. Irish J. Agric. Res., 19: 25—31.

McCabe, C.T., Sprowson, G.W. and Holness, D.H., 1980. The conductivity of cervical mucus as a predictor of ovulation in beef cows synchronized with cloprostenol. S. Afr. J. Anim. Sci., 10: 119—124.

Odde, K.G., Ward, H.S., Kiracofe, G.H., McKee, R.M. and Kittock, R.J., 1980. Short oestrous cycles and associated serum progesterone levels in beef cows. Theriogenology, 14: 105—112.

Oxenreider, J.L., 1968. Effects of suckling and ovarian functions of post-partum reproductive activity in beef cows. Am. J. Vet. Res., 29: 2099—2104.

Pace, M.M. and Sullivan, J.J., 1980. Effect of SyncroMate-B (SMB) and calf separation on beef cattle estrus and pregnancy rates. J. Anim. Sci., 51 (Suppl. 1): 312 (abstr.).

Peters, A.R., Lamming, G.E. and Fisher, M.W., 1981. A comparison of plasma LH concentrations in milked and suckling post-partum cows. J. Reprod. Fertil., 62: 567—673.

Radford, H.M., Nancarrow, C.D. and Mattner, P.E., 1978. Ovarian function in suckling and non-suckling beef cows post-partum. J. Reprod. Fertil., 54: 49—56.

Randel, R.D., 1980. Total serum estrogens before and after estrus in Brahman, Brahman × Hereford and Hereford heifers. J. Anim. Sci., 51 (suppl. 1): 2 (abstr.).

Randel, R.D. and Welker, G.A., 1976. Once daily suckling effect on cow-calf performance. J. Anim. Sci., 43: 302 (abstr.).

Reeves, J.J., Gaskins, C.T. and Coonrad, D., 1980. Rebreeding of cows nursed once a day. J. Anim. Sci., 51 (Suppl. 1): 443 (abstr.).

Rodriguez, R.A., Rodriguez, R.O., Ruiz, D.R. and Gonzalez, P.E., 1980. Effects of temporary calf removal and controlled suckling on reproduction in housed cattle in summer. Anim. Breed. Abstr., 49: 68.

Saiduddin, S.J., Riesen, J.W., Tyler, W.J. and Casida, L.E., 1968. Relation of post-partum interval to pituitary gonadotropins, ovarian follicular development and fertility in dairy cows (Effect of suckling, feeding level, breeding management and genetic level of milk production). Wisconsin Agric. Exp. Sta. Bull., 270: 15.

Short, R.E., Bellows, R.A., Moody, E.L. and Holland, B.E., 1972. Effects of suckling and mastectomy on bovine post-partum reproduction. J. Anim. Sci., 34: 70—74.

Smith, M.F., Burrel, W.C., Ship, L.D., Sprott, L.R., Songster, W.N. and Wiltbank, J.N., 1979. Hormone treatments and use of calf removal in post-partum beef cows. J. Anim. Sci., 48: 1285—1294.

PART G

ROLE OF THE MALE IN REDUCED CONCEPTION RATES

INTRODUCTION

Throughout the preceding chapters it has been assumed that low reproductive rates, particularly in Afrikaner cows, can be attributed solely to deficiencies/peculiarities on the part of the breeding females. This is obviously a one-sided view and the last part of this thesis considers the possibility that breeding males may share part of the blame. In particular, attention has been focused on the failure of males to mate oestrous females rather than on the fertilizing capacity of the ejaculate. As in the past, the initial research utilized sheep and the work was then expanded to include the species of primary importance to this thesis.

Paper 51

The occurrence of rams with impaired libido had been reported by P.S. Pretorius in South Africa and P.E. Mattner in Australia. The importance of rearing conditions viz., in monosexual groups was highlighted by J.J. Zenchak and G.C. Anderson who undertook their research at West Virginia University. Our research commenced with Ile de France rams reared under commercial conditions.

Findings :-

1. On the basis of three libido tests 25% of the rams were classified as sexually low-response animals.
2. Three of seven low-response rams failed to mate under pasture conditions.

Implications/Significance :-

1. Not all rams that avoid oestrous ewes under test conditions will perform poorly

under natural mating conditions, but there are good reasons to regard such males with suspicion.

2. Inhibited rams should be culled as there may be a genetic basis for this deficiency.

Paper 52

This paper describes an investigation into the hormones involved in male sexual behaviour in sheep. It constituted an attempt to obtain a greater understanding of the hormonal control of male sexual behaviour. Such information could be value in remedying or avoiding deficiencies. The principle of active immunization against the compound (hormone) under study was applied so as to negate the effects of the relevant substance.

Findings :-

1. Immunization against oestradiol depressed sexual behaviour towards oestrous ewes of wethers implanted with testosterone. The level of mating behaviour was then similar to that of untreated wethers.

Implications/Significance :-

1. Dihydrotestosterone was shown to potentiate the action of oestrogens and apparently had no role within the central nervous system.
2. Oestrogens do not restore mating activity to the same level as testosterone does.

Paper 53

The work of J.J. Zenchak and G.C. Anderson had shown that rearing of rams in all-male groups often led to the development of homosexual tendencies in such rams.

Beef bulls undergoing performance test at centralized centres could be exposed to the same environment that leads to deviant behaviour in rams. Virtually all breeding bulls used in beef herds undergo no evaluation as regards their mating ability and bulls with no sexual interest in oestrous cows have been reported from time to time. In view of the major impact that the use of bulls with low or absent sexual drive towards cows could have on calving rates, it was deemed necessary to give attention to performance-tested bulls.

Findings :-

1. Breed type (British beef, dual purpose or zebu) significantly influenced serving capacity.
2. Serving capacity improved with repeated testing, but not all breeds responded in this manner.
3. Of the bulls tested 27.7, 40.4 and 31.9% exhibited low, medium and high libido, respectively.
4. Brahman and Afrikaner bulls never completed service during testing.
5. Scrotal circumference and semen quality were not related to serving capacity scores.

Significance/Implications :-

1. The results support the conclusion that *B. indicus* and *B. taurus* differ in their libido under test conditions.
2. Testing under more natural mating conditions may be necessary for *B. indicus* bulls.

The impact of libido testing on buyer preference was of interest, but difficult to gauge. It was significant that bulls with a score of zero were being purchased. Clearly, farmers need to be informed of the possible consequences of such purchases.

Paper 54

In view of the poor responses observed amongst the few performance-tested Afrikaner bulls, it became evident that more critical testing was necessary. This was particularly important as the bull-factor could not be excluded as a contributor to poor reproductive rates in Afrikaner herds. Attention needed to be given to the possibility that *B. indicus* bulls were distracted by pen-mating conditions, the close proximity of an observer and the limitation of testing to daylight conditions. The latter was included as beef producers had maintained that Brahman bulls were never seen to mate with cows. Apparently, mating may be confined to hours of darkness in such breeds.

Bulls differing in "tested" libido were then allowed to,mate under pasture conditions.

Findings :-

1. No preference for nocturnal mating was seen under test conditions and presence of observer had no discernable effect.
2. The test scores varied from test to test, but scores improved with repeated testing.
3. Pen and pasture mating ability were correlated.

Significance/Implications :-

1. Bulls that showed inconsistent or poor performance in pen tests did not necessarily behave in a like manner on pasture. Thus, stimulus animals used in pen tests were apparently not ideal.
2. The specific characteristic of cows that determines their attractiveness to bulls needs to be identified in order to permit effective pen testing.

Paper 55

The aim in this study was to link pen-testing scores when Sussex bulls were tested individually (no competition as in the serving capacity test) with performance under conditions where large numbers of cows require to be mated in a short period of time.

Findings :-

1. Bull libido score had no significant effect on conception rate when bull to cow ratios of 1:35 to 1:40 were applied.

In spite of our negative results it would be foolish to ignore the contribution of bull libido to herd fertility. It must be remembered that all the bulls in this study had been scored, whereas producers purchase a bull entirely on faith, without any indication what so ever of its serving ability. With the price of breeding bulls continuing to increase, there remains an urgent need to rate bulls according to their potential mating power.

Short Communications

Identification of sexually low-response rams using a libido test and their performance when allowed to mate under field conditions

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Thirty-one Ile de France stud rams raised in monosexual groups were evaluated for sexual behaviour using three 10-minute libido tests. Rams represented two age (± SD) groups namely, 455,2 ± 6,7 (n = 10) and 352,9 ± 11,0 (n = 21) days respectively. The proportion of rams which completed service did not increase significantly over the three tests. Eight (25,8%) of the rams did not attempt to serve in any of the tests, and were termed sexually low-response. Five of these rams had a common sire. The low-response rams were mated to small flocks of ewes under pasture conditions, and two of the rams remained sexually inactive.

Seksuele gedrag in 31 Ile de France-stoetramme is deur middel van drie 10-minuut libidotoetse geëvalueer. Die ramme het twee ouderdomsgroepe (± SA) naamlik 455,2 ± 6,7 (n = 10) en 352,9 ± 11,0 (n = 21) dae verteenwoordig. Die persentasie ramme wat ooie gedek het, het nie met verloop van die toetse 'n betekenisvolle verbetering getoon nie. Agt ramme (25,8%) het nie tydens enige stadium van die toetse ooie gedek nie. Vyf van die seksueel-onaktiewe ramme het dieselfde vaar gehad. Wanneer hierdie onaktiewe ramme onder natuurlike toestande by klein groepe ooie gebring is, het twee van die sewe steeds seksueel onaktief gebly.

Keywords: Libido test, rams, field performance

The incidence of sexually low-response rams (those which fail to exhibit adequate sexual behaviour toward an oestrous ewe) in groups of rams raised under monosexual conditions after weaning has been well documented (Pretorius, 1967; Mattner, Braden & George, 1973). Zenchak and Anderson (1980) reported that low-response rams engaged in significantly higher levels of sex-like behaviour with their pen mates (monosexual groups) during rearing than did normal rams. These workers thus suggested that the poor performance of such rams when exposed to an oestrous ewe was due to their inability to interpret sexual cues correctly. Working with Karakul rams, Le Roux and Barnard (1974), demonstrated that heterosexual contact between weaning (68-days old) and 9-months old or from 9 months onwards, significantly increased the number of rams showing normal sexual behaviour at 18 months of age. Winfield and Makin (1978) speculated that short exposures to oestrous ewes as well as the periodic presence of cyclic ewes in paddocks nearby may have been sufficient to enable rams to establish a sex difference.

The incidence of sexually low-response rams in a group of 31 virgin rams from an Ile de France stud operated under intensive conditions was investigated. The rams were weaned from their dams at 8 weeks of age thereafter they were run in all-male groups on cultivated pastures. There was no certainty that rams were never grazed adjacent to camps containing ewes. The rams represented two age groups being born in the autumn (n = 10) and spring (n = 21) of 1982. Their average age ± SD at the onset of the test was 455,2 ± 6,7 and 352,9 ± 11,0 days respectively.

Rams were subjected to three 10-minute libido tests (Le Roux & Barnard, 1974), spaced 4 days apart with a recently mated oestrous ewe. Teaser ewes were synchronized using intravaginal progesterone sponges, inserted for 8 days, followed by an intramuscular injection of either PMS or prostaglandin — $F_{2\alpha}$. Rams were evaluated for courtship activity, mounting attempts, and completed services according to Zenchak (1976).

The proportion of rams serving ewes, did not improve significantly from the first to the third test (Table 1).

Table 1 The sexual activity of rams in three libido tests

	Sexual activity							
					Serving			
	Inactive		Mounting		One service		Two or more services	
Test number	n	%	n	%	n	%	n	%
1	9	29,0	2	6,5	12	38,7	8	25,8
2	8	25,8	1	3,2	12	38,7	10	32,3
3	9	29,0	1	3,2	10	32,3	11	35,5

Eight rams (25,8%) were classified as sexually low-response following the three tests. One of the eight made a vague mounting attempt in Test 1 but did not show sexual activity during the remaining two tests. Of the remaining 23 rams, two rams which were sexually inactive in Test 1, served in Tests 2 and 3, while another ram which served in the first test attempted, but failed to serve in Test 2 and was sexually inactive in Test 3.

Sexually low-response rams exhibited abnormal behaviour in the form of an odd 'baaing' sound, agitation (running around the test pen or circling the ewe), attempts to leave the pen, or simply staring through cracks in the pen. Zenchak and Anderson (1980) reported similar behaviour in low-response rams and interpreted it as a 'fear' response. In addition, two rams showed aggression toward teaser females, a phenomenon noted in some sexually inactive bulls (Crichton and Lishman, unpublished data).

All of the low-response rams were from the younger age group. Despite this, age had no significant effect on sexual behaviour as rams of 10,5–12 months should have been showing strong responses to oestrous ewes, even if incapable of serving.

On further investigation it was found that five of the eight

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Failure to demonstrate a relationship between beef bull libido and conception rate

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The relationship between beef bull libido and conception rate following a 21-day breeding period was investigated over two seasons. Libido was defined as the number of services completed during a 10-minute exposure to a restrained oestrous female. In the first year bulls which achieved a mean libido score of three or more services were compared with bulls which achieved a mean of one service. In the second year a mean score of six or more services was compared with a mean of three services. Bulls were joined as single sires at a bull to female ratio of 1:35 in the first and 1:40 in the second year. Libido, as measured in this study, did not influence conception rate significantly.

Die verwantskap tussen libido en besetting by vleisbeeste is gedurende 'n teelseisoen van 21 dae oor twee seisoene ondersoek. Libido is gedefinieer as aantal voltooide dekkings gedurende 'n 10-minute-toets saam met 'n ingeperkte koei op hitte. In die eerste jaar is bulle wat 'n gemiddelde libidotelling van drie of meer dekkings behaal het, vergelyk met bulle met 'n gemiddelde van een dekking. In die tweede jaar is 'n gemiddelde telling van ses of meer dekkings met 'n gemiddelde van drie dekkings vergelyk. 'n Enkel bul per groep is teen 'n verhouding van 1:35 in die eerste en 1:40 gedurende die tweede dekseisoen gebruik. Libido, soos uitgedruk in hierdie studie, het geen betekenisvolle invloed op besetting gehad nie.

