The influence of suckling during early lactation on the resumption of ovarian activity and oestrous behaviour in the Afrikaner cow.

by

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D E C L A R A T I O N

I hereby declare that the results presented in this thesis have not been previously submitted by me for a degree at any other university.

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GENERAL INTRODUCTION

In southern Africa, the Afrikaner has undoubtedly formed the cornerstone of the beef industry. Hardiness cannot be measured readily in any meaningful economic units, yet in Africa it is a desirable attribute in all forms of livestock.

The Afrikaner is a hardy breed. Evidence of this fact is provided by its popularity in low rainfall areas where grazing, although of good quality, is sparse and watering points are likely to be few and widely spread. In order to survive and breed under such circumstances cows need to be intelligent, mobile, heat tolerant and most important of all, be able to maintain an equilibrium between the food resources available and the nutritional demands of rearing their young.

Whether this hardiness has been gained at the expense of post-partum fertility is a question of fundamental importance to this thesis and the future of the breed.

From a purely biological standpoint, selection pressures, both natural and applied, during the development of the breed must be responsible in part for many of the characteristics of the Afrikaner today. The origin of the breed is neither clear nor well documented. Williamson and Payne (1978) proposed that the breed is derived from Hottentot cattle of the seventeenth century. This reference also comments that a portion of the breed's foundation blood may have been derived from an importation of cattle of the Alentujo breed from Portugal. Quite what selection pressures have been applied and how influential they have been in the development of the breed over the past 400

years are questions which for the present remain unanswered.

Reproductive performance, in terms of calves weaned per total number of cows exposed to the bull, is a primary component of breeding efficiency and therefore a major factor in the profitability of beef production. In this area the Afrikaner fails to equal or better other breeds in similar environments. As a general rule, conception rates in Afrikaner cows with calves at foot and a condition score of 2,5 to 3,0 (on a scale of 0.5...emaciated to 5...obese) at calving, are unlikely to be above 65% with a 90 day breeding period in consecutive years.

Calving percentages reported in the literature for this breed range from 54% to 75%, depending on the environment (Trail et al., 1977; Ward et al., 1978; Holness et al., 1980; Thorpe and Cruikshank, 1981). Of greatest concern is the finding that even in low rainfall areas (Botswana) when the breed would be expected to be operating under conditions for which it is well adapted, conception rates in the Afrikaner (67,5%) were significantly lower than those achieved by the Tuli (86,6%), Bonsmara (82,9%), Tswana (80,0%) and Brahman (72,2%), (APRU Report,1980)

In the Afrikaner a conflict of interests exists where, on one hand, the breed is well suited to extensive ranching conditions and is of outstanding value in a cross-breeding program and on the other hand has a proven record of sub-fertility.

The primary aim of this study was to attempt to establish the cause of this apparent sub-fertility and what, if any, potential exists for improving reproductive performance in this breed.

OUTLINE OF RESEARCH

Intensity of suckling has a significant effect on post-partum reproduction in the cow (Graves *et al.*, 1968; Oxenreider, 1968). For this reason the effect of suckling on the post-partum Afrikaner was of primary interest in this series of studies. Three specific areas of interest were identified for the investigation of fertility in the post-partum Afrikaner cow and included ovarian function, oestrous behaviour and suckling behaviour. An outline of the research programme used for this thesis is presented in Figure 1.

The occurrence of ovulation was monitored by changes in plasma progesterone concentrations during early lactation. From these results it was possible to establish whether post-partum reproductive performance was limited by the absence of ovulation up until 100 days after calving and what effect the suckling stimulus was having on the resumption of ovarian activity.

Patterns of suckling behaviour were investigated in order to ascertain whether or not differences in post-partum ovarian function were due to differences in levels of suckling intensity between cows.

The last area of interest was oestrous behaviour. With the exception of work by Randel (1983), there is a paucity of information relating endocrine function *post partum* and oestrous behaviour in *Bos indicus* breeds under free range conditions. Since insemination of cows prior to ovulation is essential for conception, behaviour data was indispensable for a comprehensive assessment of reproduction in the post-partum Afrikaner cow.

Figure 1. Outline of research programme into the effects of suckling on reproduction in the post-partum Afrikaner cow.

Trial 1. Determine the reproductive potential of the Afrikaner cow in the absence of the suckling stimulus.

Two suckling treatments. Two breeds. 80 cows. Oestrous behaviour recorded.

Trial 2. Determine the reproductive potential of the Afrikaner cow with a reduction in the suckling stimulus to once per 24 hours.

Two suckling treatments. Two breeds. 65 cows. Oestrous behaviour recorded.

Trial 3. Establish whether once a day suckling in the absence of the male can be used to stimulate ovulation in the post-partum Afrikaner cow.

Three suckling treatments. Two breeds. 101 cows. Oestrous behaviour recorded.

Trial 4. Establish whether exogenous progesterone will improve conception rates to the first induced ovulations following reduced suckling.

Two suckling, two PRID treatments. Two breeds. 57 cows. Oestrous behaviour rec.

Trial 5. Characterise the patterns of suckling behaviour in three breeds of cow between 0500 hours and 1900 hours over a period of 56 days.

DEFINITION OF OVULATION

In order to clarify the discussion of post-partum ovarian function two types of ovulation need to be described. 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 to 20 days post partum and suckled beef cows at 35 to 55 days post partum, (Henricks et al., 1972; Carter et al., 1980; Williams and Ray, 1980; Hinshelwood et al., 1985; Wells et al,. 1985).

Primary ovulation

Following a primary ovulation plasma progesterone concentrations exceed 0,27ng/ml for a minimum period of 72 hours. This figure was derived from the mean basal 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 0,27ng/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.

Chapter 2

A review of literature concerning the endocrine changes in early lactation with particular reference to the influence of suckling on the resumption of ovarian activity.

INTRODUCTION

Over the last decade considerable research effort has been directed toward understanding the mechanisms and key factors influencing conception rates in cattle. The bulk of this research has concerned the post-partum period in the cow, particularly the period prior to and immediately after first oestrus. The association between the cow and her calf is more than just behavioural. Endocrine changes post partum may be affected markedly by variations in the following components of this association: protective instinct in the cow, frequency of access of calf to its mother and milk production by the dam. All of these contribute to the concept of a bond between cow and calf.

This review describes the effect of suckling on the endocrine changes and interactions which precede the resumption of ovarian function and conception after calving. Where necessary, research findings concerning other species have been included where equivalent work has not been conducted in cattle.

Changes in concentrations of hormones in blood between calving and first oestrus

Prolactin

In dairy cows, concentrations of prolactin in blood increase from 50 ng/ml two to four weeks pre-partum to 234 ng/ml at calving and decline to 69 ng/ml during the following six weeks. Post-partum

prolactin concentrations do not differ between anoestrous, cyclic or pregnant cows, but the rate of metabolism of prolactin may change over the post-partum period (Edgerton and Hafs, 1973). Prolactin surges around oestrus appeared to be non-functional and related to or mediated by simple steroid dynamics (Karg and Schams, 1974). Prolactin release is not associated exclusively with suckling in the lactating beef cow but the magnitude of prolactin release at suckling is greater than at other times (Convey et al., 1982). The effect of calf removal on prolactin and cortisol concentrations is variable. When cortisol and prolactin concentrations are high, Luteinizing Hormone (LH) release is low (Amoss et al., 1981). In rats during early lactation, when the suckling stimulus is strong, serum LH is reduced directly or indirectly via high levels of serum prolactin and progesterone (Taya and Greenwald, 1982). Uilenbroek et al. (1982) suggested that high prolactin concentrations in serum enhanced luteal function. The situation in rats is apparently quite different to that in cattle where prolactin has little or no luteotropic activity (Hoffman et al., 1974). In cows, reduction of plasma prolactin concentrations by the administration of bromocryptine had no effect on post-partum interval to first ovulation (Clements et al., 1978). Similar results were obtained by Williams and Ray (1980) who concluded that prolactin is not antigonadotropic in beef cows. Carruthers and Hafs (1980) found that ad lib. suckling had no effect on basal or milking induced concentrations of prolactin in serum. Carruthers et al. (1980) concluded that prolactin does not play a role in the resumption of oestrous cycles in the post-partum cow.

Luteinizing Hormone

Concentrations of LH in blood increase from 0,5 ng/ml immediately before calving to 1,5±0,3 ng/ml six weeks later (Edgerton and Hafs, 1973). Basal LH concentrations in the plasma increase due to changes in the pattern of pulsatile release of LH which increases between 10 and 30 days post partum (Rawlings et al., 1980). Humphrey et al. (1983) found that amplitude and frequency of LH peaks increased four to eight weeks before the first oestrus post partum whereas Hansen and Hauser (1982) reported no change in basal LH or frequency and amplitude of episodic LH secretion in suckled cows during the first 7 weeks post partum.

Pulse frequency increased from 0,13±0,08 to 0,2±0,08 pulses per hour one to two weeks after calving to 0,42±0,08 to 0,59±0,08 pulses per hour three to seven weeks after calving when the higher LH levels preceding the resumption of ovarian activity induce an earlier onset of ovarian activity (Riley, 1982). In contrast Echternkamp and Hansel (1973) found little rise in LH levels until the first oestrus post partum. These highly variable results are interesting and no doubt are closely associated with the variation in post-partum reproduction between breeds and individuals within a breed. For this reason post-partum changes in gonadotropin activity are intimately involved with the work to be reported in this thesis.

Oestrogen

No consistent pattern of change in oestradiol-17-beta concentrations has been detected during the anovulatory period post partum (Rawlings et al., 1980). Oestradiol-17-beta declines from 113 ±0,54 pg/ml at calving to 7 ±3,0 pg/ml six days

later (Humphrey et al., 1983) . Similar results were obtained by Echternkamp and Hansel (1973) with concentrations declining from 68 to 10 pg/ml four days after calving. Concentrations remain low until two to three days prior to oestrus when oestradiol increases quickly and declines sharply thereafter.

Progesterone

Progesterone concentrations decline from 8±0,6 ng/ml one to two weeks prior to calving to 0,9±2 ng/ml at calving. In dairy cows, progesterone remains below 0,3 ng/ml until first oestrus after which it increases four to five days later (Echterkamp and Hansel, 1973). In beef cows, plasma progesterone declines from 2,1±1,2 ng/ml before calving to 0,1 ng/ml at calving and remains at these levels until the first oestrus. Progesterone concentrations during the first cycle post partum were 34% less than during the second cycle (Edgerton and Hafs, 1973). During this period plasma progesterone concentrations are 2-3 ng/ml for four to five days prior to the first oestrus. Progesterone priming might be necessary for the formation of a fully functional corpus luteum (La Voie et al., 1981; Williams et al., 1982b) and this is the reason for the first evidence of luteal activity post partum being atypical.

Cortisol

In dairy cows, Edgerton and Hafs (1973) have reported a threefold increase in glucocorticoid levels at calving which decline to pre-calving levels by seven days later. The pattern of plasma cortisol does not appear to change with time after calving.

(Ellicott et al., 1981). However Dunlap et al., (1981) noted that the rate of clearance of cortisol decreased as post-partum interval increased. These workers also observed that suckling did not affect the clearance rate. Within 10 minutes of the start of suckling, cortisol is released and rises to a peak, dropping to pre-suckling levels over the next 30-40 minutes (Ellicott et al., 1981). However Dunlap et al (1981) noted that the increase in serum cortisol during suckling was associated with a decrease in plasma LH concentration. This was contrary to the finding of Humphrey et al. (1983) who noted that cortisol levels were highest during the period when LH secretory episodes were highest.

General interactions

In rats, prolactin secretion has a direct action on oestrogen biosynthesis of follicle cells and delays the maturation of follicles and ovulation (Uilenbroek et al., 1982). In cattle, the situation is not as well defined for there is some evidence to indicate that prolactin may be responsible for the low levels of gonadotropins early post partum (Ehrenreich, 1981). Endogenous opioid peptides may mediate the apparent action of prolactin on gonadotropin secretion (Ehrenreich, 1981).

An increase in frequency and amplitude of episodic release of LH appears to be the primary step in the resumption of post-partum ovarian function. The onset of increased secretory activity in the pituitary varies with post-partum interval and suckling status. Oestradiol administration stimulates a higher LH release in milked cows than in suckled cows (Stevenson et al., 1982). The variable endocrine response to reduced suckling may be due to

individual variation in prolactin and cortisol levels. These hormones appear to interfere with LH release (Amoss et al., 1981).

Hypophysial activity is regulated by the hypothalamus. Since hypothalamic function is influenced by higher brain centres, here then is the route whereby certain environmental stimuli may delay or hasten the resumption of normal cyclic ovarian activity post partum.

Suckling and fertility

Reducing the suckling stimulus by either milking cows or weaning calves has been shown to reduce the interval to first oestrus after calving (Wetteman et al., 1978; Wiltbank, 1958; Reeves et al., 1980; Randel, 1981). The mean post-partum interval to first oestrus has been reduced from 116 to 69 days with once daily suckling (Randel and Welker, 1976); 81 to 46 days by weaning at 42-56 days post partum (Lusby and Parra, 1982); 82 to 72 days by separating cows and calves for 48 hours at 45 days post partum (Drew, 1979) and 67 to 31 days with twice daily suckling (Garcia-Winder et al., 1982). The variation in response to once-daily suckling appears to be due to differences in breed of cow, environment and the post-partum interval to the commencement of the reduced suckling routine. In addition, once daily suckling increased the proportion of cows exhibiting oestrus compared to their normally suckled contemporaries (Wiltbank and Cook, 1958; Laster et al., 1973; Odde et al., 1980; Fonseca et al., 1981; Bluntzer et al., 1982).

Conception rates to early ovulations appear to be generally low with the result that post-partum intervals to conception have

been unaffected by once a day suckling in a number of studies (Short et al., 1972; Trevillyan et al., 1978; Carter et al., 1980; Reeves et al., 1980).

Changes in conception rates with once daily suckling have been variable and range from no improvement (Gehlon and Sekhon, 1969; Betts et al., 1982; Bluntzer et al., 1982), to improvements ranging from 59 to 97% (Lusby et al., 1981), from 20 to 43% in the dry season and 58 to 71% in the wet season (Fonseca et al., 1981) when suckling was reduced to twice a day. Short duration calf removal (96 hours) has yielded equally variable results when used in conjunction with synchronisation of oestrus and artificial insemination. Pace and Sullivan (1980), Betts et al., (1982) and Smith et al., (1982) reported no improvement in conception rates with temporary calf removal whereas increased conception rates were reported by Peterson et al., (1980), Kiser et al., (1980) 59 to 72% and Rodriguez et al., (1980) 83 to 93%.

The effect of suckling on conception rates would appear to be additive. Two calves suckling a cow delays the onset of ovarian activity more than if one calf suckles a cow (Wheeler et al., 1982; Garcia-Winder et al., 1982). Many methods of reducing intensity of suckling have shortened post-partum interval to first oestrus, increased the proportion of cows exhibiting oestrus within 50 days of calving and, to a lesser extent, have reduced the post-partum interval to conception. The high variability in conception rate in response to reduced suckling intensities indicates that reduced suckling may interact with other factors and reduce the improvement in conception rate.

Behaviour and reproduction

A positive correlation exists between intensity of oestrus and subsequent conception rates to AI (Bostedt and Fleischman, 1981). Mawhinney and Roche (1980) commented that suckling in beef cows may shorten the length of oestrus and reduce the intensity of oestrous expression. At the same time the presence of the calf may limit female x female interaction, thus reducing general oestrous activity in nursing herds. Pope (1980) found no correlation between height or any other parameter of the pre-ovulatory oestradiol-17-beta peak and intensity of oestrus. Pope (1980) did show that the intensity of oestrus was markedly increased by the presence of a second oestrous heifer. The behavioural component of oestrus may not be influenced directly by hormonal levels or interactions, but a possible interaction between these two components cannot be ignored. The dominance heirachy in Talapoin monkeys affects both behaviour and hormone levels (Bowman et al., 1978). Higher cortisol and prolactin levels were noted in more subordinate animals. Subordination apparently prevented the LH surge normally induced by an oestrogen challenge. The question arises as to whether these hormonal anomalies were a symptom or cause of subordination.

In cattle very little is known about the mechanisms of endocrine changes and their effect on behaviour. In rats, progesterone has been shown to act upon the habenula, stimulating the proceptive component of sexual behaviour whilst the influence of progesterone on the midbrain is associated with the receptive component of oestrous behaviour (Tennent et al., 1983).

Kiddy et al., (1978) showed that specific odours are associated with oestrus in cows. These odours may be detectable (by trained

dogs) up to three days before oestrus (Kiddy and Mitchell, 1981). Such pheromones provide a mechanism whereby cows could stimulate each other without any physical interaction. De Miguel et al., (1981) noted increased plasma progesterone levels within 21 days of introducing bulls to suckled cows which were previously not cycling. This was accompanied by an increase in the proportion of cows showing oestrus. Similarly, MacMillan et al., (1979) noted a marked increase in the incidence of oestrous activity early in the bulling period following 21 days contact with the bulls. Intervals from mating to ovulation have been shortened by teasing with vasectomized bulls prior to breeding (Marrion et al., 1950). Bull receptivity in milking Criollo cows was shortened by 8 hours by natural service but not by artificial insemination (De Alba et al., 1961). This is evidence of a conscious interaction between hormonal priming and environmental stimulation.

Suckling and LH release

Two components of LH concentration in the plasma have been distinguished definitively in the literature: basal concentrations and episodic release. Characteristics of each component depend upon LH content of the pituitary and the release of LH stimulated by the hypothalamus. An inherent rhythmic pattern of LH release exists in the absence of any ovarian source of hormones (Rahe et al., 1982).

By 13 days post partum the pituitary is able to respond to exogenous GnRH and to high blood levels of oestradiol-17-beta (Webb et al ., 1977b; Inskeep and Lishman, 1981; Stevenson et al., 1982). Webb et al. (1977b) noted that suckling does not

alter the pituitary content of LH which is contrary to the finding of Carruthers et al., (1980) who commented that the releasable pool of LH in the pituitary was reduced by suckling. Since higher concentrations of LH were noted within 7 days of calving in non-suckled cows versus suckled cows (Randel et al., 1976), the absence of stimulation of the hypophysis is likely to be responsible for ovarian quiescence in early lactation. Certainly Williams et al., (1982a) suggested that pituitary responsiveness per se is not decreased in beef cows suckling a single calf. This contrasts with the findings of Carter et al., (1980) who reported that early removal of the calf post partum may lead to greater pituitary LH stores or increased pituitary sensitivity to GnRH. They found that all cows ovulated in less than two weeks with calf removal. These ovulations were frequently followed by short luteal phases.