Keywords: Libido, beef bulls, conception rate

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Introduction

Under conditions of natural mating the bull must ensure that every cow capable of producing a calf does so. In order to fertilize an acceptable number of females the bull must produce sufficient semen of good quality and possess a high level of sex-drive to seek out receptive females which are then mated with a high degree of expertise. According to Blockey (1980) farmers mate more bulls per 100 females than is necessary to ensure good herd fertility. Thus, by identifying high-libido bulls the producer can effectively reduce bull costs per calf and obtain more calves earlier in the season (and therefore improved weaning masses) as a result of higher first oestrus conception rates (Blockey, 1978).

Serving capacity, the number of services completed during a 30-minute pen test, is positively related to conception rate following veld mating (Blockey, 1978; Lunstra, 1980). Christensen, Seifert & Post (1982), however, found no such relationship.

Application of the serving capacity test (bulls tested in groups of three - five animals) to small groups of mixed-age bulls, poses a problem in that age is related to position in the social hierarchy, which is in turn related to sexual behaviour (Blockey, 1981). The libido score test (Chenoweth & Osborne, 1975) in which bulls are tested as individuals represents an alternative to the serving capacity test. Reports on the relationship between a bull's performance on the libido score test and conception rate following herd mating are limited. Sullins, Tomky, Farin, Chenoweth & Pexton (1979) found that high libido bulls (average score of 9,7 on a scale of 0 - 10) served a greater proportion of the females available and achieved better conception rates than medium libido bulls (average score 7,8). Makarechian, Farid & Berg (1983) could not confirm these results. In a trial using Santa Gertrudis bulls Smith, Morris, Amoss, Parish, Williams & Wiltbank (1981) found that libido score (number mated/ number in oestrous × 100) was positively correlated with conception rate (r=0,44).

In the light of the highly significant relationship between number of services completed in the libido score test and serving capacity (Crichton & Lishman, 1985) it seems feasible to use the individual test to predict veld mating performance expressed in terms of conception rate.

The aim of this study was therefore to link the number of services completed in the libido score test to conception rate when bulls are mated as single sires to relatively large groups of cows for a limited period of time.

Procedure

The trial was repeated over two breeding seasons, 1983/84 and 1984/85. Libido (number of completed services) was determined by exposing individual bulls to a restrained oestrus-induced ovariectomized cow for 10 minutes following at least 10 minutes of sexual stimulation (Chenoweth & Osborne, 1975). In the month prior to the start of mating in each year bulls were examined for breeding soundness. A single ejaculate was evaluated for motility and per cent abnormal sperm according to the method of Roberts (1971). Bulls were determined fertile (progressive rapid movement of sperm and less than 10% abnormalities) and had scrotal circumference greater than 35 cm.

In the first season 16 and in the second season eight Sussex bulls were subjected to three and four libido tests respectively. A given bull's libido score was determined by taking the mean of his two best scores. Six bulls were selected for mating in year 1 and four in year 2. In the first year three of the six bulls achieved mean scores of more than three services (3,5; 3,5 and 6,0), whereas the remaining three bulls achieved mean scores of one service each. Two of the four bulls in year 2 achieved mean scores of more than six services (6,5 and 7,0) and the other two bulls scored three services each. The number of bulls was reduced in the second season in order to place bulls under greater mating pressure. Bulls were joined to females in single-sire herds.

In 1983/84 the cow herd (n=230) consisted of 152 lactating and 36 dry Sussex cows together with 42 Simmentaler-cross heifers. In 1984/85 the herd (n=218) consisted of 173 lactating and 45 dry cows, no heifers were present. Bulls were thus joined with groups of 38 - 39 females in 1983/84 and 54 - 55 females in 1984/85.

Prior to the start of mating cows were evaluated for body condition (van Niekerk & Louw, 1982) and palpated rectally to determine the cyclic status of their ovaries. Cows were allocated to breeding herds in accordance with age, calving date, condition score, lactational status, and ovarian activity. In this way each bull received a uniform herd of females.

The bulls were placed with the cows for a period of 35 days. The incidence of heat cycles and of mating activity was determined by twice daily observation (5h00 and 16h30), heat mount detectors (first season only), and chinball markings from harnesses fitted to the bulls. Following termination of the trial cows were placed with clean-up bulls until the end of the season. These bulls were fitted with harnesses containing ink of a different colour to that used previously.

A rectal pregnancy diagnosis was made 4 months after the termination of the breeding season and the age of the foetus estimated. Successful conceptions were determined on the basis of heat-spotting data, pregnancy diagnosis and duration of gestation.

Cows were grazed on veld (Natal Sour Sandveld; Acocks, 1975). Veld utilization was carefully monitored and groups were most often grazed in camps adjacent to one another in order to minimize any differences in veld quality and quantity.

The majority of cows cycled within 21 days of the start of mating. For the purposes of statistical analysis cows which cycled after 21 days were omitted. Therefore bulls were evaluated over a period during which mating pressure was most intense and second oestrous conceptions were effectively excluded denying poorer bulls more than one opportunity to impregnate a female. Any changes in bull and/or cow fertility were accounted for by subdividing the season into seven 3-day intervals, conception rate being determined for each interval.

A split-plot analysis was used (Rayner, 1967). The higher and lower scores in each year were treated as whole plots and the seven time intervals as sub-plots in the design. The relationship between conception rate for each 3-day interval and the number of cows cycling, lactating, with an interval from calving to cycling of $\leqslant$75 days and an interval of $\leqslant$80 days, was examined using linear regression analysis (Rayner, 1967) for both years. The relationship between conception rate and the number of cows with condition score $\leqslant$2,0 and $\leqslant$2,5 was evaluated for 1984/85 (there being no heifers in this year). Variates which had a significant effect on conception rate were included as covariates in the split-plot analysis.

Results

The mean condition score (± SE) for lactating cows which cycled during the first 21 days of the season was 2,53 (± 0,51) and 2,55 (± 0,46) in 1983/84 and 1984/85 respectively. The mean (± SE) calving to cycling intervals for years 1 and 2 were 87,9 (± 17,5) and 84,6 (± 13,0) days respectively.

In 1983/84 the number of cows cycling in any 3-day interval and the number with a calving to cycling interval of $\leqslant$ 75 days significantly depressed conception rate ($P < 0,05$). During 1984/85 the number of cows lactating and number with a calving to cycling interval of $\leqslant$ 75 days had a significant negative effect on conception rate ($P < 0,05$), whereas number cycling, number with condition score <2,5, and number with calving to cycling interval $\leqslant$80 days tended to depress conception rate ($P < 0,10$).

A greater proportion of cows did not cycle during the second season (from the start of breeding to the start of clean-up) when compared with the first season (16,1 versus 5,2%). Furthermore, 10,6% cycled after the first 21 days in year 2 as opposed to 4,4% in 1983/84. Cows which did not cycle at all were generally in poor condition.

The mean ±SE number of services completed in the libido tests was 2,0 ±1,0 (n=16) in 1983/84 to 4,0 ± 2,0 (n=8) in 1984/85.

Bull libido score had a non-significant effect on conception rate in both years (Table 1). In the first year the interaction (score × time interval) was significant ($P < 0,05$). No trend was however evident and differences were not consistently in favour of any one group of bulls.

Conception rate increased significantly ($P < 0,05$) over time interval during 1984/85 (Table 1). The increase was not consistent. The F-value for the interaction was significant at the 10% level. Again (as for 1983/84) no trend was evident.

The increase in conception rate in 1984/85 was accompanied by a simultaneous increase in the interval from calving to cycling ($P < 0,05$). Although this interval increased ($P < 0,05$) over the 1983/84 season, conception rate did not increase concurrently.

Discussion and Conclusions

During the second season the number of cows lactating had a significant influence on conception rate. This was not the case in 1983/84. The interval from calving to cycling increased over time in both years and a longer post-partum interval would have improved the chances of conception in

Table 1 The mean conception rates adjusted for covariates for two libido score treatments over seven 3-day time intervals during two breeding seasons, 1983/84 and 1984/85[1]

Year	Libido score		Time interval 1	2	3	4	5	6	7	Treatment means
1983/84	More than 3 services (*n*=3)	Mean conception[2] rate (%)	$79,3^{c}$	$87,2^{c}$	$64,5^{c}$	$96,3^{c}$	$96,6^{c}$	$90,9^{c}$	$70,5^{c}$	83,6
	1 service (*n*=3)	Mean conception rate (%)	$74,4^{c}$	$79,9^{c}$	$89,6^{c}$	$87,4^{c}$	$61,6^{d}$	$82,9^{c}$	$88,8^{c}$	80,7
		Mean conception rate (%) for each time interval	76,9	83,5	77,1	91,8	79,1	86,9	79,7	
1984/85	More than 6 services (*n*=2)	Mean conception rate (%)	55,8	69,5	71,6	63,2	98,9	98,9	60,9	77,0
	3 services (*n*=2)	Mean conception rate (%)	64,2	68,6	48,5	59,7	99,0	84,4	82,9	72,3
		Mean conception[3] rate (%) for each time interval	$60,0^{a}$	$69,0^{a}$	$70,0^{a}$	$61,5^{a}$	$99,0^{b}$	$90,6^{b}$	$71,9^{a}$	

[1] Unless otherwise indicated means are not significantly different.

[2] LSD for score × time interval = 31,3% ($P<0,05$), means within a row are NS, and means within columns with different superscripts are significantly different.

[3] LSD for effect of time = 20,8% ($P<0,05$) and 29,3% ($P<0,01$), means within the same row without common superscripts are significantly different.

lactating females. This effect was probably masked by the greater proportion of dry cows and heifers in 1983/84, 33,9 versus 20,6% in 1984/85 (there were no heifers in this year).

In 1984/85 conception rate tended to be lower, and a greater proportion of females either did not cycle or cycled after the first 21 days when compared to 1983/84. It is suggested that limited veld availability contributed to the poorer reproductive efficiency in 1984/85. Rainfall at the peak of the growing season (December/January) was 40,4% below average. The shortage of grazing became noticeable during early January. Graham (1982) demonstrated that poor nutrition can prolong the post-partum interval. No doubt this effect is more marked in cows of sub-optimal condition. Van Niekerk (1982) considered a condition score of 3,0 at the start of mating to be ideal, whereas cows in this study had an average condition score of less than 3,0. In order to minimize the effect of grazing on conception rate, cows were grazed on veld which had been spared for winter grazing. Grazing on rested veld started on day 15 of the breeding season and was terminated at day 29.

The mean libido score increased from year 1 to year 2. The increase would appear to be due to an improvement in the performance of young bulls with no or limited previous heterosexual experience. Although bulls did not show a marked improvement in libido score over the three tests in 1983/84 it is obvious that bulls underwent some form of learning experience during the breeding season.

The insignificant effect of a bull's libido score on conception rate in 1983/84 was thought to have been largely due to insufficient numbers of cows cycling over the first 21 days. In 1984/85 an attempt was made to highlight differences in conception rate due to libido by placing bulls under greater mating pressure (the number of bulls used was decreased). The attempt did not succeed because of the larger proportion of cows which failed to cycle or cycled after 21 days.

Results from the present study indicate that a bull's libido score was not related to conception rate when bulls were mated at bull-to-female ratios of 1:35 and 1:40 (based on the mean number of cows cycling in 21 days) even though differences between the teatments were greater than 100% as regards number of services completed in 10 minutes. Furthermore, it appears as if the generally accepted bull-to-female ratio of 1:25 is conservative and that bulls of average libido are capable of covering at least 40 cows over a standard 90-day breeding season. This suggests that bulls with superior libido would be capable of impregnating more than 40 cows over a breeding season. Neville, Smith & McCormick (1979) found no difference in conception rate between bulls mated to either 25 or 40 cows for 90 days and Rupp, Ball, Shoop & Chenoweth (1977) reported that most bulls achieved good reproductive efficiency when mated to 40 or 60 heifers for 21 days.

In the light of the positive results obtained in other studies (Blockey, 1978; Sullins, *et al.*, 1979; Lunstra, 1980) the contribution of bull libido to herd fertility cannot be ignored. The need to accurately predict male fertility on the basis of semen quality, testicular size, and sex-drive is acute. To this end further work using large groups of males and females should be undertaken.

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References

ACOCKS, J.P.H., 1975. Veld types of South Africa. *Mem. Bot. Surv. S. Afr.* 40, 103.

BLOCKEY, M.A. de B., 1978. The influence of serving capacity of bulls on herd fertility. *J. Anim. Sci.* 46, 589.

BLOCKEY, M.A. de B., 1980. Getting the most out of rams, bulls and boars. *Proc. Aust. Soc. Anim. Prod.* 13, 46.

BLOCKEY, M.A. de B., 1981. Further studies on the serving capacity test for beef bulls. *Appl. Anim. Ethol.* 7, 337.

CHENOWETH, P.J. & OSBORNE, H.G., 1975. Breed differences in the reproductive function of young beef bulls in Central Queensland. Aust. Vet. J. 51, 405.

CHRISTENSEN, H.R., SEIFERT, G.W. & POST, T.B., 1982. The relationship between a serving capacity test and fertility of beef bulls. *Aust. Vet. J.* 58, 241.

CRICHTON, J.S. & LISHMAN, A.W., 1985. Libido evaluation of virgin beef bulls. *S. Afr. J. Anim. Sci.* 15, 22.

GRAHAM, J.F., 1982. The effect of body condition of beef cows at calving and post calving nutrition on calf growth rate and cow fertility. *Proc. Aust. Soc. Anim. Prod.* 14, 309.

LUNSTRA, D.D., 1980. Evaluation of libido in beef bulls. *Proc. Ann. Meet. Soc. Theriogenology* p. 169.

MAKARECHIAN, M., FARID, A. & BERG, R.T., 1983. Studies on natural service fertility of yearling beef bulls in multiple sire matings. *Agriculture & Forestry Bulletin* (Special Issue) p. 10.

NEVILLE, W.E. Jr., SMITH, J.B. & MC CORMICK, W.C., 1979. Reproductive performance of two- and three-year-old bulls assigned twenty-five or forty cows during the breeding period. *J. Anim. Sci.* 48, 1020.

RAYNER, A.A., 1967. A first course in Biometry for agriculture students. University of Natal Press. Pietermaritzburg.