Low basal LH concentrations during the first 3 weeks of lactation may be due to either inadequate stimulation of the hypothalamus or inadequate levels of GnRH in the hypothalamus. Carruthers et al (1980) found no differences between suckled and non-suckled cows in total content of GnRH in the hypothalamus as measured by radioimmunoassay. However their assay results did not provide information on LHRH release or its biological activity.

Reduction in the suckling intensity soon after calving has been associated with an increased frequency of pulsatile LH release at 20 days post partum and an earlier resumption of oestrous activity than in normally suckled cows (Walters et al., 1980). Furthermore, all cows which ovulated continued to cycle, showing that this early reproductive function was normal. Stevenson et al., (1982) noted lower serum LH levels after treatment with

oestradiol-17-beta in suckled cows compared to milked cows. The frequency of pulsatile LH release was 0,9±0,2 in suckled cows and 2,1±0,2/4hrs in non-suckled cows whilst the amplitude of this pulsatile release was 1,8±0,1 in suckled cows and 3,1±0,9 ng/ml in non-suckled cows (Carruthers et al., 1980). This decrease in frequency and amplitude of LH pulses in suckled cows was accompanied by a delay to first ovulation post partum. Milking does not appear to suppress the resumption of pulsatile LH release to the same extent as suckling (Peters et al.,1981).

These findings indicate that in general terms, stimulation of the hypothalamus from higher brain centres which monitor and respond to environmental factors may be of greater significance in the resumption of ovarian activity early post partum than the purely endocrine events associated with the hypothalamo-pituitary ovarian axis.

Stimulation of pulsatile LH release using exogenous GnRH has successfully initiated ovulation and subsequent normal oestrous cycles earlier in treated cows than in untreated contemporaries (Riley et al., 1980; Walters et al ., 1980). Apparently both frequency and amplitude of pulsatile LH release must be increased to induce folliculogenesis in anoestrous post-partum cows since an increase in pulse rate alone had no effect on the size of the largest follicle or number of follicles in treated cows (Spicer et al., 1982).

Hinshelwood et al., (1985) concluded that suckling alters the responsiveness of the pituitary to fixed amounts of GnRH

independently of the ovary. This effect also changes with interval after calving. In contrast Williams et al.,(1982a) did not find a reduction in pituitary response per se to GnRH in the beef cow suckling a single calf. Carruthers et al., (1980) concluded that a reduction in the releasable amount of LH in the pituitaries of suckled cows is not caused by a lack of receptors. The question arises as to whether the suckling stimulus could down-regulate receptors in the pituitary prior to GnRH stimulation. An initial injection of LHRH has been found to increase the release of LH from the pituitary in response to a second injection of LHRH (Foster, 1978). Thus, the greater frequency of LH pulses early post partum in non-suckled cows confirms a smaller suppressive effect of suckling on the hypothalamic-pituitary axis (Carruthers et al., 1980).

The stimuli regulating basal concentrations and those controlling the pre-ovulatory LH surge appear to have different origins (Jackson et al.,1978). In the ewe, inputs from the supra-chiasmatic and pre-optic areas appear necessary for the LH surge, whilst inputs from both the retro-chiasmatic region and medio-basal hypothalamus appear necessary for the maintenance of basal LH. This is supported by Gorski (1966) who proposed that separate hypothalamic control centres exist for tonic and episodic release of LH.

Suckling and the ovaries

Early gonadotropic stimulation following reduced suckling enables an early resumption of ovarian activity after calving. If suckling intensity is reduced 20 to 30 days post partum the first ovulation occurs earlier than in normally suckled cows. Flood et al., (1979) showed that more than 60% of cows suckled once a day

from 25 days post partum ovulated within 12 days of suckling restriction. However the corpora lutea which developed after this treatment lasted only five to twelve days (Flood et al.,1979). Lishman et al., (1979) reported that the oestrus which followed short-lived corpora lutea induced by GnRH was non-fertile and was not followed by a second increase in progesterone. This was contrary to the finding of Van der Westhuysen et al., (1980) who observed normal progesterone concentrations in the cycle following the short lived corpus luteum. This apparent discrepancy probably relates to the timing of treatment application post partum with the cows in the latter study being closer to spontaneous resumption of ovarian activity. Weaning 21 hours after birth resulted in a difference in follicular volume by day five and although plasma oestrogen concentrations were similar in weaned and suckled cows, first ovulation and first oestrus were advanced by 33 and 25 days respectively with weaning (Carter et al., 1980).

Bellin et al., (1982) reported that in suckled cows smaller follicles, fewer follicles and less intra-follicular fluid were found than in non-suckled post-partum cows. Earlier work by Wagner and Hansel (1969) and Wagner and Oxenreider (1971) demonstrated the presence of mature sized follicles in early lactation and commented that possibly LH activity was inadequate to induce ovulation. A significant feature of short luteal phases associated with early resumption of ovarian activity is the high incidence of infertile matings to first (early) ovulations. Nevertheless non-suckled cows ultimately have shorter post-partum intervals to conception than suckled cows (Carter et al., 1980).

Nutrition may have an important role in mediating the effect of suckling intensity on ovarian activity. Roussel et al., (1977) suggested that body fat may provide an extra-ovarian source of progesterone . Metabolism of fat reserves for the production of milk with insufficient energy intake may result in a release of progesterone from these reserves. Kesner et al., (1981) showed that luteal phase concentrations of progesterone inhibited surges of gonadotropin. However, progesterone released from these fat reserves is unlikely to reach luteal phase concentrations in the blood and the effects of negative energy status on gonadotropin activity are more likely to be mediated by the higher brain centres and the hypothalamus.

Thus, levels of progesterone equivalent to those of the luteal phase of the oestrous cycle suppress gonadotropin secretion. Nonetheless, progesterone priming is required prior to the resumption of normal pituitary function (La Voie et al., 1981) although no-one, as yet, has described the endocrine mechanism involved. Although the resumption of post-partum ovarian activity is dependent primarily upon stimulation by the hypothalamus and pituitary, both of these structures appear to require priming by ovarian steroids before becoming fully functional in terms of synthesis and release of gonadotropins.

The pituitary becomes responsive to GnRH before the hypothalamus is able to respond to oestrogen (Irvine et al., 1981). Elevated concentrations of oestrogen enhance the synthesis of LH and increase the number of GnRH receptors in the pituitary. At the same time oestrogen facilitates a sustained GnRH release (Beck and Convey, 1977). Short et al., (1979) found that for at least the first two weeks, suckling and/or lactation inhibited both the

release of LH in response to oestradiol and the subsequent elevation in concentrations of progesterone. They also observed that an active corpus luteum could block this effect of oestradiol. Suckling shortens the duration of the LH surge produced in response to oestradiol-17-beta (Forrest, Rhodes III and Randel, 1980; Forrest, Dunn and Kaltenbach, 1980; Randel et al., 1981; Short et al., 1974). This suppressive effect of suckling on LH secretion disappears by 42 days post partum (Echternkamp, 1978).

CONCLUSIONS

Post-partum anoestrus is prolonged by lactation and more specifically by suckling. Recent evidence indicates that prolactin levels are not involved in this effect in the cow. Furthermore, suckling would not appear to alter prolactin concentrations although it has a marked effect on duration of anoestrus.

Pituitary activity is related to post-partum interval and suckling status. The pituitary is not simply a gonadotropin reservoir and its activity can be modified by both suckling and the steroid environment. Stimulation of the hypothalamus appears to be the masterlink between the environment and endocrine activity. The situation in the early post-partum cow may be summarised as follows. GnRH concentrations in the hypothalamus are probably sufficient to sustain the pulsatile release of LH necessary for ovulation. By 20-30 days post-partum the pituitary has sufficient LH stores to respond to any GnRH stimulus. Early post-partum anoestrus would appear to be due primarily to the absence of hypothalamic stimulation. Since exteroceptors are

almost certainly a source of stimulation of the hypothalamus, this is the route by which any stimulation of early resumption of ovarian activity is likely to be effective.

Suckling then appears to be a powerful factor affecting endocrine activity in the healthy cow. By reducing the suckling stimulus (limiting access of calf to cow) it is possible to shorten post-partum anoestrus by up to 40 days, increase the intensity of oestrus and improve conception rates by up to 25%. However these responses are highly variable and are affected by breed, timing of treatment application after calving and degree of suckling reduction.

CHAPTER 3

The resumption of ovarian activity in Afrikaner and Mashona cows which were either normally suckled or weaned three days post partum.

INTRODUCTION

Suckling is known to delay the resumption of ovarian activity. This has been demonstrated by reducing suckling intensity and noting the shorter interval to first ovulation and first oestrus. (Wiltbank and Cook, 1958; Oxenreider, 1968; Graves et al., 1968; Short et al., 1972; Wetteman et al., 1978; Carter et al., 1980). These early ovulations have followed the earlier release of gonadotropins from the pituitary in cows exposed to less suckling stimulus per day than their normally suckled contemporaries (Short et al., 1972; Randel et al., 1976; Radford et al., 1978; Carruthers and Hafs, 1980; Walters et al., 1982b; Peters, Lamming and Fisher, 1981; Edwards, 1985)). This early onset of gonadotropin activity has been successfully simulated by the use of multiple low-dose injections of Gonadotropin Releasing Hormone (Riley, Peters and Lamming, 1980; Walters et al., 1982c; Edwards, Roche and Niswender, 1983).

As the aim of this thesis was an assessment of the effect of suckling on reproductive performance in the Afrikaner cow, a baseline had to be established which would indicate whether a reduction in the suckling stimulus had any value as a means of improving conception rates for the breed.

The first objective was to assess conception rates in the Afrikaner cow under experimental conditions and the second was to identify characteristics of reproductive function post partum which might be specific to the breed and which may be responsible

in part for its generally poor reproductive performance.

Relevant literature indicated that in a variety of environments the Afrikaner cow may be expected to achieve a calving percentage of the order of 55%-65% within a 90 day breeding period (Trail et al., 1977; APRU Report, 1980).

Ovarian activity after calving embraces both endocrine changes and the expression of oestrous behaviour prior to ovulation. For this reason post-partum reproductive performance was assessed in two areas. The first area monitored ovarian activity up until 100 days after calving and the second area was concerned with the behavioural manifestations of oestrus. This chapter reports the endocrine aspects of post-partum reproduction in the Afrikaner cow whilst Chapter 8 will report the findings from the behavioural studies.

In order to compare the performance of the Afrikaner cow with that of a breed with a proven record of high fertility, a small group of Mashona cows was included in this study. Mashona cows achieve higher conception rates than the Afrikaner in similar environments (Holness, Hale and Hopley, 1980; Ward and Dlodlo, 1985).

MATERIALS AND METHOD

Experimental animals

A total of sixty mature (range from 3 to 9 years), multiparous (range 1 to 5 parity) Afrikaner cows and 20 mature (range 8 to 12 years), multiparous (range 4 to 8 parity) Mashona cows were used for this study. Condition scores of these cows ranged from 2,5 to 3,0 on a scale of 0,5 (emaciated) to 5 (obese). Cows were

blocked on calving date and randomly allocated to one of two treatment groups.

Method

Cows in the control group were normally suckled and had continuous access to their calves for the duration of the trial (100 days post partum). All cows were drafted from the pregnant cow pool into the experimental groups three days after calving. Experimental groups were left intact until the last cow to calve had been in the trial for 97 days. Cows in the weaned group had their calves removed permanently 3 days after calving. All weaned calves were artificially reared.

The two treatment groups were grazed in separate paddocks to avoid contact between weaned cows and calves in the control group. Duplicate blood samples were collected at 0800 hours at 7, 14 and 21 days after calving. From day 23 to 100 post partum blood samples were collected three times each week by jugular venipuncture at 0800 hours. Plasma was extracted from the blood samples and frozen at -20 C within 120 minutes of collection.

PROGESTERONE ASSAY

Plasma samples were assayed for progesterone according to the method of Holness, Hale and Hopley (1980). Sensitivity of the assay was 200 pg/ml on a 200ul sample of plasma. For each assay 8 control plasma samples were included with 70 plasma progesterone determinations per standard curve. The control plasma samples for all assays were derived from a single plasma pool. All control plasma samples were used to calculate inter- and intra- assay variation. Intra-assay variation was $\pm$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 mean of 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 examined to ensure that there were no structural abnormalities of the genitalia. Bulls (2 per group) were rotated each week between treatment groups to minimise any sire effects on conception rates. Bulls had access to cows continuously from three days after calving until completion of the study.

Observation of oestrous behaviour

Both treatment groups were monitored by observers between the hours of 0430 and 1830 each day for the duration of the trial. Oestrous behaviour was recorded and will be presented in detail in Chapter 8.

RESULTS

Post-partum intervals to :-

Primary ovulation

Cyclic ovarian activity was estimated to have commenced when plasma progesterone concentrations rose above 0,3 ng/ml for a minimum of 72 hours. The figure of 0,3 ng/ml was derived from basal plasma progesterone concentrations during the anovulatory phase in each breed, plus 5 standard deviations from this mean.

Removal of the suckling stimulus shortened ($P<0,001$) the mean post-partum interval to the onset of ovarian activity by 30 days to 18 ±2days in Afrikaner cows weaned three days post partum

(Table 1). In the Mashona the 18 day reduction in mean post-partum interval to primary ovulation in weaned cows was not significant.

Normally suckled Afrikaner and Mashona cows did not differ significantly in mean post-partum interval to the onset of ovarian activity (Table 1). In both of the normally suckled groups a range of 60 days in post-partum interval to primary ovulation was observed.

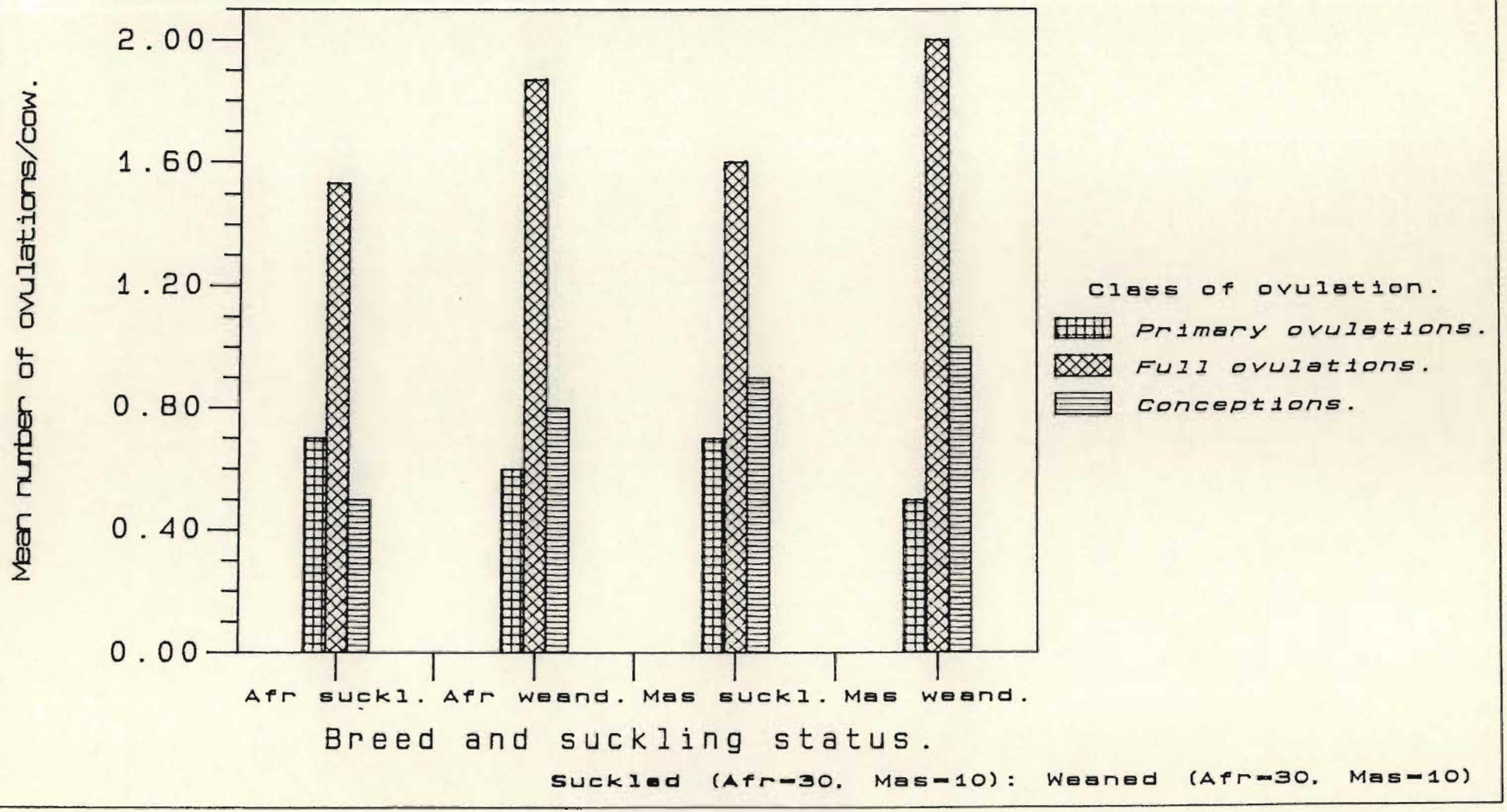
Neither breed nor suckling status had any effect on the occurrence of short cycles in this study (Figure 2). The incidence of short cycles was 70% (28/40) in normally suckled cows and 57% (23/40) in partially weaned cows. In the absence of suckling more first ovulations may have been followed by 21 day cycles but the difference was not significant.

Table 1. Mean post-partum interval (days) to primary ovulations in Afrikaner and Mashona cows which were either normally suckled or weaned three days after calving.

Suckling status / Breed	Normally Suckled	Weaned 3 days post partum	Signif. of diff.	Mean
Afrikaner				
Mean PPI	48,6±3,64	18,3±1.99	P<0,001	34,6±3,25
Sample n	21	18		
Range	19-85	8-40		
Mashona				
Mean PPI	37,6±7,48	19,2±2,20	P<0,10	29,9±5,09
Sample n	7	5		
Range	14-74	15-27		
Signif. of difference	NS	NS		
Mean PPI	45,9±3,36	18,5±1,61	P<0,01	33,5±2,75

NS Group means do not differ significantly.
PPI Post-partum interval.

Figure 2. The resumption of ovarian activity in suckled and non-suckled Afrikaner and Mashona cows



Full ovulations

Full ovulations were estimated to have occurred when either plasma progesterone concentrations exceeded 0,3 ng/ml for not less than 11 days or conception had occurred, confirmed by the birth of a calf and dated from behaviour records and plasma progesterone profiles. Mean full ovulation rate was similar in all treatment groups (Figure 2). Normally suckled Mashona cows had a significanty ($P<0,05$) shorter (16 days) interval to first full ovulation than normally suckled Afrikaner cows (Table 2). Removal of the suckling stimulus in Afrikaner cows significantly ($P < 0,001$) shortened the post-partum interval to first full ovulation by 27 days. Suckling did not appear to delay the resumption of ovarian activity in the Mashona cow to the same extent as in the Afrikaner cow. First full ovulations in suckled Mashona cows were only 8 days later (not significantly different) than in non-suckled Mashona cows (Table 2).