ROBERTS, S.J., 1971. Diagnosis of sterility and infertility in the male and evaluation of breeding soundness. In: Veterinary Obstetrics and Genital Diseases (Theriogenology).
Ed Roberts, S.J., Ithaca, New York. p. 695.

RUPP, G.P., BALL, L., SHOOP, M.C. & CHENOWETH, P.J., 1977. Reproductive efficiency of bulls in natural service: Effects of male to female ratio and single- versus multiple-sire breeding groups. *J. Amer. Vet. Med. Assoc.* 171, 639.

SMITH, M.F., MORRIS, D.L., AMOSS, M.S., PARISH, N.R., WILLIAMS, J.D. & WILTBANK, J.N., 1981. Relationship among fertility, scrotal circumference, seminal quality and libido in Santa Gertrudis bulls. *Theriogenology* 16, 379.

SULLINS, J., TOMKY, D.F., FARIN, P.W., CHENOWETH, P.J. & PEXTON, J.E., 1979. Sexual behaviour and breeding performance of bulls mated to beef cattle synchronized with PGF2α or SMB. *J. Anim. Sci.* 49 (Suppl. 1), 340 (Abst. No. 488).

VAN NIEKERK, A., 1982. The effect of body condition as influenced by winter nutrition, on the reproductive performance of the beef cow. *S. Afr. J. Anim. Sci.* 12, 383.

VAN NIEKERK, A. & LOUW, B.P., 1982. Condition scoring of beef cattle. Manual N15/1982. Dept. Agric. & Water Supply, Natal.

low-response rams had a common sire (No. 7). The remaining three inhibited rams had different sires. The small numbers of offspring from some sires did not facilitate statistical comparison of sires. It is suggested that ability to learn sexual behaviour may be genetically determined. Mattner, *et al.* (1973) found a high proportion of low response rams in one strain of Merinos.

When faced with the prospect of culling an otherwise top-quality ram, stud breeders are likely to express doubts as to the validity of pen tests. Three months after the pen tests, seven of the eight low-response rams (one culled on the basis of conformation) were each allowed access for a period of 5 days to five maiden ewes. The rams were equipped with harnesses ('sire-sine') and the ewes were checked daily for crayon markings. Two rams (2159 and 2074) did not mark any ewes, while one ram (2117) marked a single ewe indistinctly on her flank. Of these seven rams those which had marked ewes during pasture mating, actively pursued and courted ewes when confined in handling pens. Rams 2117, 2159 and 2074 stood to one side and ignored the ewes, while ram 2159 actively courted rams 2117 and 2074.

Rams 2117, 2159 and 2074 were placed as a group with 18 oestrous-synchronized maiden ewes for a further 8 days, and daily observations made for crayon markings. Ram 2117 marked two ewes while the other two appeared not to mate. Mattner, *et al.* (1973) found that the majority of low-response rams began to show sexual activity following flock mating.

It is apparent that not all rams, determined to be low-response on the basis of pen tests, remain low-response following flock mating. However, such rams should be regarded with suspicion in view of the likelihood of depressed conception rates owing to their poor mating dexterity and libido (Mattner, *et al.*, 1973). Furthermore the implication that the development of normal sexual behaviour is determined genetically suggests that inhibited sires should be culled without being given benefit of the doubt because of other superior traits.

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References

LE ROUX, P.J. & BARNARD, J.P., 1974. The effect of heterosexual contact on libido and mating dexterity in Karakul rams. *S. Afr. J. Anim. Sci.* 4, 171.

MATTNER, P.E., BRADEN, A.W.H. & GEORGE, J.M., 1973. Studies in flock mating of sheep: 5. Incidence, duration and effect on flock fertility of initial sexual inactivity in young rams. *Aust. J. Exptal Agric. Anim. Husb.* 13, 35.

PRETORIUS, P.S., 1967. Libido en dekbehendigheid by ramme grootgemaak en gehou in isolasie van ooie. *Hand. S. Afr. Ver. Diereprod.* 6, 208.

WINFIELD, C.G. & MAKIN, A.W., 1978. A note on the effect of continuous contact with ewes showing regular oestrous and of post weaning growth rate on the sexual activity of Corriedale rams. *Anim. Prod.* 27, 361.

ZENCHAK, J.J. 1976. Early socialization effect on adult ram sexual behaviour. Ph.D. Thesis, West Virginia University.

ZENCHAK, J.J. & ANDERSON, G.C., 1980. Sexual performance levels of rams (*Ovis aries*) as effected by social experiences during rearing. *J. Anim. Sci.* 50, 167.

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Role of dihydrotestosterone in the control of sexual behaviour on castrated male sheep

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Summary. Wethers (at least $2\frac{1}{2}$ years after castration) were implanted with testosterone propionate (TP), oestradiol dipropionate (ODP), dihydrotestosterone, or a combination of dihydrotestosterone and ODP Silastic capsules. Active immunization against both oestradiol and oestrone or oestradiol only was used to negate effects of oestrogens produced by aromatization of TP. On exposure to oestrous ewes, immunization of wethers implanted with TP significantly ($P < 0{\cdot}01$) reduced all components of mating behaviour (except sniffing and Flehmen) to levels seen in untreated controls. The results support the conclusion that dihydrotestosterone potentiates the action of oestrogens, particularly as regards Flehmen, and has no action on its own within the central nervous system, while oestrogens do not restore mating activity to the same level as that following treatment with testosterone.

Keywords: testosterone metabolites; immunization; sexual behaviour; castration; ram

Introduction

Male sexual behaviour in mammals is directly controlled by the hormonal secretions (androgens) of the testes (Luttge, 1979), the principal androgen being testosterone (Larrson, 1979). Testosterone is metabolized within the central nervous system (CNS) and genital tissues by aromatization to oestradiol (Naftolin *et al.*, 1972; Lieberburg & McEwen, 1975; Callard *et al.*, 1978) or 5α-reduction to dihydrotestosterone (Bruchovsky & Wilson, 1968; Whalen & Rezek, 1972; Massa *et al.*, 1975). These metabolites could mediate the function of testosterone in controlling male copulatory behaviour (Callard *et al.*, 1978).

In wethers, administration of oestradiol alone will elicit lower levels of behaviour than those seen following testosterone therapy (D'Occhio & Brooks, 1976; Mattner, 1976). The full pattern of mating arises only after administration of dihydrotestosterone in conjunction with oestradiol (D'Occhio & Brooks, 1980). It would appear that dihydrotestosterone potentiates the action of oestradiol (D'Occhio & Brooks, 1980), since administration of dihydrotestosterone alone does not give rise to sexual behaviour in wethers (D'Occhio & Brooks, 1976; Mattner, 1976; Parrott, 1978).

Support for the proposal that part of the action of dihydrotestosterone involves the central control of courtship (Parrott, 1978) is provided by the characterization of cytosolic receptors for dihydrotestosterone in addition to those for oestradiol in the pituitary and hypothalamus of rams (Thieulant & Pelletier, 1979; Pelletier & Caraty, 1981). It would therefore appear that dihydrotestosterone exerts its influence on sexual behaviour both centrally (by facilitating the action of oestradiol) and peripherally (Parrot & Baldwin, 1984). However, results of studies using aromatase inhibitors and anti-oestrogens (Larrson, 1979) have been equivocal.

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Active or passive immunization against steroid hormones has been used to create deficiencies at their sites of action and to disrupt feedback mechanisms (Nieschlag *et al.*, 1974; Wickings & Nieschlag, 1978; Sheldrick *et al.*, 1980; Thompson & Honey, 1984; Walker *et al.*, 1984). The present investigation examined the central action of dihydrotestosterone on sexual behaviour by negating, by means of active immunization, the action of oestradiol in wethers implanted with testosterone.

Materials and Methods

Implant manufacture and placement. Implants were manufactured from Silastic tubing (3·35 mm i.d., 4·65 o.d.: Dow Corning Corporation, Midland, MI, USA) according to the method of Kincl & Rudel (1970). Implants were placed subcutaneously, posterior to the shoulder, using aseptic surgical procedures.

The lengths of implants to be used were based on the results of a previous investigation (Crichton, 1989). In this study, 10 Merino wethers were used to estimate the relationship between implant length and serum hormone concentration. Wethers were treated with a given hormone implant and bled 7 days later and the implant was removed and replaced with an implant of the next hormone to be evaluated. Hormones were evaluated in the order oestradiol dipropionate, testosterone propionate and 5α-dihydro-testosterone (DHT). The implants were 13–36 mm, 176–629 mm and 27–226 mm for the three hormones, respectively. Serum samples were frozen (−20°C) and subsequently analysed for the appropriate hormone using radioimmunoassay (RIA) techniques (Abraham *et al.* 1972; Butcher *et al.*, 1974). Data from the study of Crichton (1989) suggested the following relationships between plasma hormone concentration (y) and implant length (x, mm):

for oestradiol (pg/ml)
$y = -4{\cdot}032 + 1{\cdot}290\,x\ (P < 0{\cdot}01)$,
for testosterone (ng/ml)
$y = -2{\cdot}907 + 0{\cdot}041\,x\ (P < 0{\cdot}01)$ and
for dihydrotestosterone (ng/ml)
$y = 0{\cdot}141 + 0{\cdot}001\,x\ (P < 0{\cdot}01)$.

Animals. The 43 wethers (29 Merino and 14 Dohne Merino), obtained by surgical castration at 4 weeks of age and at least $2\frac{1}{2}$ years old, were randomly allocated according to breed and body mass to the treatment groups. When treatments incorporated immunization, initially 9 animals were selected to receive antigen and the 5 animals exhibiting the highest antibody titre were retained for further immunization. Each of the 7 treatment groups thus eventually consisted of only 5 animals. Because of theft, 2 additional wethers were introduced after the initial immunization.

Treatments. The 7 implantation treatments applied were:

(a) testosterone propionate (TP; 1 × 220 mm implant),
(b) 5α-dihydrotestosterone (DHT; 1 × 100 mm implant),
(c) oestradiol dipropionate (ODP; 1 × 15 mm implant),
(d) DHT plus ODP (1 × 100 mm DHT and 1 × 15 mm ODP),
(e) TP plus immunization against oestradiol (TP + anti-E_2; 1 × 110 mm implant),
(f) TP plus immunization against both oestrone and oestradiol (TP + anti-$E_{1,2}$; 1 × 110 mm implant) and
(g) control, no implantation or immunization.

The shorter TP implant (110 vs. 220 mm) was used when the animals were immunized against oestrogens as earlier studies had shown that this procedure increased assayable concentrations of oestradiol (Crichton, 1989). Implantation occurred 48 h before the first sexual behaviour test. Implants were placed just prior to the peak of antibody production (~ 10–11 days after the final booster injection (Hurn & Chantler, 1980) thus avoiding the peak of hormone release from silicone implants which occurs within 48 h after placement (Christensen & Kesler, 1984). Sexual behaviour tests occurred within 14 days after implantation to avoid the possible effect of waning antibody concentrations.

Immunization. Antibodies which would bind oestrone and oestradiol, and thereby negate the action of these steroids, were produced by the method of Hurn & Chantler (1980). For antisera to oestradiol, the wethers were injected with conjugate of 2·5 mg 17β-oestradiol-6 and *O*-carboxymethyloxime bovine serum albumin (Sigma Chemical Company, St Louis, MO, USA). For animals in which the action of both oestrone and oestradiol were to be negated, the dose of oestradiol conjugate was halved and these animals also received 1·25 mg oestrone-3-carboxymethyloxime bovine serum albumin conjugate.

At 5 weeks after the primary immunization, the 5 wethers within treatments (e) and (f) that showed the highest antibody titre were given a booster injection. A second booster followed 30 weeks later (10 days before the first mating test).

Measuring antibody titres. At 35, 48 and 13 days after the initial, secondary and final challenges, respectively, jugular blood was obtained by venepuncture and the serum stored at −20°C. Antibody binding to [^{3}H]oestradiol and/or [^{3}H]oestrone in serial dilutions of the sera was determined using the RIA technique of Butcher *et al.* (1974).

After the final booster injection, wethers immunized against both oestrone and oestradiol (TP + anti-$E_{1,2}$) produced antisera which bound 85·7 ± 2·5% of [^{3}H]oestradiol at a 1:1000 dilution. Those immunized against oestradiol (TP + anti-E_2) bound 79·8 ± 3·2% of [^{3}H]oestradiol at the same dilution. The proportion of [^{3}H]oestrone bound (1:1000 dilution) was 58·3 ± 4·4 and 39·4 ± 6·2% for the two treatments, respectively. Cross-reaction to oestrone in the oestradiol assay was also determined and the mean cross-reaction for animals treated with TP + anti-E_2 and TP + anti-$E_{1,2}$ were 5·7 ± 1·0 and 8·0 ± 1·3%, respectively.

Assay of plasma hormone concentrations. Plasma oestrogen (all animals except controls and DHT-implanted), DHT (treatments (b) and (d)) and total androgen (all groups receiving TP) concentrations were measured in samples obtained (jugular venepuncture) 1 day before the 2nd and 3rd behavioural tests. Total androgen was measured for all animals before implantation. The procedures followed for RIA were the same as those used in the preliminary experiment. For oestrogen, testosterone and dihydrotestosterone, the respective assay sensitivities were 2·0, 2·5 and 5·0 pg/tube. The intra- and inter-assay coefficients of variation were 2·6 and 9·8% for oestrogen, 2·8 and 10·2% for testosterone and 3·6 and 17·3% for dihydrotestosterone, respectively.

Testing sexual behaviour. Oestrus was induced in ovariectomized ewes by insertion of intravaginal progestagen-impregnated sponges (Upjohn (Pty) Ltd, Isando, RSA) for 7 days followed by 1·4 mg oestradiol cypionate (Upjohn) at pessary withdrawal. The use of progesterone prior to the administration of oestradiol served to prime the ewe's reproductive system and enhance the subsequent response to oestradiol (Robinson, 1954). The dose of 1·4 mg oestradiol cypionate gave the most consistent behavioural response without masculinizing the ewes. Large numbers of ewes were used to minimize the effect of repeated doses of hormone. Intact rams with marking crayons were used to detect ewes in oestrus.

The sexual behaviour of the experimental wethers was then observed on 3 occasions after implantation. A familiarization test was applied before treatment. Tests were of 10 min duration (Parrott, 1986) and one wether was placed with 2 ewes (Parrott, 1978; Crichton & Lishman, 1984).