Table 2. Mean post-partum interval (days) to first full ovulation in Afrikaner and Mashona cows which were either suckled or weaned three days after calving.

Breed	Normally Suckled	Weaned 3 days post partum	Signif. of diff.	Mean
Afrikaner				
Mean PPI	57,2±3,56	30,4±2,85	P<0,001	43,0±2.90
Sample n	25	28		
Range	26-97	10-67		
Mashona				
Mean PPI	40,8±3,13	32,5±7,6	NS	36,4±4,27
Sample n	9	10		
Range	26-54	10-96		
Signif. of diff.	P<0,05	NS		
Mean	52,9±3,0	31,±2,84	P<0,01	41,4±2,42

NS Group means are not significantly different.
PPI Post-partum interval.

Conception

Mean post-partum interval to conception was similar in normally suckled Afrikaner and Mashona cows (Table 3). However, the removal of the suckling stimulus in Afrikaner cows significantly ($P < 0,001$) shortened the mean post-partum interval to conception by 22 days. In non-suckled compared to suckled Mashona cows, the mean post-partum interval to conception was shortened by 14 days although the difference was not significant.

In the absence of suckling, the mean post-partum interval to conception in Afrikaner and Mashona cows (47±3 days) does not differ and indicates that the temporal control of the resumption of ovarian activity in these two breeds is very similar.

Table 3. Mean post-partum interval (days) to conception in Afrikaner and Mashona cows which were either normally suckled or weaned three days after calving.

Breed	Normally Suckled	Weaned 3 days post partum	Signif. of diff.	Mean
Afrikaner				
Mean PPI	68,5±4,36	46,7±3,33	P<0,001	55,1±3,13
Sample n	15	24		
Range	45-97	22-75		
Mashona				
Mean PPI	60,0±5,76	46,2±7,18	NS	52,7±4,82
Sample n	9	10		
Range	36-93	10-96		
Signif. of diff.	NS	NS		
Mean PPI	65,3±3,51	46,5±3,10	P<0,001	54,3±2,61

NS Group means are not significantly different.
PPI Post-partum interval

Even in the absence of suckling, conception was delayed by 15 days following the first full ovulation in both Afrikaner and Mashona cows. Thus the majority of these first full ovulations in both breeds appear to have been subfertile. A similar situation existed in suckled cows where, although the first full ovulations occurred later, mean interval to conception was still delayed by 11 days in the Afrikaner and 19 days in the Mashona (Tables 2 and 3).

Anovulatory cows and conception

The absence of ovulation within 100 days of calving was not a major cause of conception failure in this study (Figure 2). Removal of the suckling stimulus had no significant effect on the proportion of Afrikaner cows which were anovulatory (13% in control cows and 3% in weaned cows) for the duration of this study (Table 4). All Mashona cows (20) ovulated within 100 days of calving.

Table 4. The resumption of ovarian activity and conception in Afrikaner and Mashona cows which were either normally suckled or weaned three days post partum.

	Afrikaner		Mashona	
	Normally suckled	Weaned 3 days PP	Normally suckled	Weaned 3 days PP
Anovulatory for 100 days post partum	4/30	1/30	0/10	0/10
Conception within 100 days post partum	15/30 **	24/30	9/10	10/10

** Treatment proportions differ significantly ($P<0,05$).

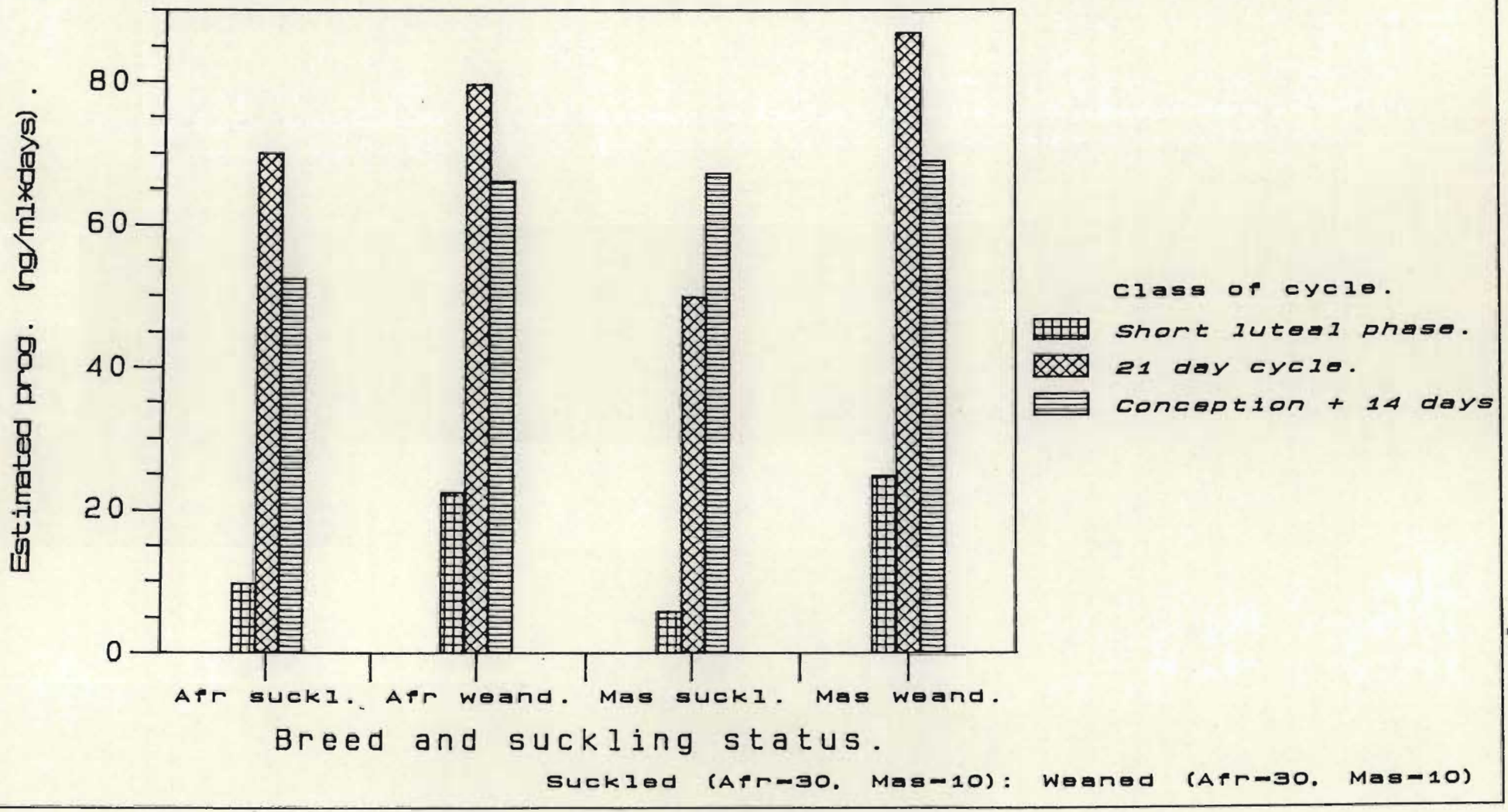
Luteal function

Primary ovulations

The unit ng/ml*days was used to describe the estimated area under a plasma progesterone curve between two ovulations. No significant breed differences were recorded in the estimated total plasma progesterone concentrations during the short cycles following primary ovulation in Afrikaner and Mashona cows (Figure 3).

Removal of the suckling stimulus enhanced plasma progesterone concentrations following primary ovulation in Afrikaner and Mashona cows. Pooling the data for the two breeds revealed that in the absence of suckling, total plasma progesterone concentrations increased (+240%) from 9±2 ng/ml*days in suckled cows to 23±4 ng/ml*days in non-suckled cows ($P < 0,01$). Luteal function is clearly not related to post-partum interval *per se* because the higher amounts of progesterone in the non-suckled cows compared to suckled cows occurred 27 days earlier in Afrikaner cows and 8 days earlier in Mashona cows. Neither suckling status nor breed had any significant effect on the duration of these short cycles which had a mean of 9,3±0,6 days (n=51; data not shown).

Figure 3. Total progesterone/cycle (ng/ml*days) in suckled and non-suckled Afrikaner and Mashona cows



Full ovulations

Neither breed nor suckling status had any detectable effect on plasma progesterone concentrations following full ovulations (Figure 3). There was a trend for non-suckled cows to have higher amounts of progesterone.

Luteal function did not differ significantly between first and second full ovulations in the Afrikaner cow (Table 5). Although there was a tendency for non-suckled cows to have higher plasma progesterone concentrations, the difference was negligible by comparison to the variation observed between cows within the same group.

Table 5. Estimated area under the progesterone curve (ng/ml*days) following first and second full ovulations in Afrikaner and Mashona cows which were either normally suckled or weaned three days post partum.

Cycle number	Normally suckled	Weaned 3 days post partum	Signif. of diff.	Mean
First				
Mean area	69,9±12,5	79,7±6,31	NS	71,7±5,7
Sample n	15	20		
Range	22-186	32-143		
PPI	51,4±4,03	27,9±3,00	P<0,001	
Second				
Mean area	79,0±15,03	84,0±15,94	NS	81,1±10,18
Sample n	11	10		
Range	25-197	19-168		
PPI	73,5±4,86	50,6±5,50	P<0,01	
Signif. of diff.	NS	NS		
Mean	73,7±9,43	81,1±6,63	NS	77,7±5,61

NS Group means are not significantly different.
PPI Post-partum interval.
Mean duration of first cycle was 20,8±0,41 days and of the second cycle was 20,7±0,49 days.

Conception

Total plasma progesterone over 14 days following conception did not differ with breed or suckling status (Figure 3). These similar amounts of progesterone were observed in suckled and non-suckled cows despite significant ($P<0,001$) differences in mean post-partum interval to conception in these two groups indicating that luteal function is more dependent upon endocrine status than post-partum interval *per se*.

DISCUSSION

From the characteristics of ovulation and conception *post partum* in suckled and non-suckled Afrikaner cows two points were highlighted. Firstly the Afrikaner cow is very sensitive (more so than the Mashona) to the suckling stimulus and secondly, in the absence of this suckling stimulus, the reproductive performance of the breed was nearly as high (80% conception rate) as the Mashona (90% conception rate). Throughout the chapter the similarity in reproductive performance of weaned Afrikaner cows and normally suckled Mashona cows indicated that sub-fertility in the Afrikaner is an active condition which can be manipulated by management.

Only after primary ovulations was there any evidence of an effect of reduced suckling intensity on luteal function. Not only was luteal activity enhanced by weaning early but post-partum interval to the start of this luteal activity was significnatly ($P < 0,001$) shortened by 30 days. The incidence of primary ovulations was about 65% overall and removal of the suckling stimulus may have reduced this figure but the difference was small. The results from this study agree with those of Hinshelwood, Dierschke and Hauser (1985) who reported that in the

absence of suckling, the incidence of short cycles was not altered. In general, not all cows exhibit short oestrous cycles early post partum (Edgerton and Hafs, 1973; Arije, Wiltbank and Hopwood, 1974; Corah et al., 1974).

These short cycles are a regular feature of the resumption of ovarian activity post partum and have been reported by numerous researchers (Oxenreider, 1968; Short et al., 1972; Radford et al., 1978; Wetteman et al., 1978; Suzuki and Sato, 1979; Carter et al., 1980; La Voie et al.,1981). The results of this study would agree with those of Peters and Lamming (1984) that short cycles (preceded by primary ovulations) are not essential for the resumption of fertile ovarian activity in all cows.

During these short cycles, total plasma progesterone in suckled cows was estimated to be 11% of that measured during a normal cycle. With the removal of the suckling stimulus, these short cycles were still 9,6±1,05 days long despite the fact that the total plasma progesterone had increased significantly (P 0,01) to 23,04 ng/ml*days or 29% of that observed during a 21 day oestrous cycle following a full ovulation. From these results it appears that the lifespan of these short cycle corpora lutea cannot be extended by higher levels of gonadotropin support which presumably were responsible for the increased luteal output noted in non-suckled cows.

These short luteal phases have been attributed to either luteinised follicles (Tribble et al., 1973), a lack of luteotropic support (Peters and Lamming, 1984) or a failure of the luteal tissue to recognise luteotropin (Schams et al., 1979) or the

secretion of a luteolytic agent (Odde et al., 1980; Ramirez-Godinez et al., 1982).

The earlier increase in luteal activity in non-suckled cows follows an earlier increase in gonadotropin activity in the absence of suckling. Randel et al. (1976) and Carruthers and Hafs (1980) working with weaned cows reported an earlier increase in amplitude and frequency of pulsatile LH release compared with normally suckled cows. Reducing the suckling frequency to once a day had the same effect on the pattern of pulsatile LH release (Walters et al., 1977; Echternkamp, 1978). A reduction in suckling intensity exerts its greatest effect prior to and immediately after first ovulation. Thereafter luteal function in the non-suckled cow is virtually indistinguishable from the normally suckled cow.

Following the substantial treatment effects on timing of first ovulation and luteal function, the absence of significant treatment effects on luteal function during the oestrous cycles which followed was most interesting. These cycles had a mean duration of 20,8±0,41 days and total of 77,7 ng/ml*days of progesterone. Breed, suckling status and cycle number had no significant effect on cycle length or estimated total plasma progesterone following a full ovulation. Although no significant differences in luteal function were recorded in weaned cows, these cows had consistently higher total amounts of progesterone than the normally suckled cows, evidence perhaps, of continued enhanced gonadotropin activity in non-suckled cows. The results of this study indicate that luteal support (in terms of total progesterone) following the first full ovulation post partum should not be a limiting factor following conception. This

finding disagrees with Ramirez-Godinez *et al.* (1981,1982) who declared that early luteal regression (with specific reference to short cycles) was responsible for conception failure following ovulations induced by weaning 30 to 41 days *post partum*. Erb *et al.* (1976) noted that pre-ovulatory endocrine asynchrony was highly correlated with infertile matings and that in 75% of these cases the subsequent luteal phase had sub-normal levels of progesterone.

Given the favourable response to weaning in terms of an earlier ovulation in non-suckled Afrikaner cows and increased luteal output, the absence of successful conception to these first ovulations was surprising. Behavioural deficiences prior to these early ovulations may have severely reduced the incidence of service prior to ovulation since La Voie *et al.* (1981) reported that first oestrus in cows suckled once a day was less intense than the first oestrus in normally suckled cows. The alternative explanation for conception failure early *post partum* rests with endocrine events after successful service and are beyond the scope of this thesis. Such problem areas would include gamete transport following service , ovum maturity at ovulation, fertilisation failure and endocrine support of the early conceptus (excluding luteal maintenance, following full ovulation).

CONCLUSION

The Afrikaner cow appears to be particularly susceptible to the suppressive effects of suckling on the resumption of ovarian activity *post partum*. Normal suckling delays the first ovulation by up to 27 days compared to the non-suckled post-partum

Chapter 4

The resumption of ovarian activity and conception in Afrikaner cows following either normal suckling or restricted suckling (partial weaning) from 28 days post partum.

INTRODUCTION

The results of the first study (Chapter 3) indicated that the Afrikaner cow is sensitive to the suppressive effects of suckling on the resumption of ovarian activity. Removal of the suckling stimulus induced primary and full ovulations 30 and 27 days earlier than in normally suckled Afrikaner cows in the same study and improved conception rates by 30%.

These improvements were established in the absence of any suckling stimulus and presumably equate to the maximum improvement in reproductive function which could be expected from manipulating suckling intensity in the Afrikaner cow. The effects of suckling post partum may not be entirely negative. Involution of the uterus may be necessary before the cow can conceive (Peters, 1984) and Riesen et al., (1968) and Wagner and Hansel (1969) commented that suckling may stimulate this involution. Conditions such as dystocia and retained placenta may inhibit involution of the uterus (Peters, 1984) and are known to prolong post-partum acyclic periods (Roberts, 1971; Laster, Glimp and Gregory, 1973; DuBois and Williams, 1980; Peters et al., 1981). Based on these reports, a limited amount of suckling during early lactation might be advantageous in shortening post-partum interval to first ovulation.

The next objective was to establish whether a similar improvement (to complete weaning) in reproductive function could be achieved

with a less severe reduction in suckling stimulus. The aim was to achieve a 20% or higher improvement in conception rate within 100 days of calving without adversely affecting calf growth or resorting to unrealistic management routines.

Under free-range conditions calves suckle their dams from 4 to 7 times per day up until 100 days post partum (Drewery et al., 1959). Reducing suckling frequency to once per 24 hours represents at least an 80% reduction in total suckling stimulus experienced by the cow. Once a day suckling in Bos taurus and Bos taurus*Bos indicus cows will reduce the post-partum interval to first oestrus (La Voie et al., 1981) without depressing milk production in the dam (Randel and Welker, 1976 and 1978; Randel, Harrison and Peterson, 1981). Once a day suckling avoids the serious management problems of rearing early weaned calves (Laster, Glimp and Gregory, 1973) and the poor growth rates of artificially reared calves (Bellows et al., 1974).

The resumption of ovarian activity after calving follows an increase in amplitude and frequency of pulsatile LH release two weeks prior to ovulation (Rawlins et al., 1980; Humphrey et al., 1983) followed by the first increases in plasma progesterone concentration post partum. The first evidence of overt oestrous behaviour after an early resumption of ovarian activity is often delayed (see review by Garcia, 1982) . Early ovulations induced by weaning are often accompanied by a low intensity of oestrus and the absence of service. The results from Chapter 3 indicated that in the absence of the suckling stimulus the mean post-partum interval to first ovulation was 30±3 days and the mean interval to conception was 47±3 days. Therefore any weaning routine

should be applied to induce the first ovulation 40 to 50 days post partum since any ovulation induced earlier than this is unlikely to be fertile.

The specific objective of this study was to establish whether once a day suckling was sufficient a reduction in the suckling stimulus to shorten the post-partum interval to first ovulation and improve conception rates in the Afrikaner cow.

MATERIALS AND METHOD

Experimental animals

At exactly 28 days after calving for each cow, 65 mature (range 4 to 10) multiparous (range 4 to 9) Afrikaner cows with a condition score of 2,5 to 3,0 were blocked according to calving date and randomly allocated to either a normally suckled (control) group or a limited suckling (partially weaned) group.

Method

Calves in the normally suckled group had continuous access to their dams for the duration of the trial whilst calves in the partially weaned group were confined to a small pen within the paddock where their dams were grazing. Partial weaning commenced 28 days after calving with cows being allowed access to their calves for thirty minute periods each day at 0800 hours and 1600 hours. From day 42 post partum cows were allowed into the calf enclosure once each day at 1600 hours to suckle their calves. When the last cow to calve had reached 100 days post partum on the trial, all calves were returned to their dams where they had continous access until complete weaning at 7 months of age.

Calves in the partially weaned group had free access to a creep feed from 28 days after calving until they were returned to their dams. During this period, the ration on offer comprised 50% milled hay, 42% milled maize and 8% cotton seed meal. Experimental cows were grazed on star grass pasture of sufficient quality to ensure that none of the cows in the control group lost live-mass over the experimental period.

Plasma samples

From 28 days to 100 days post partum blood samples were collected from all experimental cows three times each week by jugular venipuncture. Within 2 hours of collection, samples were centrifuged and plasma stored at -12 C. Progesterone content of plasma samples was determined according to the method of Holness, Hale and Hopley (1980).

Bulls

Entire bulls (2 per group) 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 minimise sire effects on conception rates in each group.

Observation of oestrous behaviour

Observers monitored experimental groups continuously each day between 0500 hours and 1800 hours. Detailed behaviour records were compiled and the analysis of this data will be presented in Chapter 9.

PROGESTERONE ASSAY

Progesterone concentration of all plasma samples was determined in two batches of 12 assays each. Details of the method have

been reported by Holness, Hale and Hopley (1980). Within each assay of 70 samples, 10 control plasma samples were included. All control samples were derived from a single pool with a mean progesterone concentration of 4,42 ng/ml of plasma. 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 plasma samples within each assay. Standard errors of the means of the control samples within each assay were 0,17ng/ml and 0,13ng/ml giving coefficients of variation of 8% and 11% respectively. The standard errors for a single observation in each batch of assays were ±0,54ng/ml and ±0,42ng/ml respectively.

RESULTS

Post-partum intervals to :-

Primary ovulation

Significantly ($P<0,001$) more normally suckled cows than partially weaned cows were anovulatory for 100 days post partum (Figure 4). There was a 26% higher incidence (per group) of primary ovulations followed by short cycles in partially weaned cows than in normally suckled cows (Figure 4). However this significant ($P<0,01$) difference was due primarily, to the higher proportion of anovulatory Afrikaner cows in the normally suckled group. Amongst cows which ovulated within 100 days of calving, a shortened luteal phase was exhibited by 87% (45/52) of cows in each group (Derived from Table 6). Reducing the suckling intensity did not shorten the mean post-partum interval to primary ovulation (Table 6).

Figure 4. Post-partum ovarian activity in normally suckled and partially weaned Afrikaner cows.

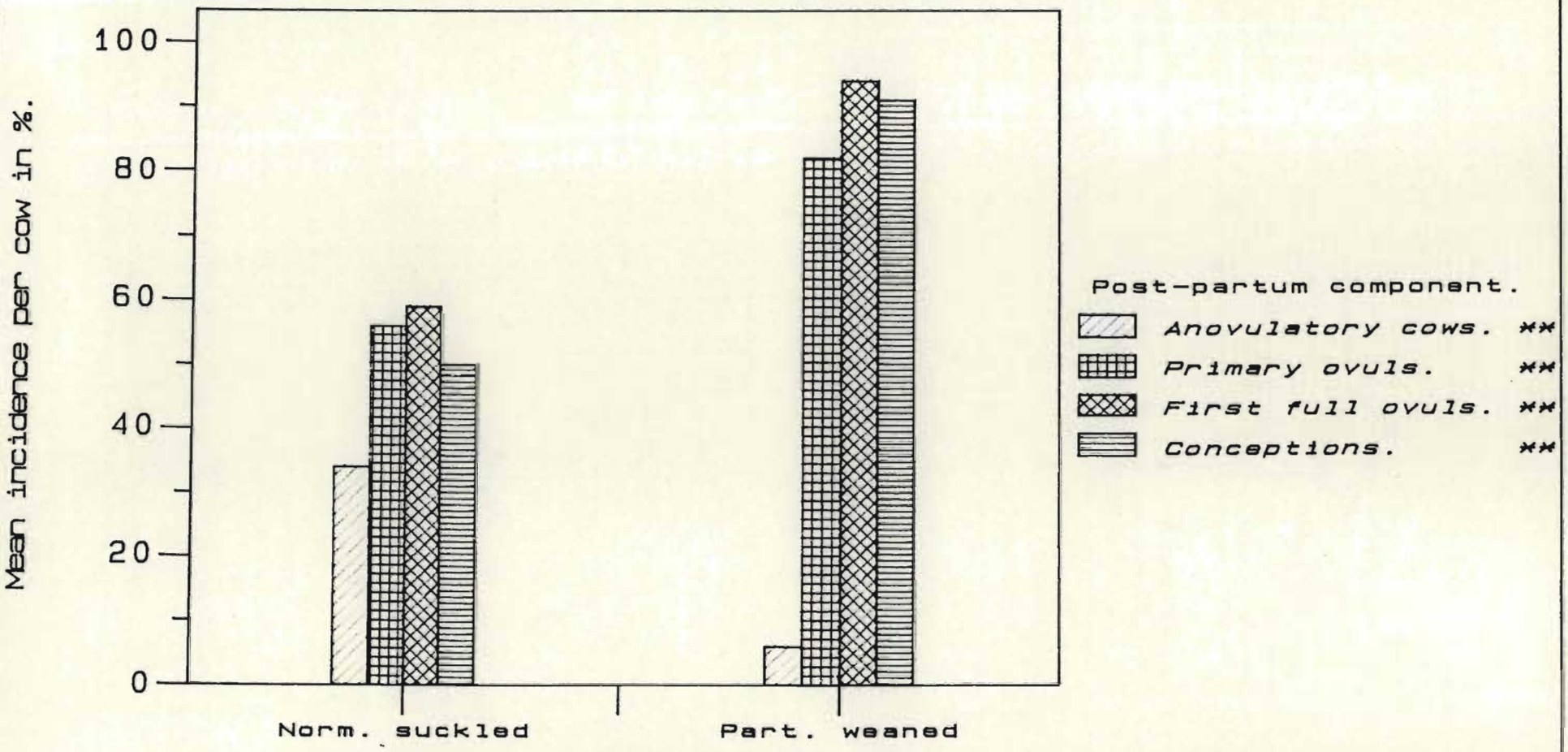


Table 6. Characteristics of primary ovulations in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

	Normally Suckled	Partially weaned	Signifi. of diff.	Mean
Anovulatory for 100 days PP	11/32	2/33	P<0,001	
Incidence of short cycles	18/32	27/33	P<0,01	
PPI (days) to primary ovulation	56,4±4,13	51,6±3,53	NS	53,5±2.68
Sample number	18	27		
Range	34-91	30-98		

NS Group means do not differ significantly.
PPI Post-partum interval.

Full ovulation

Partial weaning significantly (P<0,01) increased the incidence of full ovulations within 100 days of calving from 59% in normally suckled cows to 94% in partially weaned cows (Figure 4). Again this situation was due entirely to the complete absence of ovulation in 34% of normally suckled Afrikaner cows. 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 partially weaned cows, although this difference was not significant. 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 (P<0,01) reduced (on a herd basis) the mean post-partum interval to first full ovulation by 20 days (Table 7).

The probability of conception accompanying a full ovulation was not improved by reducing the suckling stimulus (Table 7).

Table 7. Characteristics of the first full ovulation after calving in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

	Normally Suckled	Partially weaned	Signifi. of diff.	Mean
Incidence of full ovulations	19/32	31/33	P<0,01	
PPI (days) to first full ovulation excluding anovulatory cows	64,4±4,6	53,1±3,8	NS	57,4±3,01
Sample number	21	31		
PPI (days) to first full ** ovulation including anovulatory cows	77,6±4,1	56,9±3,9	P<0,01	67,1±3.09
Sample number	32	33		
Conceptions	16	30	P<0,001	

NS Normally suckled cows do not differ significantly.
PPI Post-partum interval

** Cows which failed to ovulate within 100 days of calving were allocated a post-partum interval to first full ovulation of 100 days for this comparison.

Conception

Since a reduction in suckling intensity to one event per day virtually eliminated the incidence of cows being anovulatory for 100 days after calving, conception rates were significantly (P<0,001) improved by 41% by partial weaning (Table 8).

Assigning a mean value of 100 days to cows which failed to conceive, the analysis demonstrates how partial weaning on a herd basis significantly (P<0,01) improves reproductive performance, shortening mean post-partum interval to conception by a minimum of 20 days (Table 8).

Table 8. Characteristics of conception in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

	Normally Suckled	Partially weaned	Signifi. of diff.	Mean
Conceptions	16/32	30/33	P<0,001	
Calvings	16/32	27/33	P<0,01	
PPI (days) to conception excluding anovulatory cows	71,8±4,90	61,8±3,22	NS	65,1±2,76
Sample number *	14	29		
Range	43-100	31-100		
PPI (days) to conception ** including anovulatory cows	85,9±3,5	65,3±3,5	P<0,01	75,4±2,77
Sample number	32	33		
Range	43-100	31-100		
Duration (days) of gestation	291±1,2	290,6±1,2	NS	290,8±0,87

NS Treatment means are not significantly different.
PPI Post-partum interval.

* Two cows in the normally suckled group and one cow in the partially weaned group exhibited symptoms of either a retained corpus luteum or conception followed by embryonic death followed by reconception within 100 days of calving. Therefore their their data have been excluded from this analysis.

** Cows which failed to ovulate within 100 days of calving were allocated a post-partum interval of 100 days to conception for this comparison.

All conceptions were carried to full term in normally suckled cows whilst three conceptions were lost between 30 days after conception and the due calving date. No data were available which might account for these embryo losses which occurred more than 100 days after calving.

The majority of cows in each group, 79% of normally suckled cows and 69% of partially weaned cows, conceived to their first full ovulation (Table 9). Cows which conceived to their second ovulation, ovulated for the first time 43±9 days post partum in contrast to those cows which conceived to their first full ovulation at 63±20 days post partum (Table 9). These data confirm that ovulations occurring earlier than 50 to 55 days post partum are consistently less fertile than ovulations occurring later during the post partum period.

Table 9. Post-partum intervals (days) to first full ovulation as affected by partial weaning and post-partum interval to conception in Afrikaner cows.

	Conception at first ovululation	Conception at second ovululation
Normally suckled		
Mean PPI	68,8±21,0*	46,0±2,65
Sample number	11	3
Partially weaned		
Mean PPI	59,2±19,0	41,8±10,6
Sample number	20	8
Signif. of diff.	NS	NS
Mean PPI	62,6±20,0	42,9±9,2

NS Treatment means do not differ significantly.
PPI Post-partum interval.

Luteal function :-

Primary ovulations

Following primary ovulations, the duration of short luteal phases was unaffected by intensity of suckling (7,5±0,44 days, Table 10). However luteal activity after primary ovulation may have been altered by suckling intensity since total estimated plasma progesterone concentrations during these short cycles was higher in partially weaned cows than in control cows.

This confirms earlier results (Chapter 3) where reduced suckling intensity altered luteal function only after primary ovulation. The difference in total estimated plasma progesterone following a primary ovulation between control and partially weaned cows was substantially smaller than that observed between suckled and non-suckled cows in the last study. Presumably this follows a smaller increase in gonadotropin activity following partial weaning than following complete weaning.

Full ovulation

Luteal output following full ovulation including conception was very similar in both normally suckled and partially weaned Afrikaner cows (Table 10). Once partially weaned Afrikaner cows had ovulated (full) the similarity in their luteal function to that of control cows indicated that partial weaning was of no further benefit to the endocrine system of these weaned cows (Figure 5).

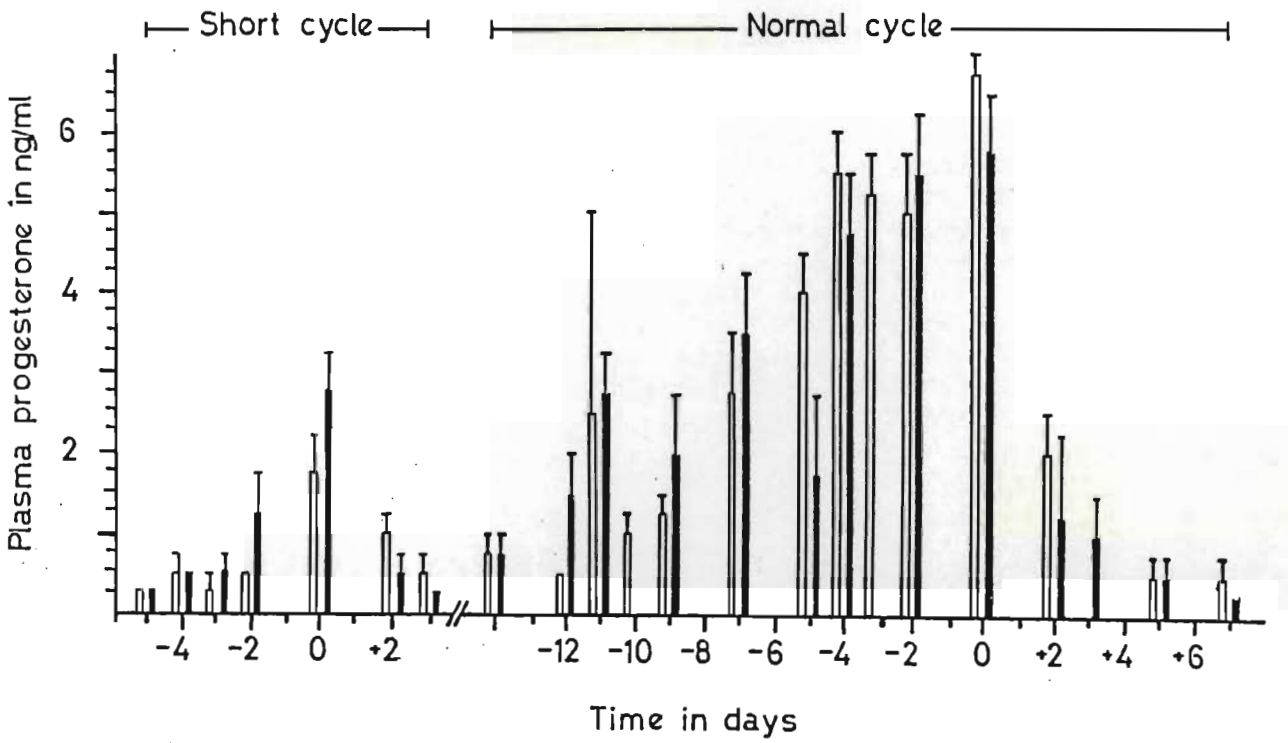
Table 10. Characteristics of luteal function following primary, first full ovulations and conception in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum. Area under the curve is described in ng/ml*days.

	Normally Suckled	Partially weaned	Signifi. of diff.	Mean
Short cycle				
Duration (days)	7,4±0,72	7,6±0,56	NS	7,5±0,44
Sample number	18	27		
Range	3-12	4-14		
Area under P4 profile	4,8±0,93	8,9±1,80	NS	7,3±1,17
Sample number	18	27		
Range	2-17	1-42		
First full cycle				
Duration (days)	21,2±0,75	21,3±0,60	NS	21,3±0,46
Sample number	21	31		
Range	18-23	17-24		
Area under P4 profile	57,8±7,38	52,6±6,26	NS	54,7±4,75
Sample number	21	31		
Range	40-84	27-89		
Conception				
Area under P4 profile *	36,3±4,33	41,5±2,64	NS	39,7±2,29
Sample number	16	30		
Range	6-60	21-86		

NS Group means are not significantly different.

* In this instance area under the plasma progesterone (P4) profile was estimated during the first 14 days following conception.

Figure 5. The mean plasma progesterone profiles (ng/ml) of normally suckled and partially weaned Afrikaner cows following primary and full ovulations. Normally suckled cows represented by open bars and partially weaned cows represented by solid bars.



Pattern of resumption of ovarian activity

A reduction in the suckling intensity failed to alter the number of primary and full ovulations prior to conception (Data not presented). The majority of conceptions in this trial were preceded by primary ovulations in normally suckled cows (81%) and in partially weaned cows (83%). Conception following one primary ovulation and one full ovulation accounted for virtually all of the remaining conceptions (3/3 in normally suckled cows and 3/5 in partially weaned cows).

DISCUSSION

Conception rates amongst Afrikaner cows in this study were 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. These results equate to those in the unsuckled cow where conception rates improved by 30% with the removal of the suckling stimulus from three days post partum.

Initially it would seem that once a day suckling improved conception rates to a greater degree than no suckling. Before this question is resolved partial weaning and complete weaning studies would have to be run concurrently. Although these complete weaning and partial weaning trials appeared similar, apart from suckling intensity, the high proportion of anovulatory control cows in the second study confirms that the control groups in these two trials were not comparable. The basic routes of improvements in conception rate differ in the complete weaning and partial weaning trial. In the former study conception was limited by conception failure in cycling cows whereas in the present study conception failure was due to the absence of ovulation for 100 days post partum in 33% of normally suckled Afrikaner cows.

Since a conception rate of 60%-67% is expected for the unselected Afrikaner herd under near optimal conditions of nutrition and management (Trail et al., 1977; Holness et al., 1980; Thorpe and Cruikshank, 1981) the breed appears to be 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 hours per day. Conception rates were 30% higher than those of their normally suckled contemporaries (Velasquez et al., 1980). In breeds which are sensitive to the suppressive effects of suckling (possibly many Bos indicus breeds) reductions in suckling stimulus are likely to be more effective in improving reproductive performance than in breeds less prone to the suppressive effects of suckling.

Prior to the resumption of ovarian activity, the pulsatile discharge of LH commences 20 to 30 days post partum (Carruthers and Hafs,1980; Peters, Lamming and Fisher, 1981). As the post partum interval increases, LH pulse frequency increases, raising mean plasma LH concentrations (Garcia-Winder et al., 1934). 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 hours of weaning. Thereafter, the steady increase in plasma LH concentrations noted (Short et al., 1972; Forrest et al., 1979a and b; Peters et al., 1981; Walters et al., 1982b) may be ascribed to an increase in pulsatile GnRH release rather than any further change in pituitary responsiveness.

In the Afrikaner cow, once a day suckling did not significantly reduce 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, work 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 and enable a full resumption of ovarian activity.

In the present study significantly more partially weaned cows were able to escape from the suppressive effects of suckling on hypothalamo-pituitary activity than their normally suckled contemporaries. At the same time, the absence of significant treatment differences on post-partum interval to primary ovulation implies that the timing of increased LH activity was similar in both groups. This was due to either the application of partial weaning coinciding with a spontaneous increase in gonadotropin activity or the possibility that minimal suckling activity is sufficient to maintain the post partum temporal controls on this increased LH activity. Where calves were weaned completely 3 days post partum the interval to primary ovulation was advanced by 34 days (Chapter 3). The degree of change in temporal components of gonadotropin activity appears to be directly proportional to suckling intensity. Thus, although partial weaning will enhance hypothalamic activity, to achieve maximum benefit, its application must be timed to coincide with, and amplify, the unprompted endogenous increase in gonadotropin concentrations 20 to 40 days post partum.