The test area was enclosed on all sides, behaviour being observed through a peep hole. Tests were spaced 3 days apart. The following forms of sexual behaviour were evaluated: investigation of the female's anogenital region (sniffing), wether stands immobile and fixates the ewes head (staring), rapid lapping of the tongue normally associated with low-pitched vocalizations (tongue flicks), repeated extension and flexion of the foreleg associated with pawing the ewes flank (foreleg kicks), abrupt forward movement resulting in shoulder contact with the ewe's flank (nudging), standing immobile with curled upper lip and head raised following sniffing of the ewes urine or genitalia (Flehmen), wether shifts his weight onto his hindlegs and moves forward without lifting his forelegs (intention to mount), wether rears up onto hindlegs but makes no contact with ewes hindquarters (attempted mount) and wether mounts clasping the ewes hindquarters with his forelegs with or without pelvic thrusting (mount).

Foreleg kicks and nudging were recorded as a single behaviour since the two most often occur simultaneously. Erection of the penis and ejaculation did not occur, hormone replacement therapy being of insufficient duration.

Statistical analysis. The data on traits of sexual behaviour were examined by means of split-plot analysis. Successive observations on the same animal (the effect of time) were treated as subplots in the design. The element of time in the statistical analysis was included in an attempt to detect any waning in antibody concentrations which in turn would give rise to an increase in the level of behaviour with the progression of testing. Differences between specific treatments were investigated using orthogonal comparisons (Genstat, version Mark 4.03 C, 1980; Lawes Agricultural Trust; Rothamsted Experimental Station).

Results

Plasma hormone concentrations

Before implantation, the mean ± s.e. total androgen recorded for wethers was 0·67 ± 0·23 ng/ml. After implantation, the wethers with implants of DHT or TP displayed dihydrotestosterone and total androgen concentrations (Table 1) lower than the anticipated 0·24 and 6·11 ng/ml, respectively. For the immunized wethers (TP implanted), total androgen values were higher than for nonimmunized TP wethers, in spite of the adjustment made to implant length (110 vs. 220 mm).

Sexual behaviour

Effect of individual hormones. Of the 11 wethers which initiated some form of mating behaviour during the acclimatization test, 7 (64%) had relatively high (3·01 ± 0·63 ng/ml) preimplantation

Table 1. Concentrations of serum hormones in wethers implanted with testosterone propionate (TP), oestradiol propionate (ODP), dihydrotestosterone (DHT) or a combination and immunized against oestradiol (anti-E_2) or oestrone and oestradiol (anti-$E_{1,2}$)

Treatment	*n*	Testosterone (ng/ml)	Dihydrotestosterone (ng/ml)	Oestradiol (pg/ml)
TP + anti-$E_{1,2}$	5	8·36 ± 2·59	—	5·51 ± 1·80
TP + anti-E_2	5	4·10 ± 0·77	—	3·06 ± 0·72
TP	5	3·13 ± 0·41	—	1·49 ± 0·25
DHT	5	—	0·12 ± 0·02	—
DHT + ODP	5	—	0·15 ± 0·03	18·29 ± 1·14
ODP	5	—	—	17·76 ± 0·57

Data are means ± s.e., for 2 sample dates.

concentrations of total androgen. Sexual behaviour before hormone treatment seldom progressed beyond the level of investigative behaviour, only 2 of the wethers showing an intention or an attempt to mount.

Significant ($P < 0{\cdot}05$) treatment effects were detected for all components of sexual behaviour (Fig. 1), except sniffing ($P < 0{\cdot}08$). The wethers implanted with TP only exhibited the highest levels of behaviour for all components of mating behaviour except Flehmen (Fig. 1). Only for tongue flicks and actual mounts was the level of response significantly ($P < 0{\cdot}01$) above that observed in animals which received both DHT and ODP. Treatment with ODP supported a higher level of Flehmen ($P < 0{\cdot}01$). In contrast, implantation with ODP alone resulted in a significantly lower level of staring ($P < 0{\cdot}05$), tongue flicks ($P < 0{\cdot}01$), nudging ($P < 0{\cdot}01$) and mounting $P < 0{\cdot}01$) than did TP (Fig. 1). Stimulation by ODP of the majority of components of sexual behaviour, when compared with untreated or DHT-implanted wethers, was significant (Fig. 1, $P < 0{\cdot}01$). The addition of DHT to ODP resulted in a significant increase in nudging and Flehmen compared with wethers receiving only ODP ($P < 0{\cdot}01$).

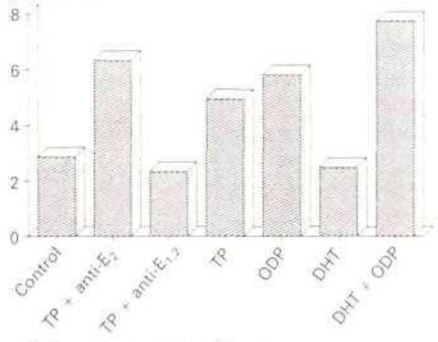
Treatment with DHT alone did not alter the behaviour from that exhibited by the untreated control (Fig. 1).

Effect of immunization. Immunization against oestradiol (TP + anti-E_2) significantly depressed ($P < 0{\cdot}01$) sexual behaviour to levels similar to those recorded for the untreated control (Fig. 1). Although immunization against both oestradiol and oestrone (TP + anti-$E_{1,2}$) appeared to be more successful in promoting binding of oestrone and oestradiol than immunization against oestradiol alone, the differences in sexual behaviour between these two treatments were not significant (Fig. 1), even though many were of the order of 100% and more. In 3 of the 5 wethers immunized against both oestrone and oestradiol, mounting was completely suppressed over all tests, while 2 of the wethers in which the effect of oestradiol was blocked never mounted. The 2 animals which mounted in the former treatment group had received only one booster injection (a result of stock theft).

Changes in behaviour over time. Two components of mating behaviour changed significantly during the course of the study. The level of sniffing declined significantly ($P < 0{\cdot}01$) from behavioural test 1 to test 2 and remained low in test 3 ($P < 0{\cdot}05$). In contrast, the incidence of mounting was significantly higher ($P < 0{\cdot}01$) in test 2 than in test 1, but then declined significantly ($P < 0{\cdot}01$) in test 3.

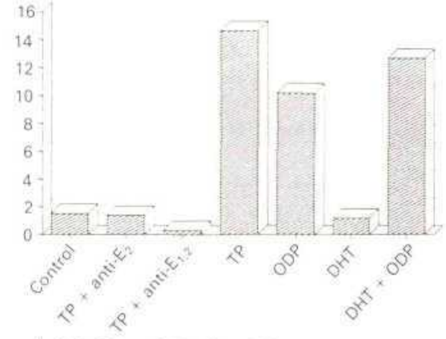
Fig. 1 Mean levels of 7 traits of sexual behaviour measured in 3 tests in wethers implanted with testosterone propionate (TP), oestradiol propionate (ODP), dihydrotestosterone (DHT) or a combination and immunized against oestradiol (anti-E_1) or oestrone and oestradiol (anti-$E_{1,2}$).

(a) Sniffing



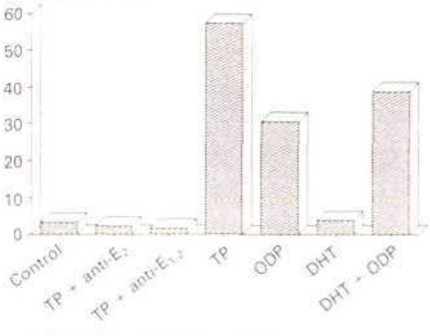
Differences not significant

(b) Staring



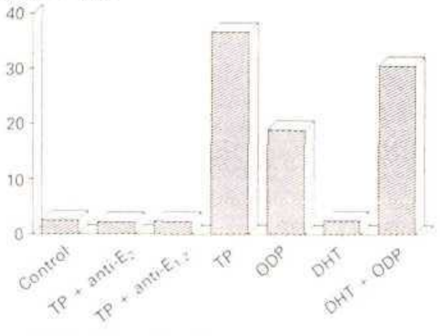
L.S.D. 5% = 3·7, 1% = 5·1

(c) Tongue flicks



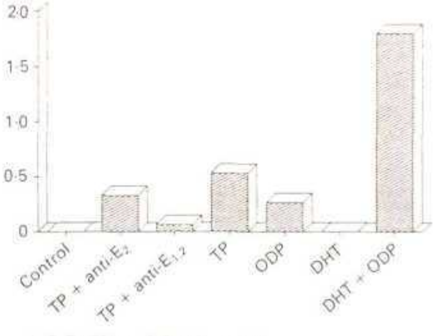
L.S.D. 5% = 9·7, 1% = 13·1

(d) Nudging



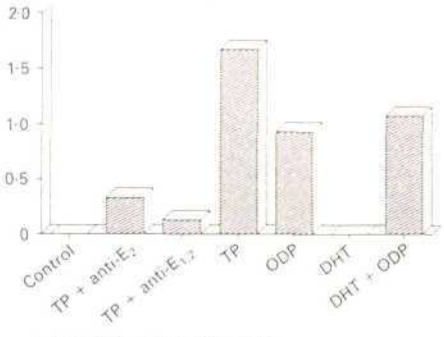
L.S.D. 5% = 9·0, 1% = 12·2

(e) Flehmen



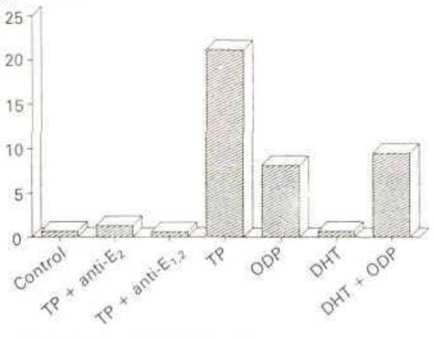
L.S.D. 5% = 0·8, 1% = 1·0

(f) Intention to mount



L.S.D. 5% = 0·9, 1% = 1·2

(g) Mount



L.S.D. 5% = 5·2, 1% = 7·1

Fig. 1.

Table 2. Changes in levels of mounting for the 7 hormone treatments over time in 3 behavioural tests in wethers implanted with testosterone propionate (TP), oestradiol propionate (ODP), dihydrotestosterone (DHT) or a combination and immunized against oestradiol (anti-E_2) or oestrone and oestradiol (anti-$E_{1,2}$)

Treatment	*n*	Behavioural test 1	 2	 3
Control	5	$0\cdot40 \pm 0\cdot40^{a}$	$1\cdot00 \pm 0\cdot55^{a}$	$0\cdot60 \pm 0\cdot40^{a}$
TP + anti-E_2	5	$1\cdot20 \pm 0\cdot97^{a}$	$2\cdot60 \pm 1\cdot29^{a}$	$0\cdot20 \pm 0\cdot20^{a}$
TP + anti-$E_{1,2}$	5	$0\cdot20 \pm 0\cdot20^{a}$	$0\cdot80 \pm 0\cdot58^{a}$	$0\cdot80 \pm 0\cdot49^{a}$
TP	5	$14\cdot40 \pm 5\cdot55^{a}$	$27\cdot40 \pm 4\cdot47^{b}$	$21\cdot80 \pm 4\cdot62^{c}$
ODP	5	$9\cdot80 \pm 2\cdot65^{a}$	$8\cdot00 \pm 2\cdot70^{a}$	$6\cdot60 \pm 2\cdot01^{a}$
DHT	5	0·00	$1\cdot80 \pm 1\cdot56^{a}$	$0\cdot20 \pm 0\cdot20^{a}$
DHT + ODP	5	$6\cdot40 \pm 1\cdot43^{a}$	$14\cdot60 \pm 4\cdot40^{b}$	$7\cdot60 \pm 1\cdot75^{a}$

Data are means ± s.e.

Means within the same row without common superscripts are significantly different.

The L.S.D. values for $P < 0\cdot05$ and $P < 0\cdot01$ were 6·67 and 9·98, respectively, for different and 5·05 and 6·73, respectively, for the same hormone treatments

The only significant ($P < 0\cdot05$) hormone × time interaction was that recorded for mounting behaviour (Table 2). The TP and DHT + ODP implanted wethers displayed a significantly higher level of mounting behaviour in test 2 than in test 1 ($P < 0\cdot01$; Table 2). In test 3, mounting behaviour was significantly lower for TP-treated and DHT + ODP-treated animals, than in test 2 ($P < 0\cdot01$). The difference in mounting between tests 1 and 3 was significant for TP-implanted ($P < 0\cdot01$), but not DHT + ODP-implanted, wethers (Table 2).

In tests 2 and 3, the level of mounting behaviour exhibited by the TP-treated wethers was significantly higher ($P < 0\cdot01$) than in animals in the other treatments (Table 2). In test 1, the difference between the TP and other treatments, with the exception of the ODP wethers, was also significant ($P < 0\cdot01$, Table 2).

The significant change in mounting behaviour over the 3 tests could not be explained in terms of any effects peculiar to either hormone treatment or declining antibody concentrations.

When the effect of treatment was examined by orthogonal comparisons (TP vs TP + anti-$E_{1,2}$ and TP + anti-E_2) it was again evident that immunization reduced ($P < 0\cdot01$) the level of all components of mating behaviour except sniffing and Flehmen. Such comparisons confirmed the earlier conclusion that administration of ODP improved ($P < 0\cdot01$) all components of sexual behaviour (DHT + ODP and ODP vs. DHT and control). The specific effect of a combination of DHT and ODP on improving Flehmen (Fig. 1) was supported by orthogonal comparisons ($P < 0\cdot01$).

Discussion

When the effects on mating behaviour of aromatization of testosterone to oestrogens, within the CNS, were negated by immunization, 5α-reduction to dihydrotestosterone were not interfered with, but behavioural levels returned to the values in castrates (Fig. 1). Furthermore, the implantation of dihydrotestosterone alone had no effect on the behavioural responses of castrates. On their own, these observations appear to contradict the conclusion that the action of androgens is not mediated exclusively by aromatization to oestrogen (Callard, 1984). However, administration of oestrogen alone was not able to support the level of behaviour achieved by testosterone propionate (Fig. 1), while the provision of dihydrotestosterone in addition to oestradiol was as effective as testosterone in all aspects of mating except tongue flicking and mounting. The lack of a strong DHT and ODP synergism, as regards mounting, might be attributed to the low, but realistic, concentrations of dihydrotestosterone. The alcohol state of dihydrotestosterone has been reported to have a less potent effect on behaviour than the propionate form (Yahr, 1979). It appears that dihydrotestosterone acts only when oestrogens are present, augmenting the action of the latter on neural tissues (Baum *et al.*, 1974).

A notable exception to this rule appears with regard to Flehmen. When dihydrotestosterone was combined with oestradiol, this markedly raised the level of Flehmen (Fig. 1), thereby supporting the contention (Hart & Leedy, 1987) that the hormonal control of this component of male mating behaviour is different from that of the other aspects. It has been suggested that CNS tissue has a lower testosterone threshold than the peripheral sex organs and that concentrations of testosterone of 0·32–0·65 ng/ml elicit nudging and mounting behaviour (centrally controlled), while full mating activity (intromission and ejaculatory reflex) is achieved with 1·26 ng/ml (D'Occhio & Brooks, 1982). On this basis 34% of the wethers should have been capable of mounting before implantation. Furthermore, oestradiol has a far greater potency than testosterone (microgram versus milligram concentrations) at the level of the CNS (Ryan *et al.*, 1972). It would therefore appear that mounting behaviour is possible in a fairly large proportion of wethers. The need for hormonal priming before the activation of mounting can, however, be questioned in view of the effect of prepubertal social interaction on subsequent sexual behaviour (Zenchak & Anderson, 1980; Thwaites, 1982). Such early behaviour, which reached a peak at 4 weeks of age (Thwaites, 1982), could have contributed to mounting without hormone treatment in the present study.

Courtship behaviour involves the testing of female receptivity and, if it is accepted that oestradiol is the primary hormone associated with initiation of mounting, it would appear that an animal motivated by increased penile stimulation (DHT + ODP vs. ODP) will indulge in higher levels of courtship, and as a result mounting behaviour, in an attempt to satisfy the urge to copulate. This agrees with the proposal by Baum *et al.* (1974) that, once an initial mount has occurred, in intact males or in castrates treated with testosterone or oestradiol and dihydrotestosterone, the neural tissues controlling this behaviour then respond at a high level because dihydrotestosterone-stimulated sensory receptors in the penis or genital region provide a heightened facilitatory input to the brain. Support for this theory comes from the work of Parrott (1975), who demonstrated that dihydrotestosterone is responsible for sensitization of the penis in rats, and Bonsall *et al.* (1985), who reported that dihydrotestosterone is preferentially bound in the male genital tract of monkeys. Katz & Price (1986), using surgical deflection and neuroectomy of the penis in bulls, concluded that sensory stimulation of the penis facilitates the expression of sexual behaviour. However, Baum *et al.* (1974) concluded that the effect of dihydrotestosterone on oestradiol-induced mounting in rats probably results from the action of dihydrotestosterone at the level of the brain and not from the stimulatory effect on genital sensory receptors.

The presence of androgen receptors capable of binding both testosterone and dihydrotestosterone, but with higher affinity for dihydrotestosterone, in the pituitary and hypothalamus of rams (Thieulant & Pelletier, 1979; Pelletier & Caraty, 1981) has been demonstrated. Therefore, it cannot be categorically stated that dihydrotestosterone has no CNS function. However, Bonsall *et al.* (1985) point out that testosterone, rather than dihydrotestosterone, is the principal androgen in brain cell nuclei and state that 5α-reductase concentrations may not produce sufficient amounts of dihydrotestosterone to compete with testosterone for binding to CNS androgen receptors.

It has been demonstrated that a low testosterone threshold exists for initiation of mounting behaviour (D'Occhio & Brooks, 1982) in wethers. Relatively high testosterone concentrations induced in wethers treated with testosterone propionate in a previous study (18 ng testosterone/ml, Crichton, 1989) and the lower values reported in the present study, were associated with low, but similar, concentrations of oestradiol (< 2 pg/ml), leading to a conclusion that the concentrations of oestradiol in the CNS required to stimulate sexual behaviour may bear little relationship to serum concentrations. These have been reported to be as high as 13 pg/ml in intact rams (Sanford *et al.*, 1982). There is a clear need to differentiate between the relative importance of testosterone, dihydrotestosterone and oestradiol at the level of the CNS.

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References

Abraham, G.E., Buster, J.E., Lucas, L.A., Corrales, P.C. & Teller, R.C. (1972) Chromatographic separation of hormones for use in radioimmunoassay. *Anal. Letters.* **5,** 509–517.

Baum, M.J., Sodersten, P. & Vreeburg, J.T.M. (1974) Mounting and receptive behaviour in ovariectomized female rat: influence of oestradiol, dihydrotestosterone, and genital anesthetization. *Horm. Behav.* **5,** 175–190.

Bonsall, R.W., Rees, H.D. & Michael, R.P. (1985) The distribution, nuclear uptake and metabolism of 5α-dihydrotestosterone in the brain, pituitary gland and genital tract of the male rhesus monkey. *J. Steroid Biochem.* **23,** 389–398.

Bruchovsky, N. & Wilson, J.D. (1968) The conversion of testosterone to 5α-androstan-17-ol-3-one by rat prostrate *in vivo* and *in vitro*. *J. biol. Chem.* **243,** 2012–2021.

Butcher, R.L., Collins, W.E. & Fugo, N.W. (1974) Plasma concentration of LH, FSH, prolactin, progesterone and oestradiol-17β throughout the 4-day estrous cycle of the rat. *Endocrinology* **94,** 1704–1708.

Callard, G.V. (1984) Aromatization in brain and pituitary: An evolutionary perspective. In *Metabolism of Hormonal Steroids in the Neuroendocrine Structures*, pp. 79–102. Eds F. Celotti, F. Naftolin & L. Martini. Raven Press, New York.

Callard, G.V., Petro, Z. & Ryan, K.J. (1978) Conversion of androgen to oestrogen and other steroids in the vertebrate brain. *Am. Zool.* **18,** 511–523.

Christensen, D.A. & Kesler, D.J. (1984) Passage of testosterone, testosterone propionate and testosterone enanthate from silastic implants and the retention of testosterone once it enters the blood of ewes. *Anim. Reprod. Sci.* **7,** 531–536.

Crichton, J.S. (1989) *Testing the libido of breeding sires to predict mating performance in the field and the hormonal control of male sexual behaviour.* Ph.D. thesis, University of Natal, Pietermaritzburg, Republic of South Africa.

Crichton, J.S. & Lishman, A.W. (1984) Identification of sexually low-response rams using a libido test and their performace when allowed to mate under field conditions. *S. Afr. J. Anim. Sci.* **14,** 177–178.

D'Occhio, M.J. & Brooks, D.E. (1976) The influence of androgens and oestrogens on mating behaviour in male sheep. *Theriogenology* **6,** 614.

D'Occhio, M.J. & Brooks, D.E. (1980) Effect of androgenic and oestrogenic hormones on mating behaviour in rams castrated before and after puberty. *J. Endocr.* **86,** 403–411.

D'Occhio, M.J. & Brooks, D.E. (1982) Threshold of plasma testosterone required for normal mating activity in male sheep. *Horm. Behav.* **16,** 383–393.

Hart, B.L. & Leedy, M.G. (1987) Stimulus and hormonal determinants of Flehmen behaviour in cats. *Horm. Behav.* **21,** 44–52.

Hurn, B.A.L. & Chantler, S.M. (1980) Production of reagent antibodies. *Meth. Enzymol.* **70,** 104–142.

Katz, L.S. & Price, E.O. (1986) The role of penile stimulation and ejaculatory experience on the development and maintenance of reproductive behaviour in the bull (*Bos taurus*). *Devl Psychobiol.* **19,** 197–209.

Kincl, F.A. & Rudel, H.W. (1970) Sustained release hormonal preparations. *Acta endocr., Copenh.* **64,** 5–30.

Larrson, K. (1979) Features of the neuroendocrine regulation of male sexual behaviour. In *Endocrine Control of Sexual Behaviour*, pp. 77–163. Ed. G. Beyer. Raven Press, New York.

Lieberburg, I. & McEwen, B.S. (1975) Oestradiol-17β: a metabolite of testosterone recovered in brain cell nuclei from limbic areas of adult male rat brains. *Brain Res.* **91,** 171–174.

Luttge, W.G. (1979) Endocrine control of mammalian male sexual behaviour: An analysis of the potential role of testosterone metabolites. In *Endocrine Control of Sexual Behaviour*, pp. 341–363. Ed. G. Beyer. Raven Press, New York.

Massa, R., Justo, S. & Martini, L. (1975) Conversion of testosterone into 5α-reduced metabolites in the anterior pituitary and the brain of maturing rats. *J. Steroid Biochem.* **6,** 567–572.

Mattner, P.E. (1976) Effect of androgens and oestradiol on libido and aggressiveness in rams castrated as adults. *Theriogenology* **6,** 612.

Naftolin, F., Ryan, K.J. & Petro, Z. (1972) Aromatization of androstenedione by the anterior hypothalamus of adult male and female rats. *Endocrinology* **90,** 295–298.

Nieschlag, E., Usadel, K.H., Kley, H.K., Schwedes, U., Schöffling, K. & Krüskemper, H.L. (1974) A new approach for investigating hypothalamo-pituitary-gonadal and adrenal feedback control mechanisms: active immunization with steroids. *Acta endocr., Copenh.* **76,** 556–569.

Parrott, R.F. (1975) Aromatizable and 5α-reduced androgens: differentiation between central and peripheral effects of male rat sexual behaviour. *Horm. Behav.* **6,** 99–108.

Parrott, R.F. (1978) Courtship and copulation in prepubertally castrated male sheep (wethers) treated with 17β-estradiol, aromatizable androgens or dihydrotestosterone. *Horm. Behav.* **11,** 20–27.

Parrott, R.F. (1986) Minimal effects of 17β-hydroxy-17α-methyl-estra-4, 9, 11-triene-3-one (R1881) on sexual behaviour in prepubertally castrated rams. *J. Endocr.* **110,** 481–487.

Parrott, R.F. & Baldwin, B.A. (1984) Sexual and aggressive behaviour of castrated male sheep injections of gonadal steroids and implantation of androgens in the hypothalamus: a preliminary study. *Theriogenology* **21**, 533–542.

Pelletier, J. & Caraty, A. (1981) Characterization of cytosolic 5α-DHT and 17β-oestradiol receptors in the ram hypothalamus. *J. Steroid Biochem.* **14**, 603–611.

Robinson, T.J. (1954) The necessity for progesterone with oestrogen for the induction of recurrent oestrus in the ovariectomized ewe. *Endocrinology* **55**, 403–408.

Ryan, K.J., Naftolin, F., Reddy, V., Flores, F. & Petro, Z. (1972) Oestrogen formation in the brain. *Am. J. Obstet. Gynec.* **114**, 454–460.

Sanford, L.M., Simaraks, S., Palmer, W.M. & Howland, B.E. (1982) Circulating oestrogen levels in the ram: influence of season and mating, and their relationship to testosterone levels an mating frequency. *Can. J. Anim. Sci.* **62**, 85–93.

Sheldrick, E.L., Mitchell, M.D. & Flint, A.P.F. (1980) Delayed luteal regression in ewes immunized against oxytocin. *J. Reprod. Fert.* **59**, 37–42.

Thieulant, M. & Pelletier, J. (1979) Evidence for androgen and oestrogen receptors in castrated ram pituitary cytosol: influence of time after castration. *J. Steroid Biochem.* **10**, 677–687.

Thompson D.L. & Honey, P.J. (1984) Active immunization of prepubertal colts against oestrogens: hormonal and testicular responses after puberty. *J. Anim. Sci.* **59**, 189–196.

Thwaites, C.J. (1982) Development of mating behaviour in prepuberal rams. *Anim. Behav.* **30**, 1053–1059.

Walker, M.P., Thompson, D.L., Godke, R.A. & Honey, P.G. (1984) Active immunization of prepubertal bulls against testosterone: seminal and testicular characteristics after puberty. *Theriogenology* **22**, 269–278.

Whalen, R.E. & Rezek, D.L. (1972) Localization of androgenic metabolites in the brain of rats administered testosterone or dihydrotestosterone. *Steroids* **20**, 717–725.

Wickings, E.J. & Nieschlag, E. (1978) The effects of active immunization with testosterone on pituitary-gonadal feedback in the male rhesus monkey (*Macaca mulatta*). *Biol. Reprod.* **18**, 602–607.

Yahr, P. (1979) Data and hypotheses in tales of dihydrotestosterone. *Horm. Behav.* **13**, 92–96.

Zenchak, J.J. & Anderson, G.C. (1980) Sexual performance levels of rams (*Ovis aries*) as affected by social experiences during rearing. *J. Anim. Sci.* **50**, 167–174.

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Libido evaluation of virgin beef bulls

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Fifty-four beef bulls with an average age of 21 months and representing nine breeds were evaluated for libido using two types of tests. The serving capacity (SC) test was repeated five times on groups of bulls and was followed by an individual test. In the SC test, breed significantly influenced the number of services completed in 30 min. Bulls showed a highly significant improvement in their SC as their experience increased but this did not apply to all breeds. There was thus a significant breed by test interaction. Agonistic interactions had no influence on SC although exceptions were noted. Age, mass, physical size, scrotal circumference, semen quality, and environmental temperature (10,5 – 26,5°C) did not influence SC. There was a highly significant correlation (r = 0,664) between SC and the number of services completed on the individual test. Of the bulls tested, 27,7%, 40,4%, and 31,9% were classified as having a low, medium, and high libido.