The net effect of reducing the suckling stimulus is that it shortens the interval to first ovulation by a removal of the suppression of GnRH and gonadotropin activity as distinct from a positive stimulation of these factors early post partum. For this reason partial weaning will probably not stimulate the

resumption of ovarian activity in all cows unless they are within 10 to 30 days of ovulating under a normal suckling intensity.

Once ovarian activity commenced, all parameters used to describe luteal function measured in this study did not differ significantly between the two treatment groups. Although progesterone concentrations following primary ovulations tended to be higher in weaned cows than in control cows the difference was not significant. In the absence of suckling however, progesterone concentrations after primary ovulations were more than 200% higher than in suckled cows even though the duration of these short cycles was unchanged. These very high levels of progesterone are evidence of an abnormal endocrine environment. In the light of the poor conception rates to first full ovulations less than 50 days post partum in the weaned and partially weaned cows, the higher luteal activity after primary ovulation in weaned cows must be viewed with some suspicion. Certainly Henricks, Dickey and Hill (1971) found that higher plasma progesterone concentrations just prior to oestrus reduced the number of blastomeres in the embryo on day three and proposed that this might reduce fertility. High progesterone levels around oestrus have been shown to adversely affect gamete and zygote transport (Russell et al., 1980; Newcomb and Rowson, 1975). Abnormal patterns of change in progesterone and tonic LH concentrations may be involved in the impairment of sperm transport and oestradiol secretion (Maresign, 1985).

In the Afrikaner cow, after primary ovulations, shortened luteal phases were 7,5±0,6 days long and progesterone output was estimated to be about 8%-17% of that measured during a normal luteal phase in this study. The role of these short cycles is

uncertain. Shortened luteal phases have been reported by others (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. Shortened luteal phases would seem to occur at the resumption of ovarian activity in all cows. Webb et al., (1977b) and La Voie et al. (1981) considered this luteal activity essential for the resumption of full ovarian activity.

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 (Chapter 3) indicate that the probability of conception is highest 58 to 70 days after calving. In this study conception at first ovulation occurred 63 days post partum whilst conception to second ovulation occurred at about the same time, 64 days post partum (Table 9). 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.

CONCLUSION

In terms of conception rate, reducing the sucking stimulus to twice a day (28 to 42 days post partum) and then once a day thereafter was as effective as eliminating the suckling stimulus in improving reproductive performance in the Afrikaner cow. The present study indicated that the improvement in reproductive performance was due largely to the induction of ovulation in

anovulatory Afrikaner cows. Reducing the suckling stimulus releases the hypothalamus and pituitary from the suppressive effects of suckling on gonadotropin activity up until 40 days post partum. At first ovulation in weaned cows this increased gonadotropin activity induces higher plasma progesterone concentrations following primary ovulation. These higher steroid levels may be a symptom of the abnormal endocrine milieu responsible for the subfertility of these early ovulations in weaned cows.

In Afrikaner cows in this trial, the major hurdle to the resumption of ovarian activity was the absence of any ovarian activity. Having achieved this threshold, the probability of conception was not improved by the continued reduction in suckling intensity. A question which arose from the results of these last two trials was whether partial weaning in the absence of the bull could be used to "tease" cows prior to the breeding season as a means of hastening the resumption of ovarian acitivity.

Chapter 5

The resumption of ovarian activity and conception in Afrikaner and Mashona cows after partial weaning either before or during the breeding period.

INTRODUCTION

A considerable degree of improvement in post-partum reproductive function (particularly conception rate, +30%) was achieved in the Afrikaner cow with either an elimination of suckling or a reduction in the frequency of suckling to once a day. Normal suckling intensities delay 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.

Both of the previous studies in the Afrikaner cow have 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 reproductive function 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 or allelomimetic cues or pheromones (Baker, 1984).

The ram effect in sheep is well documented (Schinkel, 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 vasectomised 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, 1982b).

If a reduction in suckling stimulus per se was responsible for the improvements in reproductive performance observed in the preceding two chapters, 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.

If partial weaning alone proved to be effective in inducing ovulation in the anovulatory Afrikaner cow and since regular ovarian activity has always followed these induced ovulations (Chapters 3 and 4) 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 will increase gonadotropin activity and induce ovulation in 50% of anovulatory cows (Edwards, 1985). Unfortunately, restoration of normal suckling levels after a period of weaning has been shown to depress gonadotropin activity within 4 hours of calf return (Walters et al., 1982a; Edwards. 1985). Edwards (1985) reported, however, that this only occurred in cows which were anovulatory. In cyclic cows no such reduction in gonadotropin activity was observed. Thus the effect of returning calves to their dams upon the introduction of bulls might 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. Nevertheless if partial weaning was to be used commercially there could be no question of weaning each calf at a specific age.

MATERIALS AND METHOD

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.

Method

Suckling intensity was reduced 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 although 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. No adverse effects were noted with either calf growth or cow stress. With good handling, cows learnt the system within three to four days. Calves began consuming concentrate within a week and consumed approximately 640 gms per day. This ration comprised 40% milled hay, 50% maize meal and 10% milled cotton seed meal.

The three treatment groups used in this trial consisted of two partial weaning groups which differed in the timing of partial weaning application in relation to the breeding period and one control group in which cows were normally suckled for the duration of the trial.

A...Partial weaning during the breeding period:

Twenty two Afrikaner cows and sixteen 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 thirteen Mashona cows which were respectively 61±23 days and 77±9 days post partum 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±15 days post partum 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.

Plasma samples

Blood samples were collected three times each week at 0800 hours from the 30 November until the 5 March. Plasma was extracted from these samples and stored at -20 C for determination of plasma progesterone concentration.

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 will be presented in detail in Chapter 10.

PROGESTERONE ASSAY

Plasma progesterone determinations were carried out according to the method of Holness, Hale and Hopley (1980). Assay repeatability was monitored using eight control plasma samples per assay of 120 tubes. In all, 39 assays were carried out and all control plasma samples were derived from a single plasma pool with a mean progesterone concentration of 4,69±1,01 ng/ml (Mean ± Standard Deviation). Coefficients of variation were estimated from a simple one way analysis of variation of all control plasma samples. Within assay variation was 22% based on the Standard Error of a single determination and the between assay variation was 15%. The standard error for a single observation in an assay was ±0,42ng/ml.

RESULTS

Anovulatory cows

Partial weaning as against normal suckling before the breeding period did not significantly reduce the incidence of cows which were anovulatory for the duration of the trial. However partial weaning applied with the introduction of the bulls significantly ($P<0,05$) decreased the proportion of anovulatory cows from 50%, in cows weaned before the breeding period or normally suckled throughout the trial to 18% in cows partially weaned during the breeding period (Table 11). There were no instances of anovulatory Mashona cows in this study.

Table 11. The number of Afrikaner and Mashona cows in each treatment group which ovulated within the experimental period from 21 December to 5 March.

		Afrikaner		Mashona
A	Partial weaning with breeding	18/22		16/16
			P<0,05	NS
B+C	Normally suckled + Partial weaning before breeding	20/40		23/23
Totals for each breed		38/62	P<0,001	39/39

* 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.

NS Treatment means do not differ significantly.

Resumption of ovarian activity

By the start of the trial on 21 December, 19% of Afrikaner cows and 80% of Mashona cows had ovulated (Table 12). Within each breed a similar proportion of cows in each of the treatment groups had ovulated at the start of the study.

Table 12. The distribution of the resumption of ovarian activity in relation to the application of partial weaning and the introduction of bulls.

Treatment group	Number in Group	Commencement of ovarian activity (Cumulative totals)			
		Before 21 Dec	21 Dec–25 Jan	25 Jan–5 Mar	No ovarian activity
Afrikaner					
A Weaned during breeding	22	4	3 (7)	11 (18)	4
		NS	NS	P<0,05	P<0,05
B Weaned prior to breeding	25	5	5 (10)	3 (13)	12
		NS	NS	NS	NS
C Control	15	3	2 (5)	2 (7)	8
Mashona					
A Weaned during breeding	16	12	1 (13)	3 (16)	0
		NS	NS	NS	NS
B Weaned prior to breeding	13	11	2 (13)	3 (13)	0
		NS	NS	NS	NS
C Control	10	9	1 (10)	0 (10)	0

NS Treatment means do not differ significantly.

Since the majority of Mashona cows had ovulated before the beginning of the trial, too few cows remained to assess whether partial weaning alone would have stimulated ovulation in the anovulatory Mashona cow (Table 12).

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 12).

In sharp contrast to this lack of response to partial weaning in the absence of bulls, partial weaning together with the introduction of bulls significantly ($P < 0,05$) increased the proportion of cows which ovulated for the first time during the breeding period from 33% of normally suckled Afrikaner cows to 73% in partially weaned Afrikaner cows (Table 2).

Ovulations during the pre-breeding and breeding periods

Figures 6 and 7 clearly illustrate the differences in endocrine status 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 and 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

Figure 6. Mean number of ovulations/cow prior to and during the breeding period in Afrikaner cows.

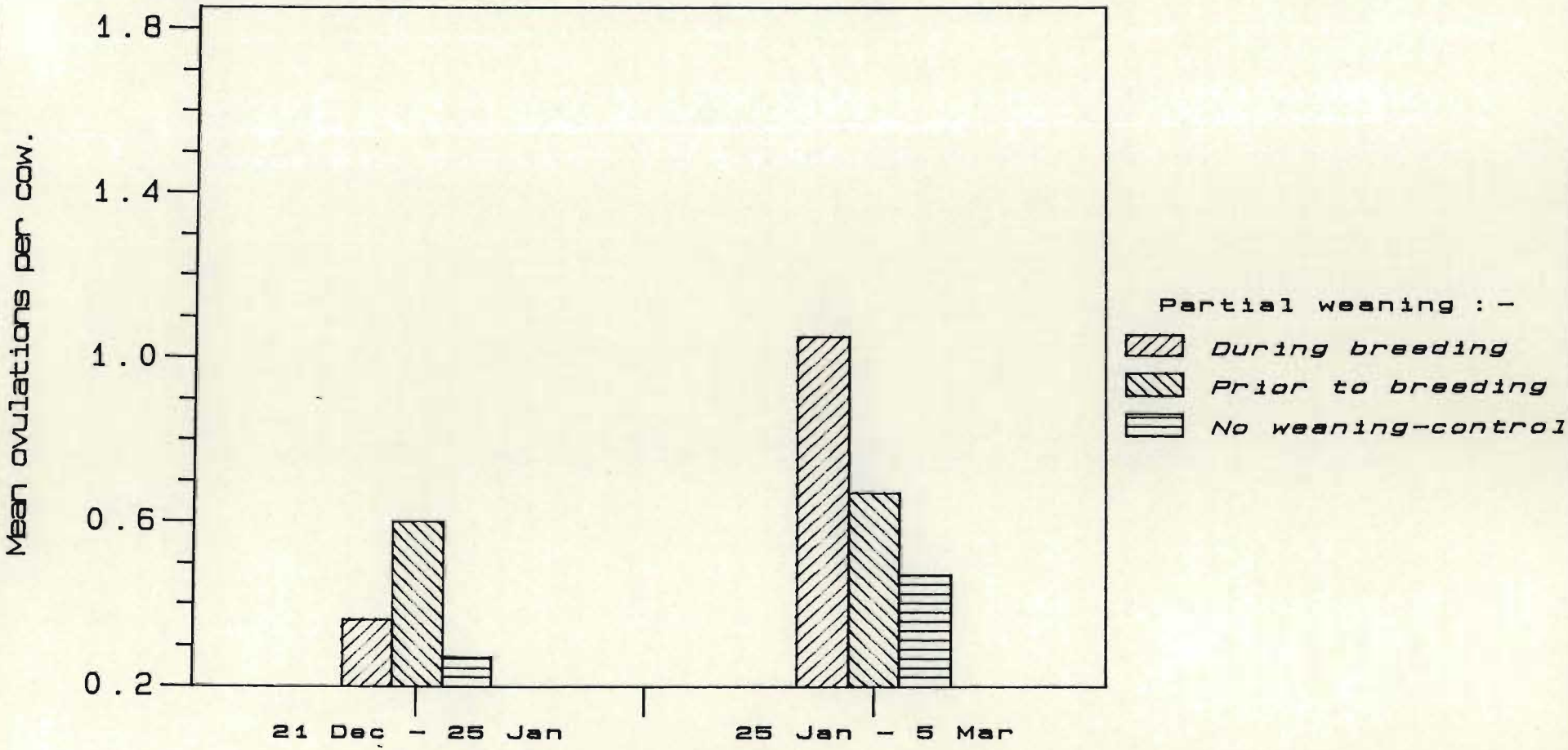
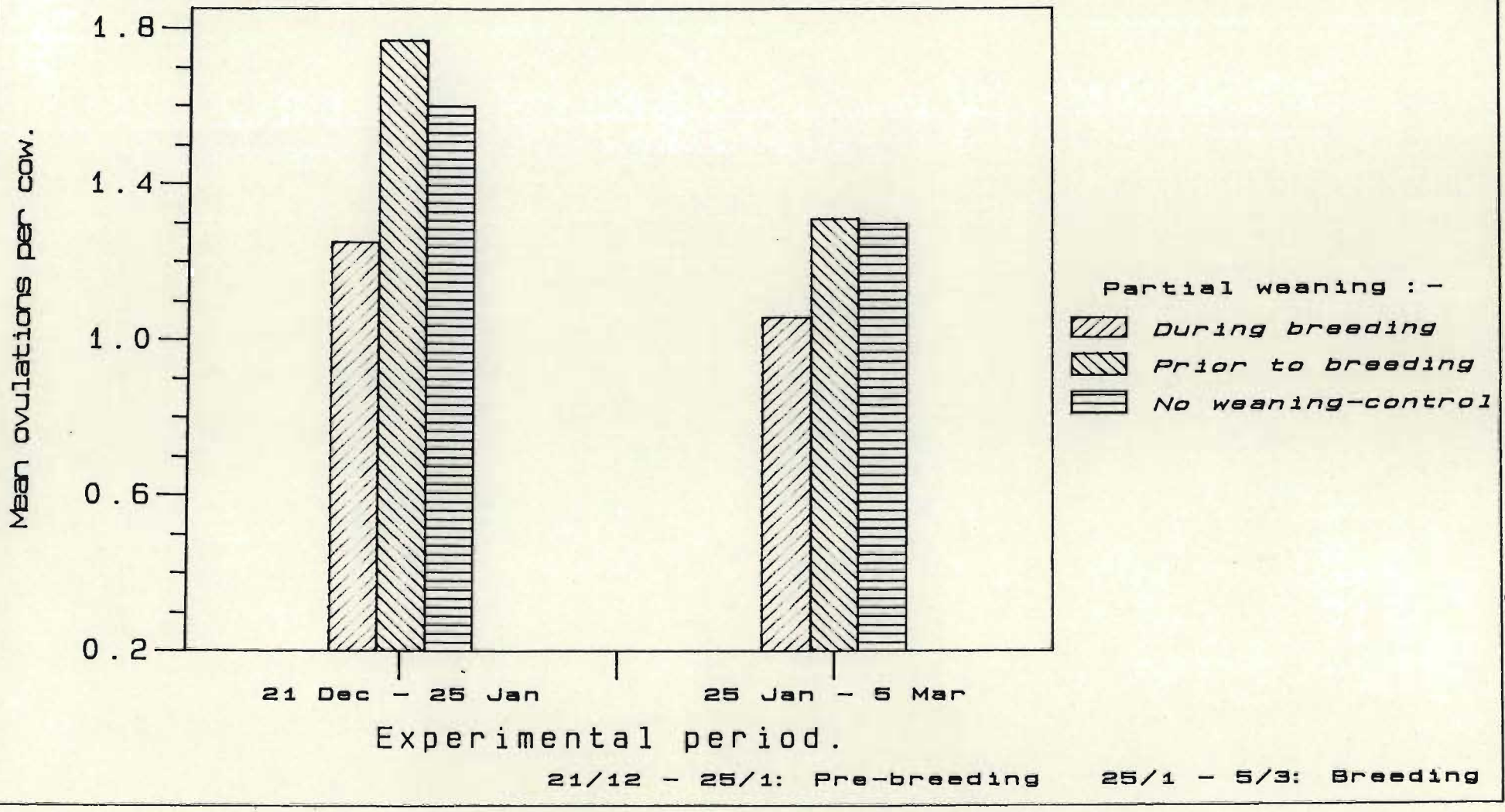


Figure 7. Mean number of ovulations/cow prior to and during the breeding period in Mashona cows.



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 (Figure 7) and appears to have arisen from the fact that these cows may have been close to spontaneous ovulation initially and with the reduction of the suckling stimulus, ovulation was advanced 10 to 20 days.

During the breeding period, partial weaning and the introduction of bulls significantly ($P<0,05$) increased the mean ovulation rate per cow (Figure 5). In both control cows and cows weaned before breeding there were a number of cows which failed to ovulate after the introduction of the bulls. Clearly, weaning before breeding had not had much impact on the majority of anovulatory Afrikaner cows. This finding points to the necessity of partial weaning cows with the simultaneous introduction of bulls for a maximal effect. This statement is qualified by the fact that treatment application in this study was confounded with post-partum interval.

In the Mashona cows, the mean ovulation rate appeared to decline when cows were partially weaned with the introduction of bulls (Figure 6). This apparent anomaly was due to earlier conceptions in cows weaned during the breeding period compared to their normally suckled contemporaries.

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 13).

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. This situation was similar to that reported in Chapter 4, where conception rates were limited by a high (34%) incidence of anovulatory cows (Table 6). 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 92% in the Mashona cows (Table 4).

Table 13. Conception rates following suckling once each day either before or during the breeding period in Afrikaner and Mashona cows .

Time of weaning	Afrikaner			Mashona		
	With breed.	Before breed.	Control	With breed.	Before breed.	Control
Number in group	22	25	15	16	13	10
Number showing resumption of ovarian activity	18	13	7	16	13	10
Pregnancies	13 NS	9 NS	7	15 NS	11 NS	9
Breed totals	29/62			35/39		

NS Total conceptions between groups within each breed do not differ significantly.

Although partial weaning during the breeding period stimulated the resumption of ovarian activity in 82% (18/22) of Afrikaner cows only 72% (13/18) of these cows which ovulated conceived. In the control group however, the pattern of conception differed in that 100% (7/7) of the cows which ovulated during the breeding period conceived (Table 13). Sample numbers were too small to confirm 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 analysed 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 14).