Vier-en-vyftig vleisrasbulle verteenwoordigend van nege rasse en met 'n gemiddelde ouderdom van 21 maande, is deur middel van twee afsonderlike toetse vir libido geëvalueer. Die dekkapasiteit (DK)-toets wat vyf keer herhaal is, is deur 'n individuele toets opgevolg. By die DK-toets is die aantal volledige dekkings per 30 min. betekenisvol deur ras beïnvloed, terwyl 'n hoogsbetekenisvolle vermeerdering in DK gevind is namate bulle ondervinding opgedoen het, alhoewel rasverskille wel voorgekom het. Strydlustigheid het op enkele uitsonderings na, nie die DK beïnvloed nie. Die DK is ook nie deur ouderdom, massa, grootte, skrotumomtrek, saadkwaliteit en omgewingstemperatuur (10,5 – 26,5°C) beïnvloed nie. Tydens die individuele toets is 'n hoogs betekenisvolle positiewe korrelasie (r = 0,664) tussen aantal volledige dekkings en DK gevind. Van die getoetste bulle, is 27,7%, 40,4% en 31,9% as lae, gemiddelde en hoë libido geklassifiseer.

Keywords: Libido evaluation, beef bulls

With increasing attention being given to the unsatisfactory reproductive rates of beef herds, the need has arisen to evaluate the mating performance of bulls used in such herds. One facet which appears to warrant attention is that of the libido of such bulls. High libido bulls significantly increased first oestrus conception rates in cows and heifers over those achieved by low libido bulls (Blockey, 1978; Lunstra, 1980). Furthermore, high libido bulls can be successfully mated to more females over a limited breeding season (Blockey, 1980).

Fifty-four virgin beef bulls undergoing a phase D2 performance test were evaluated for libido at the end of their test. Nine breeds were represented. Their average age ± SE was 629 ± 24 days (approximately 21 months).

Bulls were subjected to two types of tests. The first was a group test, the serving capacity (SC) test, designed to predict veld mating performance (Blockey, 1981b). Bulls were classified in three breed type groups, British beef (Sussex, Red Angus, and Hereford), dual purpose (Simmentaler and South Devon) and zebu types (Drakensberger, Afrikaner, Brahman, and Santa Gertrudis). At the end of the feedlot stage of the performance test each breed type was subjected to five SC tests (breed types did not finish the feedlot stage simultaneously, they finished in the following order, British beef, dual purpose, and zebu). One test involved the use of non-oestrus heifers (Tests 2, 3, and 4 for the zebu, dual purpose, and British beef breed types respectively). Wherever possible, three bulls of the same breed and of similar mass were placed in a pen with two ovariectomized heifers in which oestrus had been induced. Oestrus was induced by a series of three injections (Lunstra, 1980):

(a) Day 1, 50 mg progesterone (Depogest, Centaur).
(b) Day 2, 25 mg progesterone.
(c) Day 3, 4 mg estradiol cypionate (ECP, UpJohn).

All injections were intramuscular and administered at 10h00 each day. The heifers were restrained in service crates. As soon as a heifer became unreceptive she was replaced by another. Bulls were scored on the basis of the number of services completed in 30 min, i.e. their serving capacity. Prior to test, bulls were sexually stimulated for 30 min by observation of the previous group on test. Bulls which had been culled for various reasons formed 'stimulator' groups which were allowed to mate for 30 min prior to the first test on any given day. Tests were spaced 2 – 3 days apart. Agonistic interactions were recorded as having either no definite outcome or resulting in a win or lose. Disrupted mounts were also recorded as was the environmental temperature during each test.

In the second type of test used to evaluate individual bulls, bulls were exposed to a restrained, oestrus-induced, ovariectomized heifer for 10 min following at least 10 min of sexual stimulation (Chenoweth & Osborne, 1975). Libido was scored using the following system (Chenoweth, Brinks & Nett, 1979):

0 = showed no sexual interest
1 = sexual interest shown only once
2 = positive sexual interest in the female more than once
3 = active pursuit of the female with persistent sexual interest
4 = one mount or mounting attempt, no service
5 = two mounts or mounting attempts, no service
6 = more than two mounts or mounting attempts, no service
7 = one service followed by no further sexual interest
8 = one service followed by further sexual interest including mounts or mounting attempts
9 = two services followed by no further sexual interest
10 = two services followed by sexual interest including mounts, mounting attempts or further services.

Thus a completed service was not necessary for bulls to achieve a score. Only one such test was applied to each bull and it occurred once all the group tests had been completed.

The scrotal circumference, body length, and height (cm) of the bulls were measured in the month before the start of the SC tests. One ejaculate of semen (electroejaculation) was obtained just prior to the start of the SC tests and was evaluated for motility and percentage abnormalities.

Data from the SC tests were analysed using successive observations on the same animal as sub-plots in a split-plot design. The effects of agonistic interaction and environmental temperature on SC were evaluated using linear regression analysis. The influence of age, mass, length, height, scrotal circumference, and semen quality on SC was evaluated using covariate analysis.

The bulls showed a highly significant improvement in their SC as the tests progressed (Figure 1). This agrees with Lunstra (1980) who reported an increase over the first three tests after which scores stabilized (yearling bulls). Blockey (1981b) stated that 2-year-old virgin bulls did not undergo a learning process. He did however note that a few bulls improved their scores in their second test.

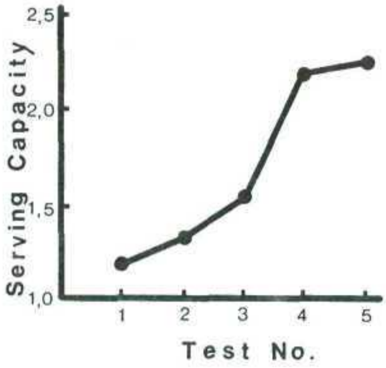

Figure 1 The improvement in serving capacity (SC) over five tests, expressed as the mean number of services completed in 30 min (n = 54).

There were significant breed influences on the number of services completed in 30 min (Table 1) and although the overall tendency was for SC to improve with experience (Figure 1), a significant breed by test interaction occurred.

The small number of bulls in some breeds (Sussex, South Devon, and Santa Gertrudis) contributed to their inconsistent scores, since a good or poor performance by one or two bulls on any given test had a drastic influence on the breed score. The Simmentaler and South Devon showed a slight but non-significant increase with time (Table 1).

The Brahman and Afrikaner breeds did not complete service at any time during the five tests, despite a willingness to mount (bear in mind the small numbers of Brahmans (n = 5) and Afrikaners (n = 2) evaluated). Two Afrikaner bulls, one in a 'stimulator' group and one which did not complete any SC tests owing to intestinal ulcers but partook in the individual test, completed service. Differences in libido among bulls representing *B. indicus* and *B. taurus* breeds are well documented (Chenoweth, 1975; Chenoweth & Osborne, 1975; Chenoweth, 1981). Indeed, Chenoweth (1981) states that, 'zebu bulls have been reported to exhibit marked sexual sluggishness and a tendency only to mount cows in full oestrus'. The latter suggests that zebu bulls are more sensitive to the oestral stimuli elicited by the teaser females than are *B. taurus* bulls. A test under more natural conditions and of longer duration is suggested for zebu bulls. Chenoweth & Osborne (1975) reported that individual Brahman bulls (16 – 31 months of age) achieved libido scores equal to the best of other breeds (Afrikaner, Hereford, and various crossbreds). It was suggested that selection against deficient or delayed libido could improve the reproductive performance of this breed. The possibility that the zebu bulls in this study were still undergoing pubertal changes cannot be discounted in the light of work carried out by Fields, Burns & Warnick (1979) on 16 – 20-month-old Angus, Hereford, Brahman, and Santa Gertrudis bulls. Furthermore, some bulls can show temporary

Table 1 Mean number of services completed by each breed over the five serving capacity tests, breed means included

Serial no	Breed	n	Serving capacity test* 1	2	3	4	5	Breed** mean
1	Sussex	5	$2,20^{a}$	$4,00^{b}$	$2,40^{ac}$	$2,60^{ac}$	$3,60^{bc}$	2,96
2	Red Angus	4	$0,75^{a}$	$2,25^{ac}$	$2,00^{a}$	$4,50^{b}$	$3,75^{bc}$	2,65
3	Hereford	9	$0,33^{a}$	$1,11^{ab}$	$1,56^{bc}$	$1,22^{bc}$	$2,22^{c}$	1,29
4	Simmentaler	14	$2,57^{ab}$	$2,07^{a}$	$2,50^{ab}$	$3,21^{b}$	$2,86^{ab}$	2,64
5	South Devon	4	$2,25^{a}$	$0,50^{b}$	$2,50^{a}$	$3,25^{a}$	$3,50^{a}$	2,40
6	Drakensberger	9	$0,44^{a}$	$0,22^{a}$	$1,00^{ab}$	$1,89^{b}$	$1,78^{b}$	1,07
7	Santa Gertrudis	2	$0,00^{a}$	$1,50^{a}$	$0,00^{a}$	$0,50^{a}$	$0,50^{a}$	0,50
8	Brahman	5	0,00	0,00	0,00	0,00	0,00	0,00
9	Afrikaner	2	0,00	0,00	0,00	0,00	0,00	0,00

*Means without common superscripts within breeds are significantly different ($P<0,05$). In Tests 2, 3, and 4 non-oestrus females were used for the zebu, dual purpose and British beef breeds respectively

**Significance of breed means: 2,4,5>8 ($P<0,05$); 4>3 ($P<0,05$); 1,4>6,9 ($P<0,05$); 1>8 ($P<0,01$)

psycogenic or central nervous inhibition of libido (particularly *B. indicus* bulls) and commence copulatory behaviour unpredictably at a later stage (Chenoweth, 1975).

All forms of agonistic interaction (no definite outcome, wins/losses and disrupted services) had a significant stimulatory effect on the number of mounts in 30 min (r = 0,242). This is not surprising, since Lunstra (1981) found that group evaluation was more effective than individual evaluation in terms of number of mounts and matings achieved per bull. The number of services achieved was not influenced detrimentally by agonistic interactions in general. The number of encounters won represented 2,5% of all agonistic interactions. Blockey (1979) reported that the social dominance order (SDO) in groups of 2-year-old bulls was unstable and had no significant influence on the number of cows a given bull mounted or served.

Despite the fact that agonistic interactions did not have a statistically significant effect on SC (all breeds considered), the performance of the Simmentaler breed decreased markedly though non-significantly in Tests 2 and 5 (Table 1). Analysis of the data for this breed revealed a significant negative association between all forms of agonistic interaction and SC in Test 5 (r = −0,605), and a strong tendency in Test 2 ($P<0,10$; r = −0,470). Also the performance of one South Devon bull was decreased (six vs one service) by continual harassment from another bull which did not serve. This significantly decreased the breed average for Test 2 (Table 1).

Neither age, mass, length, height, scrotal circumference, semen quality, or environmental temperature (range 10,5 to 26,5°C) were related to SC. Furthermore, it was not necessary for heifers (hindquarters immobilized by the service crate) to be in oestrus (Table 1). This confirms the work of Chenoweth, Brinks & Nett (1979) and Blockey (1981a). They stated that an immobilized set of hindquarters is the most important stimulus causing a bull to mount and complete service.

The chance of bulls injuring themselves as a result of agonistic interaction while undergoing the SC test is always prevalent. Thus the idea of an individual test is appealing. Total SC over five tests was highly significantly correlated with the number of services completed during the individual test (r = 0,664).

Based on the means for the five SC tests, excluding Brahmans and Afrikaners, 27,7% of bulls had a low (less than one service), 40,4% had a medium (one or two services), and 31,9% had a high libido (more than two services).

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References

BLOCKEY, DE B.M.A., 1978. The influence of serving capacity of bulls on herd fertility. *J. Anim. Sci.* 46, 589.

BLOCKEY, DE B.M.A., 1979. Observations on group mating of bulls at pasture. *Appl. Anim. Eth.* 5, 15.

BLOCKEY, DE B.M.A., 1980. Getting the most out of rams, bulls and boars. *Proc. Aust. Soc. Anim. Prod.* 13, 46.

BLOCKEY, DE B.M.A., 1981a. Modification of a serving capacity test for beef bulls. *Appl. Anim. Eth.* 7, 321.

BLOCKEY, DE B.M.A., 1981b. Further studies on the serving capacity test for beef bulls. *Appl. Anim. Eth.* 7, 337.

CHENOWETH, P.J., 1975. Bull behaviour and management. *Proceedings, the Range Beef Cow A Symposium on Production.* 4, 17.

CHENOWETH, P.J., 1981. Libido and mating behaviour in bulls, boars and rams. A review. *Theriogenology.* 16, 155.

CHENOWETH, P.J., BRINKS, J.S. & NETT, T.M., 1979. A comparison of three methods of assessing sex-drive in yearling beef bulls and relationships with testosterone and LH levels. *Theriogenology.* 12, 223.

CHENOWETH, P.J. & OSBORNE, H.G., 1975. Breed differences in the reproductive function of young beef bulls in Central Queensland. *Aust. Vet. J.* 51, 405.

FIELDS, M.J., BURNS, W.C. & WARNICK, A.C., 1979. Age, season and breed effects on testicular volume and semen traits in young beef bulls. *J. Anim. Sci.* 48, 1299.

LUNSTRA, D.D., 1980. Evaluation of libido in beef bulls. *Proc. Ann. Meet. Soc. Theriogenology* p. 169.

LUNSTRA, D.D., 1981. Effect of male-to-female ratio on assessment of sexual aggressiveness in yearling beef bulls. *J. Anim. Sci.* 53 (Suppl. 1), 104.

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Factors Influencing Sexual Behaviour of Young *Bos indicus* Bulls Under Pen and Pasture Mating Conditions

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ABSTRACT

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Some of the factors which influence the pen and subsequent pasture mating activity of young (20 months of age) Afrikaner bulls were investigated. The bulls ($n=18$) were subjected to 12 Blockey-type pen tests. Eight of these bulls, four high and four low serving capacity, were subsequently mated to small groups of oestrous-synchronised females under pasture conditions. During the serving capacity tests, bulls showed no preference for nocturnal sexual behaviour and were not hindered by the presence of personnel scoring their activity. Many of the bulls did not serve consistently from one test to another. However, their serving capacity improved with progression of the tests. The inconsistent serving behaviour of the bulls suggests that restrained females do not present the ideal mating stimulus. Furthermore, agonistic interactions had a depressive effect on sexual behaviour. There was, however, a significant relationship between pen and pasture mating behaviour; high-serving-capacity bulls achieved superior conception rates to low-serving-capacity bulls. Pasture mating performance was significantly influenced by the degree of cow receptiveness, the bull's previous activity and duration of his exposure to an oestrous female within a given observation period. The need to identify the specific oestral characteristics which attract and promote copulatory behaviour in *Bos indicus* bulls clearly exists.

INTRODUCTION

Although *Bos indicus* breeds have been used widely in cross-breeding programs, some of these breeds have a record of sub-fertility relative to that in *Bos taurus* breeds (Seebeck, 1973). Thus, Wells (1986) maintained that Afrikaner cow herds seldom achieve conception rates > 65% in a 90-day breeding

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season and Van Niekerk et al. (1985) obtained between 5 and 10% fewer calves from Afrikaner cows than from Simmentaler cows, even when the breeds were compared under an environment to which Afrikaners are said to be well adapted. Similarly, Brahman cross genotypes developed in Australia display a reproductive performance which is in some cases lower than *B. taurus* breeds (Seebeck, 1973) and a male contribution to lowered fertility has been suggested. The latter might be explained by the finding that *B. indicus* bulls have less sperm per gram of testicular tissue and a lower sperm production potential than *B. taurus* bulls (Entwistle et al., 1980). Since bull libido is also a factor influencing fertility (Blockey, 1978), *B. indicus* bulls are at a further disadvantage because they display marked sexual sluggishness in pen libido tests (Chenoweth, 1981; Crichton and Lishman, 1985). There is, perhaps, a need to modify accepted pen tests since Chenoweth (1981) suggested that *B. indicus* bulls prefer to mount cows which are in full oestrus and Price (1985) speculated that the use of unrestrained oestrous females is preferred when conducting pen libido tests with *B. indicus* bulls. Furthermore, in the tests conducted by Crichton and Lishman (1985), the *B. indicus* bulls often appeared to be more aware of the observer and/or aggressive threats made by other bulls (bulls tested in groups of three) than of the oestrous females.

Commercial producers believe that *B. indicus* bulls are more likely to engage in sexual behaviour during the hours of darkness. Such a belief, while apparently unsubstantiated and based largely on casual observation, cannot be ignored, particularly when the reproductive characteristics of *B. indicus* females are considered. Oestrous behaviour is less intense (Dobson and Kamonpatana, 1986), of shorter duration (Baker, 1969) and more silent ovulations are noted (Van Der Westhuysen, 1972) than in *B. taurus* cows.

In the light of the differences between *B. taurus* and *B. indicus* females, it may be speculated that in the ontogeny of the latter breed little emphasis has been placed on fertility in the male, thereby leading to sub-optimal fertility.

The aim of the present study was to investigate whether *B. indicus* bulls mate more frequently at night than during the day, to determine the effect of the presence or absence of an observer on performance during the pen test (Blockey, 1981) and to determine the extent to which the pen test is able to predict sexual behaviour under more natural conditions in *B. indicus* bulls.

MATERIALS AND METHODS

Animals

The trial was conducted using 18 heterosexually inexperienced Afrikaner stud bulls with a mean age of 601.7 ± 9.3 days. Bulls were raised primarily on natural grazing (Northern Tall Grassveld; Acocks, 1975) during summer and maize residues with limited maize silage during winter. Their mean mass and scrotal circumference were 351.0 ± 7.4 kg and 31.2 ± 0.4 cm, respectively.

Night versus day

The effect of night or day on sexual behaviour was evaluated over eight serving-capacity tests (Blockey, 1981) in a cross-over design. Bulls were subdivided into six groups of three, blocked for body mass, with three groups being allocated to each treatment (night or day). Bulls were tested under conditions of either night or day for four serving-capacity tests, following which treatments were switched for a further four tests. Each group of three bulls was exposed to two restrained, ovariectomised, oestrous-induced (Lunstra, 1980) cows for 30 min following a minimum of 10 min sexual stimulation. Cows were mildly sedated (Rompun, Bayer) and were replaced by fresh cows once they refused to remain immobile during mating. The frequency of mounts (without ejaculation) and completed services was recorded by means of a tape recorder.

For the purpose of night tests, the bulls were marked with luminous paint numbers. The position of bulls relative to one another and the teaser females was ascertained at the start of a test using a flashlight. Thereafter, use of the flashlight was kept to a minimum to avoid disturbing the bulls excessively. Tests (both night and day) were conducted with the observer located in a hide near the pen.

Observer versus no observer

The effect of the presence or absence of an observer was evaluated over four serving-capacity tests; these tests followed the initial series of eight tests. Bulls were re-allocated to six groups in accordance with their mass and serving capacity (mean for the first eight tests). Treatments were switched alternately in a cross-over design. The test procedure was the same as for the first eight tests. During the "no observer" tests, personnel hid in a hut on one side of the pen. In addition to a record of sexual behaviour, agonistic interactions were also noted. The following agonistic activities were recorded: disrupted mounts; encounters won or lost; encounters with no outcome. Encounters usually took the form of physical contact, but could take the form of threats with no physical interaction.

Natural mating

Following termination of the pen tests, a sub-sample of eight bulls was selected for evaluation under conditions of natural mating. Bulls were selected on the basis of their mean serving capacity over the 12 pen tests. The four "high" bulls achieved a mean serving capacity of 2.0 ± 0.5 services, while the four "low" bulls achieved 0.3 ± 0.1 services.

A group of 72 females (heifers and dry cows) was used during this phase of the trial. Oestrus was induced by administering a single injection of prostaglandin (Lutalyse, Upjohn). The trial began 48 h after injection and was terminated 72 h later. The females were kept in a single group, separate from the bulls, with oestrus detection being carried out between 04.30 and 18.30 h daily. The recording of sexual behaviour amongst the bulls was carried out during two periods each day. The first period began at 05.00 h with the allocation of oestrous females and ended at 10.00 h. The second period began at 16.00 h (fresh oestrous females allocated) and ended at 19.00 h. Once allocated, a female remained with a particular bull until conclusion of the trial.

In order to facilitate observation, this phase of the trial was spread over 2 weeks with four bulls being evaluated in each week. Thus, the females to be synchronised were sub-divided into two groups of 36 each. Bulls were evaluated as single sires in four adjacent paddocks. The number of mounts (without ejaculation) and services were recorded for each bull.

Within a given observation period, oestrous females were allocated to bulls on a random basis. Oestrous females were not always available in sufficient numbers to allow simultaneous allotment to bulls at the start of an observation period. Furthermore, bulls were not necessarily exposed to a fresh oestrous female in every observation period. This led to some bulls being mated to more females than others, and the mating pressure varied between four and seven cows over 3 days. With bulls not being exposed to a fresh oestrous female in every period, a bull's previous activity could have influenced his performance at any time. Previous activity was quantified as follows: 1 = bull exposed to no female on heat or showed no sexual behaviour; 2 = bull exposed to previously mated female at the end of her oestrus; 3 = bull exposed to a fresh female at the start of her oestrus. A bull's previous activity was included as a variate in the analysis of data.

Oestrous females showed varying degrees of receptiveness (in spite of being prepared to stand for other females). Cow receptivity was quantified as follows: 1 = female at the end of her heat, previously mated, willing to stand; 2 = female at the start of her heat, partially receptive, stood when clasped or female at the end of her heat, not previously mated, but partially receptive; 3 = female freshly on heat, willing to stand. Cow receptivity was also included as a variate in the analysis of data.

Following completion of the pasture mating, females were joined with bulls which were fitted with chinball harnesses and were checked daily for signs of oestrus and paint marks. Conception rates were evaluated on the basis of returns to service.

TABLE 1

The seven combinations of variates X_1 (cow receptivity) and X_2 (bull's previous activity) used in the multiple regression analysis

Combination	X_1	X_2
1	1	3
2	2	1
3	2	2
4	2	3
5	3	1
6	3	2
7	3	3

Statistical analysis

The night/daylight and observer/no observer evaluations were analysed by means of analysis of variance for a cross-over design (Genstat V Mark 4.03C, 1980, Lawes Agricultural Trust, Rothamsted Experimental Station). The effect of agonistic behaviour on serving capacity was analysed by means of correlation techniques (Rayner, 1967). Data from the evaluation of sexual behaviour under natural mating conditions were subjected to multiple regression analysis (Genstat V Mark 4.04B, 1984). Services per 100 min (Y), cow receptivity (X_1) bulls previous activity (X_2), duration of exposure (X_3) and serving capacity (X_4, expressed in terms of 0 = high and 1 = low serving capacity) were included as variables in the model.

The qualitative nature of the variates X_1 and X_2 necessitated the creation of dummy variates in the analysis (Draper and Smith, 1966). These variates served to establish whether or not the relationship between Y and X_3 and X_4 changed for the various combinations of X_1 and X_2 (Table 1). There were nine possible combinations of X_1 and X_2, two of which were excluded from analysis (no observations were recorded for these two combinations).

RESULTS

Neither time of day (night versus daytime) nor the presence of an observer had any measurable effect on the serving capacity of the bulls (Table 2) and data were pooled for further analysis.

The bulls did not serve consistently from one test to another (Fig.1). On average, only 43.05 ± 1.69% of the bulls served in any test. Seven bulls (38.9%) served in less than four tests, 27.8% ($n=5$) in between five and eight tests and 33.3% ($n=6$) served in 9–12 tests. Only one bull served in all the tests, while two bulls failed to serve in any test.

Despite some marked fluctuations, serving capacity increased significantly

TABLE 2

Treatment means for mounting (without ejaculation) and serving behaviour recorded during the night/daytime and observer/no observer comparisons[1]

Sexual behaviour	Night versus day		Observer versus no observer	
	Night	Day	Observer	No observer
Mounts	12.360 ± 0.890	11.510 ± 0.890	12.560 ± 1.590	11.580 ± 1.560
Services	0.694 ± 0.121	0.708 ± 0.111	0.917 ± 0.270	0.861 ± 0.200

[1] All differences between means are non-significant.

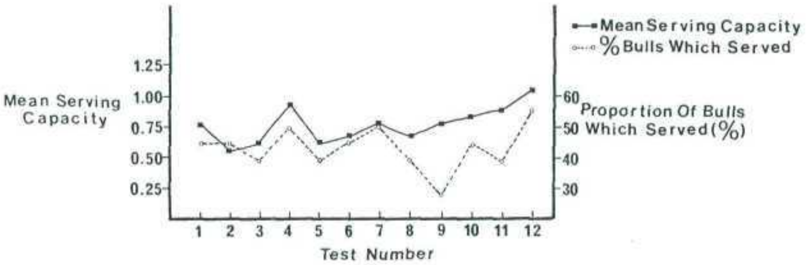

Fig. 1. The mean serving capacity and proportion of bulls which served over the 12 serving-capacity tests

($r=0.59$, $P<0.05$) with increasing number of exposures to oestrous females. Fluctuations in serving capacity tended to be associated with changes in the proportion of bulls mating (Fig. 1). A notable exception was in Test 9, where the smallest proportion (27.8%) of bulls served. This test was the first of the last four (observer versus no observer) tests and bulls had been allocated to new groups. Re-allocation appears to have stimulated agonistic behaviour and 35.8% of all such interactions recorded during the last four tests occurred during Test 9. This undoubtedly led to the reduction in the proportion of bulls serving. However, mean serving capacity for Test 9 was not similarly depressed because one bull doubled his serving capacity (two services in Test 8 versus four in Test 9), while another bull increased from zero to two services.

The number of interactions won, expressed as a proportion of the number of interactions in any given test (percentage wins), had a low positive effect on serving capacity ($r=0.19$, $P<0.10$). The number of mounts (without ejaculation) completed by a given bull was positively associated with percentage wins ($r=0.28$, $P<0.02$). The number of occasions on which mounting behaviour was disrupted had a negative effect on serving capacity ($r=-0.28$, $P<0.02$). In a number of groups, a clear dominance order emerged. In most cases, two of the bulls effectively gained access to the two cows, thereby excluding the third bull. Furthermore, bulls tended to concentrate on individual cows.

Under pasture mating conditions, the bulls with high serving capacity displayed a higher level of serving behaviour ($P < 0.05$) and a lower, non-significantly different, level of mounting behaviour than low-serving-capacity bulls (Fig. 2). Thus high-serving-capacity bulls showed a superior, but non-significantly different, degree of serving efficiency (Lane et al., 1983) when compared with low-serving-capacity bulls (Fig. 2). The higher level of mounting behaviour and poorer serving efficiency of the low-serving-capacity bulls may be largely attributed to two bulls, which displayed poor sexual technique. At times, these two bulls displayed high levels of mounting behaviour (between 21 and 25 mounts per 100 min), but ejaculated infrequently.

As each pen test was completed, the results were combined with those from previous tests and a mean score was calculated. The relationship between means calculated in this way and the mean number of services completed per 100 min of pasture mating was tested. Following seven tests, a significant association was obtained (Table 3).

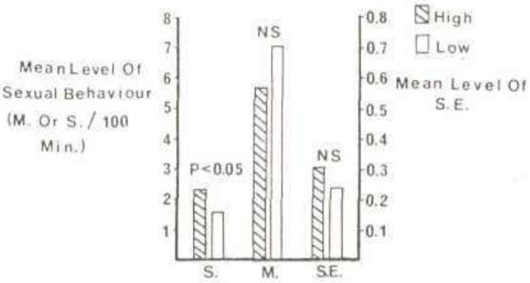

Fig. 2. Mean serving (S) and mounting (M) response per 100 min and level of serving efficiency (SE) for high and low-serving-capacity bulls under pasture mating conditions

TABLE 3

The relationship between mean number of services per 100 min of pasture mating (y) and mean serving capacity (x) with increasing number of serving capacity tests

	Pen test number											
	1	2	3	4	5	6	7	8	9	10	11	12
r	0.450[a]	0.592[a]	0.637[b]	0.665[b]	0.630[b]	0.693[b]	0.718[c]	0.718[c]	0.737[c]	0.721[c]	0.712[c]	0.677[b]
a	1.747	1.547	1.522	1.492	1.521	1.464	1.465	1.474	1.470	1.491	1.513	1.521
b	0.176	0.393	0.431	0.430	0.416	0.495	0.497	0.477	0.479	0.463	0.445	0.403
r^2	20.250	35.046	40.577	44.222	39.690	48.025	51.552	50.694	54.317	51.984	50.694	45.833

[1] Mean serving capacity for individual bulls ($n=8$) derived on the basis of scores obtained in preceding tests.
[a] Not significant.
[b] $P < 0.10$.
[c] $P < 0.05$.