Total estimated plasma progesterone in the Afrikaner cow did not differ during either short luteal phases or during the first four oestrous cycles across the three treatment groups (Table 14). 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 14. 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	With breeding	Before breeding	Control	Sig. of Differ.
Short luteal phase				
Afrikaner	5,5±0,7 (15)	4,7±2,2 (10)	2,3±1,3 (3)	NS
Mashona	5,4±1,2 (9)	4,8±0,3 (3)	7,6±3,3 (3)	NS
First four oestrous cycles (pooled)				
Afrikaner	54,4±4,5 (23)	50,5±3,3 (26)	56,7±6,6 (10)	NS
Mashona	77,2±4,6 (37) [a]	60,5±3,6 (43) [b]	78,5±3,8 (28) [a]	P<0,01
Conception + 17 days				
Afrikaner	77,4±7,2 (10) [a]	50,9±5,4 (9) [b]	58,8±4,1 (7) [b]	P<0,05
Mashona	94,4±9,4 (15) [c]	64,5±6,6 (9)* [d]	93,0±5,0 (7) [c]	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.

Significance of the differences between treatment means:
a - b P<0,01 c - d P<0,05

NS Treatment means do not differ significantly between groups.

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 15).

Table 15. 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.

Luteal type	With breeding	Before breeding	Control	Signif. of diff.
Short luteal phase				
Afrikaner	89,8±7,6 (15)	89,5±7,8 (10)	94,7±12,7 (3)	NS
Mashona	46,2±5,0 (9)	72,7±3,0 (3)	54,0±15,0 (3)	NS
First oestrous cycle				
Afrikaner	79,6±8,9 (9)	73,6±7,1 (10)	63,5±9,5 (2)	NS
Mashona	49,8±3,0 (12)	57,6±4,5 (12)	57,1±5,9 (8)	NS
Conception				
Afrikaner	108,2±8,3 (13)	118,0±3,2 (9)	121,1±9,3 (7)	NS
Mashona	107,5±5,5^{c} (15)	128,5±4,0^{d} (11)	123,7±6,9^{d} (9)	P<0,05
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)	

Significance of the differences between treatment means:
a - b = $P<0,05$ c - d = $P<0,01$
NS Means do not differ significantly between treatment groups.

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 by 16 days to 107±6 days compared with 123±7 days in normally suckled control cows and 128±4 days in cows which had been partially weaned prior to breeding (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 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

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% (Tables 4 and 8). 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.

These results support the findings from the second study (Chapter 4) in which 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 (Table 6). With complete weaning during breeding (Chapter 3) however, the mean interval from calving to primary ovulation was significantly ($P < 0,001$) shortened by 35 days in the Afrikaner (Table 1). 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. (1982) 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.

Following 35 days of partial weaning, normal suckling would have simulated an increase in the intensity of suckling. 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. Increased gonadotropin concentrations may only be of value in the anovulatory cow and might even be undesirable

following primary ovulations when the concomitant increase in luteal activity appears to be associated with low conception rates during the subsequent oestrus.

Since conception followed significantly different total amounts of plasma progesterone in the preceding cycle, it would appear that the endocrine changes necessary for a fertile ovulation may not be strongly dependent upon total progesterone concentrations prior to ovulation. In the Afrikaner cow, once a day suckling during the breeding period stimulated seven primary ovulations followed by one short luteal phase and then conception out of 13 previously anovulatory cows. During these short cycles total plasma progesterone concentrations measured 5,6±1,1 ng/ml*days or less than 10% of the total amount available during a normal oestrous cycle. If progesterone priming is essential prior to the first ovulation post partum then this small (relative to a normal cycle) amount was sufficient to achieve a fertile ovulation accompanied by conception.

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 their first ovulation. This is the first strong evidence that partial weaning during oestrus has improved the probability of conception. Chapter 10 should indicate whether or not this improvement in conception was due to a higher probability of service during oestrus or whether these cows were more fertile to the first ovulations after the introduction of the bulls.

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.

CONCLUSIONS

All earlier work with partial weaning in the Afrikaner and Mashona was conducted with simultaneous exposure to the bull. Reduced suckling alone, prior to breeding would appear to have little or no potential as a means of stimulating ovulation in the anovulatory Afrikaner cow. This statement is qualified by noting that cows which have experienced a spontaneous endogenous increase in gonadotropin activity prior to the start of partial weaning may be induced to ovulate sooner than normally suckled cows. This advance in first ovulation would probably be no more than 10 to 20 days. The use of partial weaning with the simultaneous introduction of bulls into the Afrikaner herd with a high proportion of anovulatory cows is an effective tool for dealing with the quiescent ovary. In fertile breeds such as the Mashona or in cows which are already cycling, the use of partial weaning during the breeding period can improve conception rates to first ovulations within the breeding period.

Chapter 6

The use of exogenous progesterone as a means of improving conception rates to first full ovulations in Afrikaner and Mashona cows suckled once a day.

INTRODUCTION

Reductions in the intensity of suckling in the presence of bulls in the post-partum Afrikaner cow have proved very effective in stimulating the early (less than 50 days post partum) resumption of ovarian activity (Chapters 3 and 4). A consistent feature of these early ovulations however was the high incidence of conception failure as a result of ovulations which based on their subsequent luteal activity, appeared quite normal. This finding is in agreement with Ward et al. (1979), Odde et al. (1980) and Ramirez-Godinez et al. (1981 ,1982). These low conceptions rates do not appear to have been a result of inadequate luteal support following ovulation and attention must now be focused on pre-ovulatory events.

Whether first ovulations post partum are spontaneous (Short et al., 1974) or induced by either gonadotropins (see Roberts, 1971) or gonadotropin releasing hormone (Britt et al., 1974; Britt et al., 1975; Lamming and Bulman, 1976; Webb et al., 1977b) they are invariably infertile and the corpora lutea which follow are short lived (Marion and Gier, 1968; Lishman et al., 1979; Pratt et al., 1982; Sheffel et al., 1982). Cyclic ovarian activity may or may not follow these induced ovulations. In contrast to the findings of Webb et al., (1977b) and Bulman and Lamming (1978) who induced ovulations followed by normal cyclic activity with the use of Gonadotropin Releasing Hormone and Progesterone

Releasing Intravaginal Devices respectively, neither of these methods induced fertile and persistent ovarian activity in the Afrikaner cow (Holness and Hale, 1980).

In order to improve both the fertility of induced (by any means) ovulations and the persistence of the subsequent corpus luteum the hypothalamo-pituitary axis would appear to need prior exposure to progesterone for at least the duration (7-9 days in the Afrikaner) of a short cycle (La Voie et al., 1981; Ramirez-Godinez et al., 1981). At the ovarian level improper follicular development has been considered to be a factor that could contribute to the shorter life span or low function of some induced corpora-lutea (Marion and Gier, 1968; Webb et al., 1977b), although Sheffel et al. (1982) do not agree.

Knowing that partial weaning (±20 days post partum) will induce anovulatory Afrikaner cows to ovulate by 45 days post partum and that exposure of the reproductive system to progesterone prior to first ovulation post partum might improve the lifespan of the subsequent corpus luteum or probability of conception, the following study was conducted.

The objective was simply to improve the probability of conception with first ovulations post partum in partially weaned Afrikaner cows by the use of exogenous progesterone from a Progesterone Releasing Intra-vaginal Device (PRID). Mashona cows were also included in this study not only to assess treatment effects in a fertile breed but also because there were sufficient numbers of Mashona cows to use as a non-PRID control group.

MATERIALS AND METHOD

Experimental animals

A total of 22 Afrikaner and 35 Mashona multiparous (range 3 to 6) cows were considered suitable for this study. Of these, all of the Afrikaner cows and 23 Mashona cows received progesterone for 9 days. In general, cows were not in ideal condition for this study with condition scores ranging from 2 to 2,5.

Experimental conditions

Rainfall was below average and grazing although adequate was sparse. Cows were obliged to range over unsatisfactorily large areas to graze, drink, suckle calves and muster three times each week for blood sampling.

Method

With the aid of a speculum, Progesterone Releasing Intra-vaginal Devices (PRID's) were inserted into each cow in the PRID group 35 days after calving and removed 9 days later. Since these PRID's were used as an exogenous progesterone source only, no oestradiol was administered to the cows.

Of the 24 Afrikaner cows, 10 were partially weaned 25 days post partum and their calves were permitted to suckle once each day at 1600 hours. Of the 35 Mashona cows 5/12 in the no-PRID group and 12/23 in the PRID group suckled their calves once each day. All experimental cows were run as two herds, normally suckled cows and partially weaned cows.

All calves were partially weaned until the last cow to calve was 100 days post partum. After this all calves were returned to their dams until weaning at seven months of age. Whilst calves

were held in weaning pens, they had free access to a ration comprising 42% maize meal, 50% roughage and 8% cotton seed meal.

Bulls

Fertile Mashona bulls (two per group) were run with all cows from 25 days after calving. Bulls were examined and declared sound for breeding prior to their introduction into the experimental herds. During the study all bulls were rotated between the two treatment groups at weekly intervals to minimise bull effects on cow performance.

Plasma samples

Blood samples were collected via jugular venipuncture three times each week at 0800 hours from 25 days post partum until 100 days post partum for each cow. Plasma was extracted and stored at -12 C until being assayed for progesterone.

PROGESTERONE ASSAY

Plasma progesterone concentrations were determined by the method of Holness, Hale and Hopley (1980). A total of 2382 samples were analysed in 18 assays. In each assay 8 control plasma samples were included from a common plasma pool. Over the whole assay run, the control plasma pool had a mean concentration of 4,1 ng/ml. Standard error of a mean (of 8 samples) within an assay was 0,41 ng/ml (10%) whilst the standard error of the mean between the 18 assays was 0,48 ng/ml (12%). Within and between assay variation was estimated using a simple two way analysis of variation with no replications.

RESULTS

Total plasma progesterone during PRID treatment

Estimates of total plasma progesterone concentrations over the nine days that progesterone releasing intravaginal devices (PRID) were *in situ*, indicated that significantly ($P < 0,01$) more progesterone was present in Mashona cows over this period than in Afrikaner cows (Table 16).

Table 16. Estimated total plasma progesterone concentrations (ng/ml*days) following the intra-vaginal insertion of a PRID for 9 days in Afrikaner and Mashona cows which were either normally suckled or partially weaned from 25 days *post partum*.

Suckling treatment	Afrikaner	Mashona	Signif. of diff.	Suckling mean
Normally suckled				
Mean area	15,1±1,13	19,8±1,73	P<0,05	17,5±1,13
Range	9,8-20,0	6,1-29,4		
Sample number	12	12		24
Partially weaned				
Mean area	17,1±1,59	26,6±3,30	P<0,05	22,1±2,19
Range	9,8-24,6	15,9-53,0		
Sample number	10	11		21
Significance of difference	NS	P<0,10		P<0,10
Breed means	16,0±0,95	23,1±1,92	P<0,01	

NS Treatment means do not differ significantly.

These breed differences are consistent and significant ($P<0,05$ and $P<0,05$) within each of the two suckling treatment groups. Cows in the partially weaned group had been suckled once a day for 11 days prior to insertion of the PRID's. Across breeds, partially weaned cows had ($P<0,05$) higher total plasma

progesterone concentrations during the nine days of progesterone priming than their normally suckled contemporaries. Results within each breed are inconclusive since differences in suckling status were not significant in Afrikaner cows and only approached significance (P<0,10) amongst Mashona cows (Table 16).

Primary ovulation

No significant breed differences were recorded in the proportion of cows exhibiting primary ovulations at the resumption of ovarian activity in cows treated with progesterone (Table 17).

Table 17. Incidence and characteristics of short cycles following primary ovulations in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with exogenous progesterone for nine days from a PRID inserted 35 days post partum.

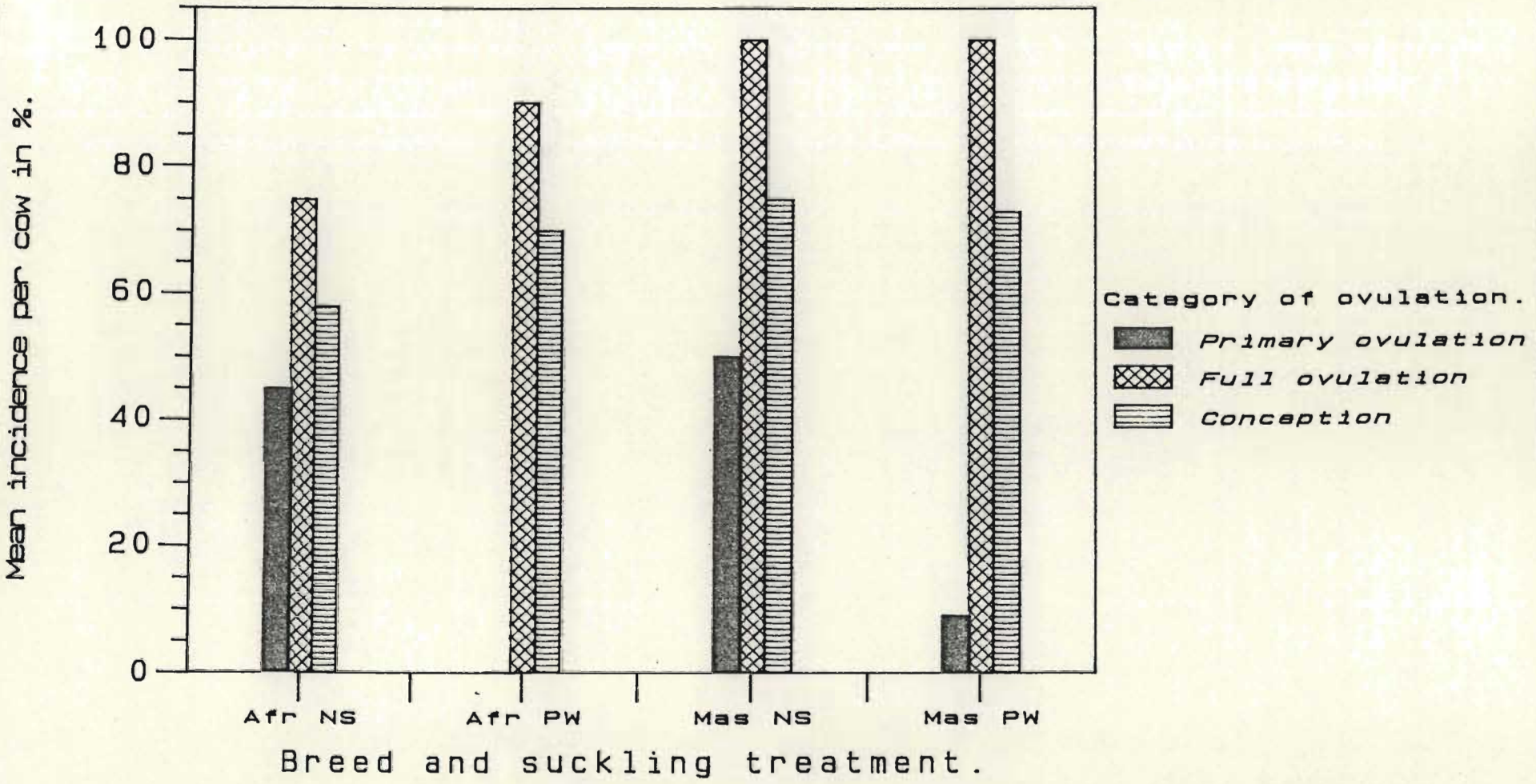
Suckling status	Afrikaner	Mashona	Signif. of diff.	Suckling Mean
Normally suckled				
Incidence	5/11 1*	6/12	NS	11/23 c
Mean PPI to start (days)	76,8±4,26	69,8±3,53	NS	73,0±2,81
Duration (days)	5,0±0,63	6,5±0,72	NS	5,8±0,52
Area in (ng/ml*days)	5,4±1,25	3,5±1,13	NS	4,3±0,85
Peak in (ng/ml)	2,3±0,26	0,97±0,18	P<0,01	1,5±0,25
Partially weaned				
Incidence	0/10	1/11 4*	NS	1/21 d

NS Treatment means do not differ significantly.
c--d Proportions differ significantly P<0,05.
* These cows ovulated prior to the insertion of the PRID.

Partial weaning 25 days post partum along with progesterone 10 days later significantly (P<0,02) reduced the incidence of short cycles following first ovulation in both Afrikaner and Mashona cows (Figure 8). Progesterone treatment had assumed the role of the short luteal phase since previous studies have indicated (Figures 2 and 4) that partial weaning did not reduce the incidence of short cycles following first ovulation.

Normally suckled Afrikaner and Mashona cows exposed to progesterone 35 days post partum did not differ in post-partum interval to primary ovulation (73±3 days), duration of short cycle (6±0,5 days) or total plasma progesterone during the short cycle (4,3±0,9 ng/ml*days). Only peak plasma progesterone was significantly higher in Afrikaner than Mashona cows after primary ovulation (Table 17). The delayed (+17 days) post-partum interval to primary ovulation confirms that the cows in this study were under stress, since normally suckled Afrikaner and Mashona cows should ovulate for the first time 49 to 56±4 days and 37±8 days after calving respectively.

Figure 8. Post-partum ovulation and conception in NS and PW Afr. and Mas. cows < 100 days of calving



NS Norm. suckled (n = 24): PW Part. weaned (n = 21)

In Mashona cows, exogenous progesterone treatment did not shorten the post-partum interval to primary ovulation although it significantly (P<0,01) reduced the incidence of short cycles in Mashona cows (Table 18). Despite the environmental stresses of this trial partial weaning (in cows not treated with progesterone) shortened (P<0,01) the post-partum interval to primary ovulation by 32 days to 39±6 days.

Table 18. The incidence and characteristics of short cycles following primary ovulations in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with progesterone for nine days beginning 35 days post partum.

Suckling status	No PRID	PRID	Signif. of diff.	Mean
Normally suckled				
Incidence	5/5	6/12	NS	11/17
PPI to start (days)	71,2±6,12 a	69,8±3,53	NS	70,4±3,20
Duration (days)	7,0±1,38	6,5±0,72	NS	6,7±0,73
Area (ng/ml*days)	7,2±2,09	3,5±1,13	NS	5,2±1,22
Peak (ng/ml	2,3±0,48	0,97±0,18	P<0,01	1,57±0,37
Partially weaned				
Incidence	6/7	1/7 4*		7/14
PPI to start (days)	39,0±5,94 b			
Duration (days)	7,7±1,76			
Area (ng/ml*days)	8,9±3,88			
Peak (ng/ml	2,4±0,41			

NS Treatment means do not differ significantly.
PPI Post-partum interval.
* These cycles commenced prior to the insertion of PRIDs and have therefore been excluded from the analysis.
a--b These means differ significnatly (P<0,01)

General resumption of ovarian activity

Following the low incidence of short cycles in partially weaned cows treated with progesterone, Table 19 was compiled to investigate the effects of breed and suckling status on the resumption of ovarian activity, including both primary and full ovulations.

Progesterone treatment did not alter the effects of suckling status and breed on post-partum interval to the resumption of ovarian activity (Table 19).

Table 19. Mean intervals from calving to first rise in plasma progesterone above 0,30 ng/ml (including primary and full ovulations) in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with exogenous progesterone for nine days from a PRID inserted 35 post partum.

Suckling status	Afrikaner	Mashona	Signif. of diff.	Suckling mean
Normally suckled				
Mean (days)	70,3±5,13	58,4±4,26	P<0,10	63,5±3,46
Range	35-88	31-84		
Sample number	9	12		21
Partially weaned				
Mean (days)	49,4±3,08	37,8±4,2	P<0,05	43,1±2,94
Range	40-73	49-89		
Sample number	9	11		20
Significance of difference	P<0,01	P<0,01		P<0,001
Breed mean (days)	59,9±3,85	48,6±3,7	P<0,05	53,6±2,17

Partial weaning significantly (P<0,01) shortened the post-partum interval to the resumption of ovarian acitivity by 20 days to 43±3 days. Mashona cows , as usual, ovulated for the first time

49±4 days after calving, 11 days earlier (P<0,05) than the Afrikaner cows. There was no apparent interaction of breed and suckling status on the resumption of ovarian activity amongst cows given progesterone 35 days post partum (Table 19).

With normal suckling, progesterone treatment tended to shorten the interval to first ovulation in Mashona cows (Table 20). Progesterone therapy after partial weaning in Mashona cows had no effect on post-partum interval to the resumption of ovarian activity which at 37±3 days must be the minimum for once a day suckling. This short interval to first ovulation is remarkable considering the post-partum intervals to conception to be presented in Table 25.

Table 20. Mean intervals from calving to first rise in plasma progesterone above 0,3 ng/ml in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with progesterone for nine days from 35 days post partum.

Suckling status	No PRID	PRID	Signif. of diff.	Suckling Mean
Normally suckled				
Mean (days)	71,2±6,12	58,4±4,26	NS	62,2±3,70
Range	57-90	31-84		
Sample number	5	12		17
Partially weaned				
Mean (days)	37,6±5,22	37,8±4,2	NS	37,8±2,66
Range	26-61	22-70		
	7	11		18
Significance of difference	P<0,01	P<0,01		P<0,001
PRID mean	51,6±4,9	48,6±3,7	NS	49,6±2,53

NS Treatment means do not differ significantly.

Full ovulations

The incidence of first full ovulations after progesterone therapy did not differ significantly between breeds or between suckling treatments within each breed (Table 21).

First full ovulations were significantly ($P<0,001$) earlier (by 23 days) in partially weaned cows compared to control cows. Within treatment the resumption of ovarian activity in partially weaned Afrikaner and Mashona cows was very similar at 45±2 days whereas in normally suckled cows the Afrikaner was 14 days later at 75±5 days post partum than the Mashona (P 0,10 Table 21).

Although first full ovulations were significantly ($P<0,01$) later in control cows (+23 days), a significantly ($P<0,01$) higher proportion (+45%) of these ovulations were accompanied by conception in normally suckled cows (55%) than in partially weaned cows (10%, Table 21). Breed had no significant effect on the incidence of conception to first full ovulation.

In all, 12/12 non-PRID Mashona cows and 23/24 PRID Mashona cows exhibited full ovulations (Table 22). Interestingly, progesterone may have shortened ($P<0,10$) the interval to first full ovulation in normally suckled Mashona cows by 17 days (Table 22). In partially weaned cows in the absence of progesterone the mean post-partum interval to first full ovulation was 44±5 days, thus progesterone for 9 days at 35 days could not have shortened this interval.

Table 21. Mean post-partum intervals and conception rates to first full ovulations in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with exogenous progesterone for nine days from a PRID inserted 35 days post partum.

Suckling status	Afrikaner	Mashona	Signif. of diff.	Suckling mean.
Normally suckled				
Mean PPI (days)	75,5±5,00	61,7±5,01	P<0,10	67,2±3,85
Range	47-91	31-89		
Sample number	8/12	12/12	NS	20/24
Conceptions to first full ovul.	5/8	6/12 d	NS	11/20 a
Partially weaned				
Mean PPI (days)	47,1±1,09	43,2±4,37	NS	44,9±2,44
Range	40-52	23-77		
Sample number	9/10	11/11	NS	20/21
Conceptions to first full ovul.	1/9	1/11 c	NS	2/20 b
Significance of difference	P<0,001	P<0,01		P<0,001
Breed mean	60,4±4,24	52,8±3,82	NS	56,0±2,87

NS Treatment means do not differ significantly

PPI Post-partum interval.

a...b Proportions differ significantly P<0,01.

c...d Proportions differ significantly P<0,05.

Table 22. Mean post-partum intervals to first full ovulation in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with progesterone for nine days starting 35 days post partum.

Suckling status	No PRID	PRID	Signif. of diff.	Suckling mean
Normally suckled				
Mean PPI (days)	78,2+6,37	61,7+5,01	P<0,10	66,5+4,32
Range	62-97	31-81		
Sample number	5/5	12/12	NS	17/17
Conceptions to first full ovul.	0/5	6/12 c	NS	6/17 a
Partially weaned				
Mean PPI (days)	44,1+4,74	43,2+4,37	NS	43,5+3,20
Range	29-66	23-77		
Sample number	7/7	11/12	NS	18/19
Conceptions to first full ovul.	0/7	1/11 d	NS	1/18 b
Significance of difference	P<0,001	P<0,01		P<0,01
PRID means	58,3+6,24	52,8+3,82	NS	54,7+3,27
Sample number	12/12	23/24	NS	
Conceptions	0/12	7/23	P<0,05	

NS Treatment means do not differ significantly.

PPI Post-partum interval.

a..b Proportions differ significantly (P<0,05).

c..d Proportions differ significantly (P<0,01).

Conceptions to first full ovulations occurred in 50% of normally suckled Mashona cows treated with progesterone (Table 22). Thus progesterone may have improved conception rate to first full ovulation but only in normally suckled cows. First full ovulations in partially weaned cows in this study were generally infertile with a success rate of only 8%.

Luteal function after full ovulation

Estimates of total plasma progesterone during all complete oestrous cycles in this study confirm earlier findings (Chapters 3 to 5) that partial weaning had no significant effect on luteal function following full ovulation (Table 23).

Table 23. Characteristics of luteal function following all full ovulations in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with progesterone for nine days from 35 days post partum.

Suckling status	Afrikaner	Mashona	Signif. of diff.	Suckling Mean
Normally suckled				
Mean area (ng/ml*days)	50,8±3,60	46,8±4,85	NS	47,8±3,70
Range	46,3–57,9	26,9–76,8		
Sample number	3	9		12
Partially weaned				
Mean area (ng/ml*days)	34,1±2,08	45,8±3,63	P<0,05	40,8±2,48
Range	25,1–48,3	23,0–70,8		
Sample number	12	18		20
Significance of difference	P<0,01	NS		NS
Breed mean	37,4±2,50	46,2±2,85	P<0,05	42,9±2,10

NS Treatment means do not differ significantly.

The present estimates of total progesterone confirm the findings from Chapter 4, that progesterone concentrations in the Afrikaner are 20% lower than in the Mashona (Table 23).

In the Mashona cow, exposure to exogenous progesterone 35 days after calving appears to have significantly (P<0,05) depressed subsequent luteal function from a total of 55±2 ng/ml*days in cows not exposed to exogenous progesterone to 46±3 ng/ml*days in cows treated with progesterone (Table 24).

Table 24. Characteristics of luteal function following all full ovulations in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with progesterone for nine days from 35 days post partum.

Suckling status	No PRID	PRID	Signif. of diff.	Suckling mean
Normally suckled				
Area mean (ng/ml*days)	54,5±7,33	46,8±4,85	NS	48,7±4,04
Range	45,6-89,0	26,9-76,8		
Sample number	3	9		12
Partially weaned				
Area mean (ng/ml*days)	54,9±3,63	45,8±3,63	P<0,05	50,2±2,42
Range	34,9-71,3	23,0-70,8		
Sample number	15	18		33
Significance of difference	NS	NS		NS
PRID mean	54,9±2,42	46,2±2,85	P<0,05	49,8±2,03

NS Treatment means do not differ significantly.
PRID Progesterone releasing intra-vaginal device.

Conception

By the conclusion of the trial (100 days post partum) conception rates did not differ significantly amongst normally suckled cows, although Afrikaner cows tended to have a lower conception rate (58%) than Mashona cows (75%, Table 25). Mean post-partum intervals to conception were similar in the two breeds at 77±3 days after calving. Amongst partially weaned cows the picture was very similar both in terms of conception rates and post-partum intervals to conception. This was the first time in this series of studies that partial weaning had failed to make a significant impact on conception in the post-partum cow. Considering the fact that first full ovulations were 22 days earlier in partially weaned cows than in normally suckled cows (Table 21) conception rates to these first full ovulations in weaned cows were clearly infertile. On average partially weaned cows did not conceive for another 30 days after first full ovulation whereas normally suckled cows conceived 10 days after their first full ovulation (from Tables 21 and 25).

Estimates of total plasma progesterone over the 14 days after conception followed the two trends observed with luteal activity after full ovulations reported earlier. First of all, partial weaning had no effect on luteal function within 14 days of conception and second Afrikaner cows tended to have less progesterone than Mashona cows, although in this study the difference was not significant (Table 25.)

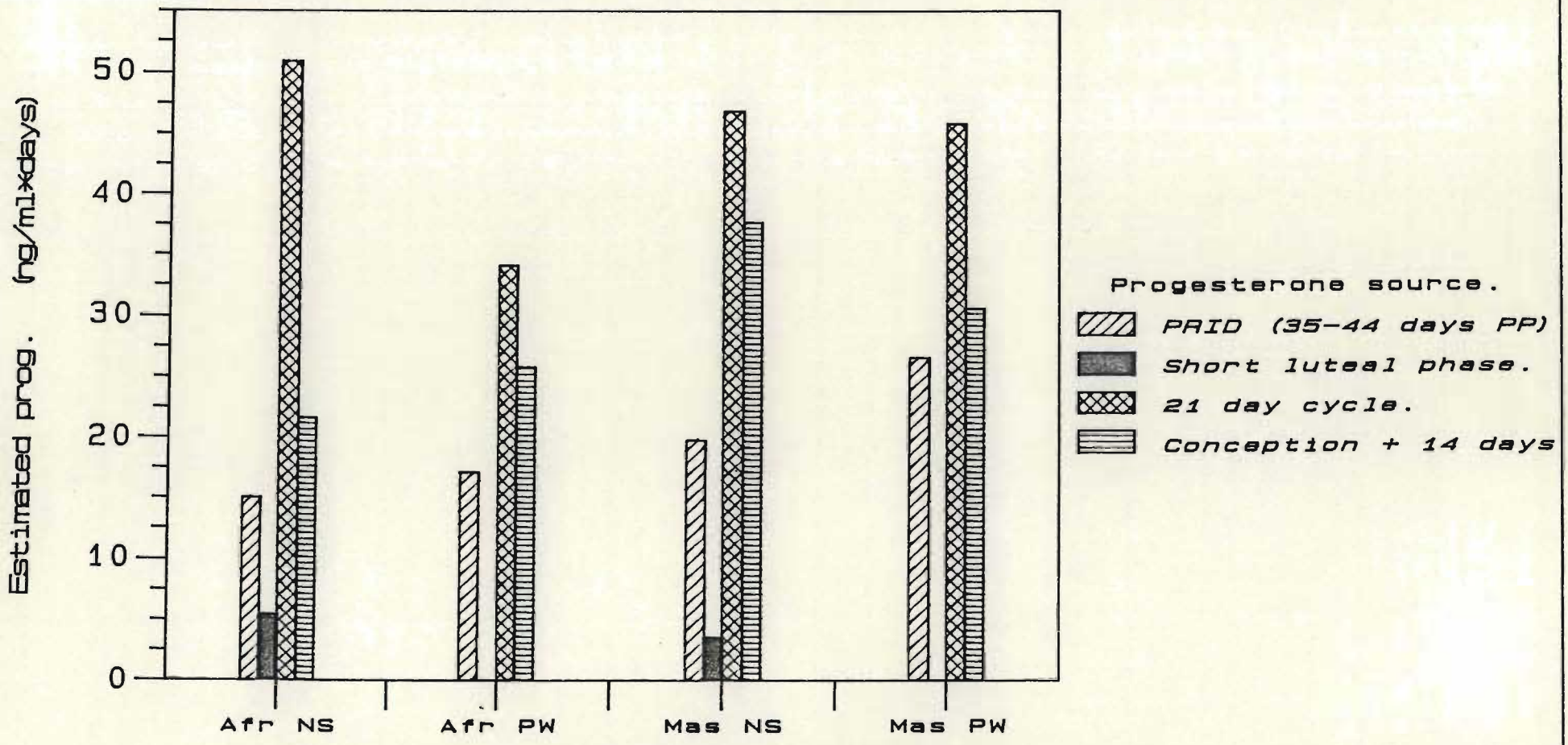
Table 25. Mean intervals from calving to conception and estimated total plasma progesterone (ng/ml*days) during the first 14 days after conception in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with exogenous progesterone for nine days from a PRID inserted 35 days post partum.

Suckling status	Afrikaner	Mashona	Signif. of diff.	Suckling mean
Normally suckled				
Mean PPI to conception (days)	80,9±4,14	73,4±3,98	NS	76,8±2,94
Range	66-92	49-89		
Sample number	7/12	9/12	NS	16/24
Partially weaned				
Mean PPI to conception (days)	76,4±5,52	72,3±4,46	NS	74,3±3,42
Range	50-90	50-89		
Sample number	7/10	8/11	NS	15/21
PPI to conception Significance of difference	NS	NS		
Breed means	78,6±3,37	73,0±2,88	NS	75,5±2,22
Breed sample number	14/22	17/21	NS	

NS Treatment means do not differ significantly.
PPI Post-partum interval to conception.

The relative amounts of total plasma progesterone during the four classes of luteal function discussed in this chapter have been summarised in Figure 9. Total estimated plasma progesterone during the first fourteen days after conception tended to be lower in Afrikaner cows than in Mashona cows (Figure 9), a finding which agrees with the differences recorded in these two breeds in Chapter 5.

Figure 9. Total progesterone/cycle (ng/ml*days) in control and partially weaned Afr. and Mas. cows.



Normally suckled (n = 24): Part. weaned (n = 21)

DISCUSSION

In the absence of suckling, the mean interval from calving to first full ovulation in the Afrikaner is shortened by 27 days to 30±3 days whilst a reduction in the suckling stimulus to once a day shortens this interval by 11 days to to 53±1 days compared to the normally suckled cow. In the present study, the use of progesterone and partial weaning together shortened the post-partum interval to first ovulation by 22 days to 47±1 days.

The present study confirms that partially weaned Afrikaner and Mashona cows have similar reproductive potentials post partum. Thus 20% of Afrikaner cows which would have been anovulatory for 100 days post partum can be induced to ovulate and conceive within this period by once a day suckling. The Mashona under similar circumstances will achieve an 80% conception rate without any manipulation of suckling status. The endocrine system of the Mashona is certainly not immune to the effects of suckling but post-partum hypothalamo-pituitary activity is certainly not suppressed to the same degree as in the normally suckled Afrikaner cow. The fact that post-partum intervals to first ovulation were shortened by 25 days with once a day suckling is evidence of the suppressive effects of suckling in the Mashona when environmental stresses delay the resumption of ovarian activity in the normally suckled cow. (Table 20).

In the present study partial weaning 25 days post partum would have increased gonadotropin activity 10 days later (Walters et al., 1980; Stevenson et al., 1982). followed by ovulation, which in about 60% to 80% of cases would have induced short cycles (MacMillan and Watson, 1971; Hansen and Hauser, 1980;

Odde et al., 1980; La Voie et al., 1981; Ramirez-Godinez et al., 1980,1981; Peters and Riley, 1982).

Since this short increase in plasma progesterone is detected more often than not prior to oestrus (Donaldson et al., 1970; Corah et al., 1974; Peters and Riley, 1982) it has been proposed as an essential step in the restoration of normal cyclic ovulatory activity post partum (La Voie et al., 1981). Carrick and Shelton (1969) proposed that a refractoriness to oestradiol during the early post-partum period was responsible for the low incidence of oestrus with first ovulation. Short-term exposure to progesterone restores sensitivity to oestradiol which could account for the higher incidence of oestrus with the second ovulation post partum.

The use of PRIDs in the present trial was designed to mimic these short cycles. The total plasma progesterone measured in cows during treatment with PRIDs differed between breeds with Mashona cows having about 30% more progesterone than the Afrikaner cows. This may have arisen for one or all of three reasons. Either the difference is due to a dilution factor based on total body mass differences between the breeds or clearance rates are higher in Afrikaner cows or exogenous progesterone was augmented by endogenous luteal output. Nothing can be concluded from the data available but it was interesting to note that endogenous plasma progesterone concentrations were 20 to 30% lower in Afrikaner cows than in Mashona cows after both full ovulations and conception.

Total concentrations of progesterone released from the PRID for nine days from day 35 post partum induced higher plasma

progesterone concentrations (16±1 ng/ml*days, Table 16) than those observed during the short cycles following primary ovulations (5±1 ng/ml*days, Table 17). In previous chapters higher total plasma progesterone concentrations have not been associated with increased fertility at first full ovulation.

Despite having programmed these Afrikaner and Mashona cows with some success and largely eliminated the short cycle, the majority of Afrikaner and Mashona cows failed to conceive to these first full ovulations (2 conceptions out of 20 ovulations). This is contrary to the findings of Ramirez-Godinez *et al.* (1981) who reported an improvement in conception rates to first oestrus *post partum* following partial weaning and the provision of progesterone. The question arises as to whether the provision of exogenous progesterone 35 days *post partum* might have been in some way responsible for this poor performance. In a recent publication Haresign (1985) has proposed that abnormal patterns of change of progesterone concentrations (particularly after oestrus synchronisation) can adversely affect oestrus control and sperm transport. Since no conceptions occurred prior to seven first full ovulations in partially weaned non-PRID Mashona cows with a mean post-partum interval to first full ovulation of 44±5 days other factors are almost certainly involved. In the light of the these findings and comments by Haresign (1985) the results of the behaviour analysis of this trial will be of interest to establish whether or not progesterone treatment had any affect on the expression of oestrus.