TABLE 4

The multiple regression equations for each of the seven combinations of X_1 and X_2 expressed in terms of Y and X_3 and X_4

Combination	Regression equation		Level of significance
1	$Y=(369.3-160.2)$	$-0.486X_3-83.3X_4$	***
2	$Y=(369.3+47.8)$	$-0.486X_3-83.3X_4$	NS
3	$Y=(369.3-148.2)$	$-0.486X_3-83.3X_4$	**
4	$Y=(369.3-116.4)$	$-0.486X_3-83.3X_4$	**
5	$Y=(369.3+92.9)$	$-0.486X_3-83.3X_4$	*
6	$Y=(369.3-0.2)$	$-0.486X_3-83.3X_4$	NS
7	$Y=369.3$	$-0.486X_3-83.3X_4$	–

*Denotes $P<0.05$; ** denotes $P<0.01$; ***denotes $P<0.001$; NS denotes not significant. In each case, significance was measured relative to Combination 7.

The multiple regression analysis accounted for 66.9% ($P<0.01$) of the variation in serving activity under pasture mating conditions. Duration of exposure (X_3) had a significant negative effect on services per 100 min (partial regression coefficient $=-0.486$, $P<0.05$). The bulls showed their highest rate of work during the first hour in which they were exposed to a fresh oestrous female. Consequently, bulls with a shorter duration of exposure (females were not necessarily allocated to all bulls at the start of a given observation period) achieved more services per unit time. Serving capacity (X_4), coded 0 for high and 1 for low serving capacity, also contributed significantly to the regression equation (partial regression coefficient $=-83.3$, $P<0.001$). Arising from the analysis were seven equations, one for each possible combination of X_1 and X_2, expressed in terms of Y and X_3 and X_4 (Table 4). The significance of these regression equations was measured relative to the seventh combination of X_1 and X_2 (Table 1). It is evident (Table 4) that Combination 5 represented the most ideal and Combination 1 the least favourable mating situation.

The high-serving-capacity bulls achieved a mean conception rate of $67.1\pm5.3\%$, which was significantly ($P<0.05$) higher than the $46.5\pm2.1\%$ measured for the low-serving-capacity bulls.

DISCUSSION

There was no evidence to support the popularly held belief that *B. indicus* bulls mate with greater frequency at night than during daylight hours. Should more *B. indicus* cows conceive as a result of nocturnal sexual behaviour, it is probably the result of cows coming into oestrous at night, rather than enhanced bull activity, a possibility suggested by Wells (1986). Furthermore, the low incidence of homosexual and heterosexual behaviour associated with oestrous in Afrikaner cows (Wells, 1986) could readily lead the casual observer to believe that sexual behaviour occurs exclusively at night.

The absence of any detrimental effect of the presence of an observer (if such an effect exists) suggests that bulls used in this study were accustomed to the presence of people. In the period leading up to the pen tests, bulls were mustered daily for the purposes of feeding. At the termination of their pen tests, certain bulls showed a reluctance to leave the test area, despite attempts to remove them.

Bulls which performed inconsistently or did not serve at all during the pen tests did not necessarily continue to perform poorly under pasture mating conditions. This is indicated by the magnitude of the constant term (a) in the regression equations for the relationship between pasture and pen mating (Table 3). Thus, a bull with a pen test score of 0 is capable of attaining a score of ~ 1.5 serves per 100 min under pasture conditions. Clearly then, the teaser females used in this study did not represent an ideal stimulus for bulls. Garcia et al. (1986) have recently demonstrated that in restrained cows the stage of oestrous, relative to ovulation, had a bearing on the behavioural response elicited in Santa Gertrudis bulls. Cows which were at the pre-ovulatory stage of oestrus offered a better stimulus than post-ovulatory cows. This is contrary to the findings of Blockey (1981), who worked with *B. taurus* bulls, and Crichton and Lishman (1985), who used both *B. taurus* and *B. indicus* bulls. These researchers reported that adequately restrained non-oestrous cows elicited the same behavioural response, in bulls, as oestrous cows. The latter two studies made no attempt to differentiate between ovulatory status and cows were utilised as teasers when they displayed "standing" oestrous. While the serving behaviour of the *B. indicus* bulls, in the study conducted by Crichton and Lishman (1985), appeared to be unhindered by the use of non-oestrous teasers, the low level of serving behaviour recorded for these bulls and the high proportion (55.6%) of bulls which did not serve may have limited the value of the oestrous/non-oestrous comparison. Garcia et al. (1986) speculated, as did Chenoweth (1981), that *B. indicus* bulls are more discriminating with respect to stage of oestrus than are *B. taurus* bulls.

The significant improvement in serving capacity, with the progression of tests, can be attributed to a few bulls which showed a gradual improvement with increasing number of exposures and to some bulls which began serving, although erratically, at a relatively later stage. The improvement in serving capacity suggests some form of adaptation to the test situation as well as improved copulatory technique.

Clearly, agonistic behaviour exerted a detrimental effect on the success of sexual behaviour. Blockey (1979) studied *B. taurus* bulls and found that agonistic behaviour did not influence serving capacity in 2-year-old bulls since such bulls did not have a stable social dominance order. Crichton and Lishman (1985) confirmed these findings.

It is possible, therefore, that the bulls in the present study could have been forced to establish a social structure during the period of feeding, just prior to the serving-capacity tests, when trough space and/or silage may have been limiting. A social dominance order develops more slowly in young animals which are kept in intact peer groups where resources are not limited (Craig, 1986). However, where feed was limiting, social rank influenced weight gain in 15–19½-month-old Hereford bulls (Blockey and Lade, 1974). A further consideration could be that *B. indicus* bulls, with their higher threshold for sexual behaviour (Houpt and Wolski, 1982), are more readily put off by agonistic behaviour than are *B. taurus* breeds, suggesting that *B. indicus* bulls would be tested more effectively in a single-sire situation.

The significant association between serving capacity and pasture mating score was obtained in spite of the inconsistent performance of bulls in the pen tests and the depressive effect of agonistic behaviour. Following pasture mating, the bulls remained within either the high- or low-serving-capacity groups with the exception of one animal which moved into the high scoring category, displacing the lowest rank bull within this group. This bull did not serve in any but the last pen test. Chenoweth (1975) referred to bulls, particularly *B. indicus* bulls, which show temporary psychogenic or central nervous inhibition of libido and commence copulatory behaviour unpredictably at a later stage.

The establishment of a significant effect of serving capacity on conception rate was unexpected in view of the relatively small (0.78 services per 100 min), although significant, advantage maintained by the high-serving-capacity bulls during pasture mating and the small numbers of bulls and cows evaluated. The mating load of between four and seven oestrous females over 3 days is similar to that observed in a study conducted by Crichton et al. (1987), where bulls mated as single sires covered between 35 and 40 cows over 21 days and should, therefore, be considered realistic. While the beneficial effect of high serving capacity on conception rates has been clearly demonstrated by a number of workers (Blockey, 1978; Lunstra, 1980; Birkner et al., 1984), others have reported little or no effect (Makarechian and Farid, 1985; Makarechian et al., 1985; Crichton et al., 1987). The aforementioned trials were carried out using *B. taurus* bulls and only limited information on the relationship in *B. indicus* bulls exists. Christensen et al. (1982) could find no positive relationship, whereas Smith et al. (1981) and Post et al. (1987) concluded that mating behaviour assessed under field rather than pen conditions provided a useful estimate of bull performance. In the present study, bulls were selected on the basis of a large number of pen tests (most other studies used one or two such tests). This meant that successive tests resulted in an improved mean estimate of serving capacity (Table 3). These data suggest that a minimum of seven tests is needed for *B. indicus* bulls, which is hardly practical for routine testing of serving capacity.

The inconsistent serving behaviour of *B. indicus* bulls during pen tests suggests that unrestrained oestrous cows are to be preferred. The problem which presents itself in such a test is the difficulty of selecting cows which are properly receptive to the bull. The degree of cow receptivity clearly influenced bull behaviour (Table 4). Thus, large numbers of intact females are necessary to evaluate sexual behaviour under pasture conditions, since in the present study females did not always come on heat in sufficient numbers to ensure that each bull received a fresh oestrous female at the start of or during every observation period. Since this could not be met in the present trial, an attempt was made to allow for "bull's previous activity" and "duration of exposure" by the inclusion of dummy variates in the multiple regression analysis (Table 4). Continuous observation represents another alternative, but is clearly costly in terms of manpower needs.

The findings of Garcia et al. (1986) regarding the differences between *B. taurus* and *B. indicus* bulls further complicate the conduct of sexual behaviour tests. Clearly the specific characteristics which make oestrous females attractive to *B. indicus* bulls need to be identified before pen tests can be used effectively to measure serving capacity in these breeds.

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REFERENCES

Acocks, J.P.H., 1975. Veld types of South Africa. Mem. Bot. Surv. S. Afr., 40: 103.

Baker, A.A., 1969. Post-partum anoestrous in cattle. Aust. Vet. J., 45: 180–183.

Birkner, J.E., Garcia Vincent, J.E., Alberio, R.H. and Butler, H., 1984. The effect of bulls libido on heifers conception rates over a 21 days breeding period. Rev. Argent. Prod. Anim., 4: 1149–1155.

Blockey, M.A. De B., 1978. The influence of serving capacity of bulls on herd fertility. J. Anim. Sci., 46: 589–595.

Blockey, M.A. De B., 1979. Observations on group mating of bulls at pasture. Appl. Anim. Ethol., 5: 15–34.

Blockey, M.A. De B., 1981. Further studies on the serving capacity test for beef bulls. Appl. Anim. Ethol., 7: 337–350.

Blockey, M.A. De B. and Lade, A.D., 1974. Social dominance relationships among young bulls in a test of rate of weight gain after weaning. Aust. Vet. J., 50: 435–437.

Chenoweth, P.J., 1975. Bull behaviour and management. Proc. The Range Beef Cow: A Symposium on Production, Denver, CO, Vol. 4, pp. 17–24.

Chenoweth, P.J., 1981. Libido and mating behaviour in bulls, boars and rams. A review. Theriogenology, 16: 155–177.

Christensen, H.R., Seifert, G.W. and Post, T.B., 1982. The relationship between a serving capacity test and fertility in beef bulls. Aust. Vet. J., 58: 241-244.

Craig, J.V., 1986. Measuring social behaviour: Social dominance. J. Anim. Sci., 62: 1120-1129.

Crichton, J.S. and Lishman, A.W., 1985. Libido evaluation of virgin beef bulls. S. Afr. J. Anim. Sci., 15: 22-24.

Crichton, J.S., Lishman, A.W. and Lesch, S.F., 1987. Failure to demonstrate a relationship between beef bull libido and conception rate. S. Afr. J. Anim. Sci., 17: 27-30.

Dobson, H. and Kamonpatana, J.M., 1986. A review of female cattle reproduction with special reference to a comparison between buffaloes, cows and zebu. J. Reprod. Fertil., 77: 1-36.

Draper, N.R. and Smith, H., 1966. The use of "dummy" variables in multiple regression. In: Applied Regression Analysis. Wiley, New York, London, Sydney, 134 pp.

Entwistle, K.W., Winantea, A. and Holroyd, R.G., 1980. Sperm production in *Bos indicus* strain bulls. Proc. Aust. Soc. Anim. Prod., 13: 68-70.

Garcia, M.C., McDonnell, S.M., Kenney, R.M. and Osborne, H.G., 1986. Bull sexual behaviour tests: stimulus cow affects performance. Appl. Anim. Behav. Sci., 16: 1-10.

Houpt, K.A. and Wolski, T.R., 1982. Sexual behaviour. In: Domestic Animal Behaviour for Veterinarians and Animal Scientists. Iowa State University Press, Ames, IA, 112 pp.

Lane, S.M., Kiracofe, G.H., Craig, J.V. and Schalles, R.R., 1983. The effect of rearing environment on sexual behaviour of young beef bulls. J. Anim. Sci., 57: 1084-1089.

Lunstra, D.D., 1980. Evaluation of libido in beef bulls. Proc. Society for Theriogenology, Omaha, NE, pp. 169-179.

Makarechian, M. and Farid, A., 1985. The relationship between breeding soundness evaluation and fertility of beef bulls under group mating at pasture. Theriogenology, 23: 887-898.

Makarechian, M., Farid, A. and Berg, R.T., 1985. Evaluation of bull and cow fertility at pasture in single-sire mating. Can. J. Anim. Sci., 65: 799-809.

Post, T.B., Christensen, H.R. and Siefert, G.W., 1987. Reproductive performance and productive traits of beef bulls selected for different levels of testosterone response to Gn Rh. Theriogenology, 27: 317-328.

Price, E.O., 1985. Sexual behaviour of large domestic farm animals: An overview. J. Anim. Sci., 61 (Suppl. 3): 62-74.

Rayner, A.A., 1967. A first course in biometry for agriculture students. University of Natal Press. Pietermaritzburg, pp. 325.

Seebeck, R.M., 1973. Sources of variation in the fertility of a herd of zebu, British and zebu × British cattle in Northern Australia. J. Agric. Sci., 81: 253-262.

Smith, M.F., Morris, D.L., Amoss, M.S., Parish, N.R., Williams, J.D. and Wiltbank, J.N., 1981. Relationship among fertility, scrotal circumference, seminal quality and libido in Santa Gertrudis bulls. Theriogenology, 16: 379-397.

Van der Westhuysen, J.M., 1972. Differences in progesterone synchronised oestrous and ovulation in three cattle breeds. S. Afr. J. Anim. Sci., 2: 91-92.

Van Niekerk, A., Lishman, A.W. and Lesch, S.F., 1985. The reproductive responses of two breeds of cows and the performance of their progeny in two contrasting environments. S. Afr. J. Anim. Sci., 16: 209-214.

Wells, P.L., 1986. The influence of suckling during early lactation on the resumption of ovarian activity and oestrous behaviour in the Afrikaner cow. Ph.D. Thesis, University of Natal, Pietermaritzburg.

CONCLUDING STATEMENT

Circumstances have changed considerably since my research career began and it seems a great pity that many of the questions raised along the way may never be answered. Perhaps somewhere, sometime, someone will be inspired by this thesis to continue the journey of exploration.