Luteal function must be viewed ultimately as a crude indicator of quality of ovulation. Thus although progesterone profiles

confirm that partial weaning will induce luteal activity which is apparently normal, they cannot provide any information on whether or not the rest of the endocrine and uterine environment has been able to match these accelerated changes. The confirmed pregnancies less than 20 days after calving in Afrikaner and Mashona cows reported in Chapter 3 demonstrate the tremendous flexibility of the reproductive system of breeds such as the Afrikaner and emphasise the significance of suckling on the resumption of ovarian activity.

CONCLUSIONS

Ultimately the use of exogenous progesterone and reduced suckling did not improve conception rates. At the same time conception rates and mean post-partum intervals to conception did not differ significantly between breed or suckling treatment. However the mean interval to conception of 75±2 days is longer than would have been expected and supports the proposal that adverse trial conditions may have delayed the resumption of ovarian activity in all cows in this study. The later application of treatments would almost certainly have yielded more favourable results.

Thus, the provision of progesterone in partially weaned cows appears to have exacerbated the problem of poor conception rates to ovulations occurring before 50 days post partum. The lack of success using exogenous progesterone in this study implies that insufficient steroid priming was not entirely the cause of sub-fertility of ovulations within 50 days of calving. Since luteal function following first full ovulations would appear to have been normal, insufficient luteal support following ovulation is unlikely to have caused conception failure. Earlier results (Chapter 3) indicated that uterine environment should not be

limiting conceptions before 50 days post partum which leaves the possibility of problems with either poor expression of oestrus or post-insemination malfunctions. The latter area is quite beyond the scope of this thesis and the behavioural component of low conception rates within 50 days of calving will be considered in detail in Chapters 8 to 11.

Chapter 7

Suckling behaviour during daylight hours in Afrikaner, Mashona and Hereford cows.

INTRODUCTION

In contrast to the Afrikaner with its low (60%) conception rate (Wells *et al.*, 1985) and strong maternal instincts (aggressive defence of new-born calf) the Mashona is more fertile (Ward and Dlodlo, 1985) and yet exhibits the same strong bonding with its calf. Work described in Chapters 3 and 5 demonstrated the significant differences in the reproductive performance of these two breeds and between suckled and non-suckled cows within each breed. The results of the past four chapters have also confirmed that a strong correlation exists between suckling intensity and post-partum reproductive function. This finding prompted the question of whether or not significant differences exist in any aspect of suckling behaviour in the Afrikaner and Mashona cow and if they do exist, whether they might be associated with differences in reproductive performance between these two breeds.

Whilst there is a paucity of studies in the literature relating suckling behaviour to reproductive function, there are a number of reports of studies investigating patterns of suckling behaviour of both *Bos taurus* and *Bos indicus* cows and calves (Drewry *et al.*, 1959; Odde *et al.*, 1985 (*Bos taurus*) and Reinhardt and Reinhardt, 1981; Hutchinson *et al.*, 1962, (*Bos indicus*)).

Since lactation in the beef cow is thought to reach a maximum four weeks *post partum* (Cole and Johansson, 1933) 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 and Lowman (1979) observed no relationship between age of calf and suckling frequency. This apparent contradiction may have been a result of different levels of milk production of cows in each of these latter studies since Odde et al. (1985) reported that milk production of the cow and live mass of calf were related to suckling incidence whereas age of calf was not. These researchers 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.

None of the studies reported above contained any reference to specific activities of the calf during each suckling bout. For this reason the primary objective of the present study was to describe in detail, levels of udder stimulation, specifically teat changes and udder butts during each suckling bout. From the foregoing literature it would appear that the duration of suckling bouts and the frequency of suckling during a 24 hour period would not differ sufficiently between Afrikaner and Mashona cows to account for the significant breed differences in reproductive performance between these two breeds. However the possibility that calf activity during each suckling bout might be related to breed of cow and milk yield, needed investigation and formed the second objective in this study.

MATERIALS AND METHOD

Compilation of detailed data on suckling behaviour under free-range conditions is laborious by virtue of the fact that during a 24 hour period the total time spent suckling will range from 27±11 minutes to 55±15 minutes (Drewry *et al.*, 1959). With one suitable observer per cow-calf pair per eight hour period, sample numbers were small. This situation was unavoidable if detailed records were to be compiled.

Experimental animals

A total of 15 mature, multiparous cows were available for this study and comprised five animals from each of three breeds Afrikaner, Mashona and Hereford. Mashona cows were again included in this study to provide a model of events in a fertile breed against which the characteristics of the Afrikaner could be assessed. Hereford cows were included to provide some indication of behaviour in *Bos taurus* cows under similar conditions. Unfortunately, whereas the calving dates were closely matched for the Afrikaner and Mashona cows, the Hereford cows available at the time calved significantly earlier and their data are included for comparative purposes only.

Method

Behaviour records were compiled between the hours of 0500 and 1900 for each observation day. All the cows used in this study were run over an 8 hectare area sub-divided into 2 hectare paddocks. Cows were supplemented with maize meal and cotton seed meal to increase live mass by 2% of calving mass over the experimental period. There was sufficient grazing available for cows to graze normally throughout the trial.

Preliminary observations during a 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 these two phases may be estimated within 10 to 30 seconds by a significant increase in teat change rate. 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, total number of udder butts and the time at end of suckling.

All cows were observed at least 3 times over a total of 56 observation days. The order of observation was determined prior to the commencement of the trial.

Following the initial analysis of data and as a result of an independent study on the milk yield of suckled Nashona cows a further study was initiated. With the specific objective of relating suckling behaviour to estimated milk intake by a calf a detailed data base was compiled with accurate estimations of teat change and udder butting rate within each of the two phases of suckling. Penned cows enabled close monitoring and control of calf to dam access.

Statistical analysis

All data pertaining to suckling events were analysed using a simple one way analysis of variance (Rayner, 1967).

RESULTS

General characteristics of a single suckling event

Within each breed cows varied widely in all components of suckling behaviour recorded in this study. For this reason no significant breed differences were recorded in the duration of the extraction phase with a mean duration of 5,9±0,3 minutes or the stripping phase with a mean duration of 8,5±0,3 minutes.

Although the stripping phase (9 minutes) lasted only 50% longer than the extraction phase (6 minutes) nearly seven times as many teat changes occurred during stripping (135±4 teat changes) than during extraction (24±2 teat changes, Table 26).

Table 26. Characteristics of suckling behaviour during a single suckling event in Afrikaner, Mashona and Hereford cows.

Suckling component	Afrikaner	Mashona	Hereford	Mean	Signif. of diff.
Sample number	65	60	48	173	
Extraction phase teat changes Range	25±2,1 5-80	25±2,0 5-75	22±3,2 6-110	24±2,1	NS
Duration of phase one in minutes Range	6,3±0,5 1-27	5,6±0,3 2-25	5,8±0,6 0-25	5,9±0,3	NS
Stripping phase teat changes Range	145±6,8 a 26-305	133±5,9 a,b 18-240	123±7,7 b 35-266	135±4,0	(a-b= P<0,10)
Duration of stripping phase in minutes Range	8,9±0,5 3-21	8,3±0,7 2-30	8,4±0,6 3-20	8,5±0,3	NS
Udder butts Range	34±2,9 a 0-102	22±2,3 b 0-89	27±3,3 a,b 0-94	28±1,7	(a-b= P<0,01)

NS Means within a row do not differ significantly.

a-a, b-b Means with common superscripts do not differ significantly.

Thus, during the extraction phase, calves change teats every 15 seconds whilst during the stripping phase teat changes occurred every 4 seconds. There was a tendency ($P<0,10$) for Afrikaner and Mashona calves to have a slightly higher number of teat changes than Hereford calves during stripping (Table 26).

The total number of udder butts per suckling event appeared to differ significantly between breeds. Amongst Mashona and Hereford cows the total number of udder butts per suckling was similar, 22 ± 2 and 27 ± 3 respectively but in the case of the Afrikaner the total number of udder butts per suckling was significantly (P 0,05) higher at (34 ± 2) than in the remaining two breeds.

Diurnal variation in patterns of suckling behaviour

Within each breed the total number of teat changes during either the extraction phase or stripping phase did not appear to change with sequential sucklings through the day (Table 27). In Afrikaner cows however there were significantly ($P<0,01$) fewer udder butts during the fourth and fifth suckling events than during the first two. Similarly, amongst Hereford cows, the first suckling in the day recorded significantly ($P<0,01$) more udder butts per suckling than during the second or third sucklings of the day.

Table 27. An analysis of the number of teat changes and udder butts with sequential suckling events through each day.

Suckling event	Afrikaner	Mashona	Hereford	Signif. of diff.
First				
Extraction	22±2,3	23±3,3	27±6,7	NS
Stripping	144±12,0	134±8,7	134±8,7	NS
Udder butts	40±4,6	29±4,9	41±7,5 a	NS
Sample number	22	19	15	
Second				
Extraction	30±4,9	24±3,6	23±5,3	NS
Stripping	149±16,7 a	140±12,2 a	109±11,8 b	a-b = P<0,05
Udder butts	41±6,9 a	20±3,3 b	17±3,2 b	a-b = P<0,05
Sample number	20	18	15	
Third				
Extraction	24±5,8	28±5,2	19±6,7	NS
Stripping	138±8,4	124±12,2	127±17,2	NS
Udder butts	24±2,9	18±5,0	17±2,6 b	NS
Sample number	12	14	13	
Fourth				
Extraction	24±5,3	25±6,4		NS
Stripping	150±13,0	128±22,3		NS
Udder butts	21±5,2	18±3,3		NS
Signif. of differences in suckling events 1-3.				
Extraction	NS	NS	NS	
Stripping	NS	NS	NS	
Udder butts	NS	NS	a-b = P<0,01	

NS Means within a row or column do not differ significantly.

a-a, b-b Means with common letters within a row or column do not differ significantly.

Changes in suckling behaviour with age of calf

Although suckling data for the Herefords were compiled simultaneously with the Afrikaner and Mashona data, Hereford cows calved too early for their data to correspond closely to the post-partum intervals of the Afrikaner and Mashona cows. For this reason no valid comparisons could be made between the Herefords and the other two breeds.

Between 29 days and 70 days post partum the total number of teat changes during the extraction phase did not differ significantly between breed or between observation periods within each breed (Table 28).

Only amongst Afrikaner cows did the number of teat changes during the stripping phase differ significantly ($P<0,05$) with post-partum interval. No consistent trend with increasing post-partum interval was observed and the highest number of teat changes ($181\pm16,0$) was recorded at $53\pm1,0$ days post partum.

Amongst Mashona cows, teat changes during stripping declined with post-partum interval but the differences were not significant. Within Hereford cows there was some evidence of a decline with increasing post-partum interval but again the changes were not significant.

Only amongst Mashona cows there was a significant ($P<0,01$) and consistent decline in the number of udder butts per suckling bout with increasing post-partum interval. In the Afrikaner cows stripping phase teat changes increased to a peak ($P<0,05$) 53 days post partum at 181 ± 16 and declined thereafter. The incidence of udder butting in Hereford cows differed significantly ($P<0,05$) with post-partum interval but no consistent trend was observed.

During the first observation period (29-36 days post partum) Afrikaner and Mashona cows did not differ significantly in any of the three components of suckling behaviour studied. At the second observation period (53 to 63 days post partum) significantly

Table 28. Characteristics of suckling behaviour in Afrikaner, Mashona and Hereford cows with increasing post-partum interval.

Observation number	Afrikaner		Mashona	Hereford
First				
Post-partum interval	29±2,4		36±2,6	
Extraction phase teat changes	26±4,4	NS	26±3,4	
Stripping phase teat changes	139±12,7	NS	142±7,9	
Udder butts	43±7,8	NS	34±5,3	
Sample number	17		19	
Second				
Post-partum interval	53±1,0		64±3,4	63±0,6
Extraction phase teat changes	22±3,0	NS	23±3,0	28±7,2
Stripping phase teat changes	181±16	<.02	134±10,1	142±12,8
Udder butts	42±6	<.01	21±3	34±5
Sample number	17	<.001	18	19
Third				
Post-partum interval	70±2,6		80±3,2	91±1,0
Extraction phase teat changes	29±5,6	NS	22±5,4	14±1,5
Stripping phase teat changes	133±9,9	NS	113±13,2	98±10,5
Udder butts	29±5	<.01	13±2	15±2
Sample number	16	<.001	15	14
Fourth				
Post-partum interval				111±0,7
Extraction phase teat changes				20±4,5
Stripping phase teat changes				122±14,0
Udder buts				28±6,1
Sample number				15
Significance of effects of age of calf on:				
Extraction phase teat changes	NS		NS	NS
Stripping phase teat changes	P<0,05		NS	NS
Udder butts	NS		P<0,01	P<0,05

(P<0,05) less stimulation was recorded amongst Mashona cows than in Afrikaner cows. Stripping phase teat changes totalled 181±16 and 134±10 and udder butts totalled 42±6 and 21±3 in Afrikaner and Mashona cows respectively. During the third observation interval at 70 days (post partum) the total number of extraction phase teat changes was similar in Afrikaner and

Mashona cows. However, significantly ($P<0,01$) more udder butts were again recorded for Afrikaner cows (29±4) than for Mashona cows (13±2) at this period. The net effect was that the Afrikaner cow was subjected to significantly more udder stimulation than the Mashona cow over the period 53 to 70 days post partum. In the normally suckled Afrikaner cow the first evidence of the resumption of ovarian activity and oestrus will be occurring during this period.

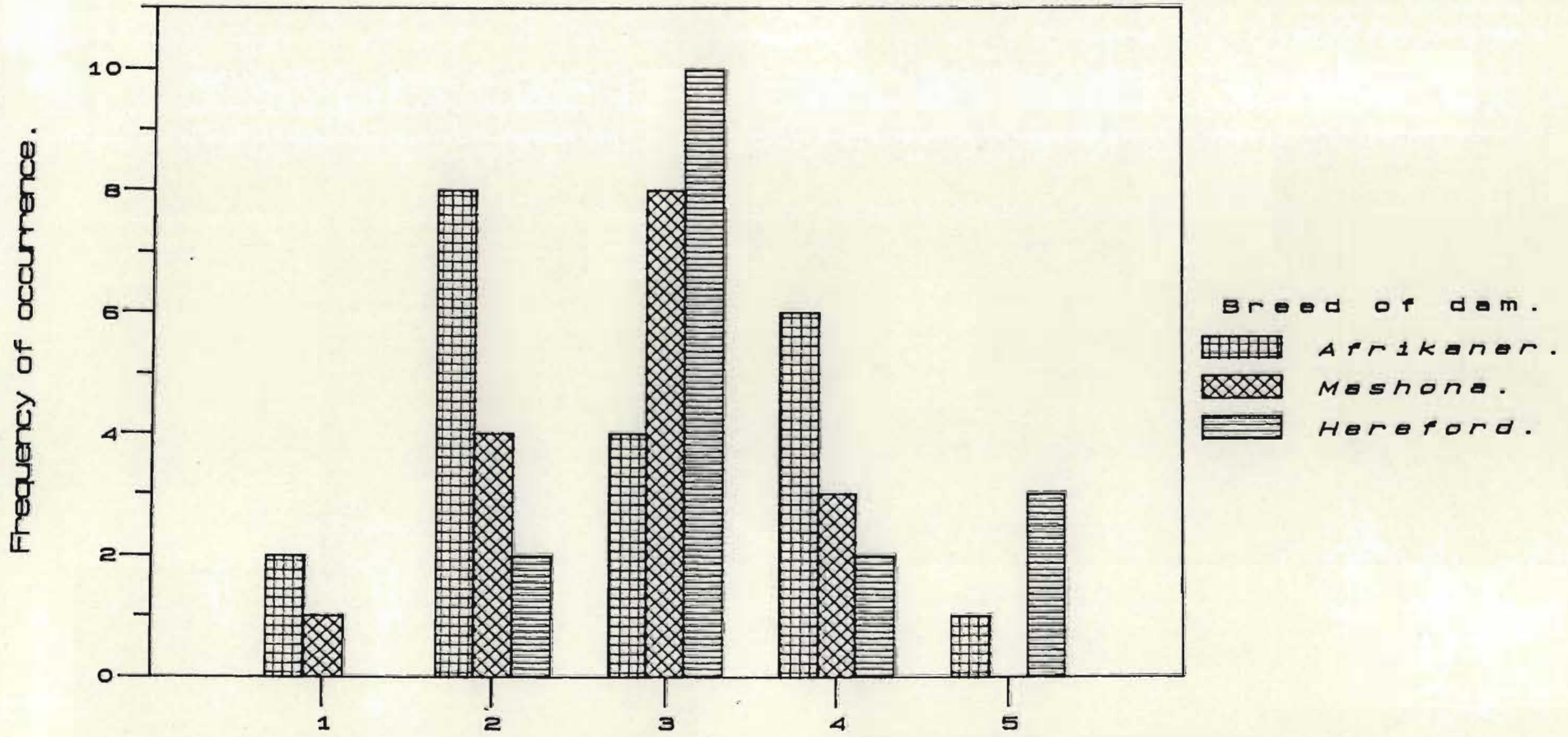
Breed effects on the number of suckling bouts per 24 hours.

Analysis of data presented in Figure 10 indicated the absence of any significant breed differences in the proportion of cows which suckled their calves from one to six times each day. In 14/56 and 22/56 observation days, cows nursed their calves two and three times per day respectively between the hours of 0500 and 1900. There was limited evidence that the Afrikaner may have a higher variability in daily suckling frequency than either the Mashona or the Hereford.

The diurnal distribution of suckling bouts

Intervals between suckling events varied according to the number of times a calf suckled during the day (Table 29). Thus, mean starting times for each suckling event were fairly regularly distributed over the day but considerable individual variation was observed between cows in the same sub-class. From the analyses presented above, extraction phase teat changes and udder butts are the two components of suckling most likely to change under different circumstances. In order to simplify this analysis only these two factors have been assessed in Table 29.

Figure 10. The influence of breed of dam on the number of suckling bouts between 0500 and 1900 hrs



No significant breed differences exist.

Table 29. The distribution of suckling bouts and patterns of behaviour with Afrikaner, Mashona and Hereford cows suckled from one to five times, between 0500 and 1900 hours each day.

	Sequence of suckling events					
	First	Second	Third	Fourth	Fifth	Signif. of diff.
Suckled once/14 hours.						
2 Afrikaner, 1 Mashona.						
Start (hours)	1312					
SE	±108m					
Range	6 hrs					
Teat changes	129±15					
Udder butts	38±9					
Suckled twice/14 hours.						
8 Afrikaner, 4 Mashona, 2 Hereford.						
Start (hours)	1006	1506				
SE	±48m	±40m				
Range	7 hrs	9 hrs				
Teat changes	127±16	158±20				NS
Udder butts	26±6	34±9				NS
Suckled three times/14 hours.						
4 Afrikaner, 8 Mashona, 10 Hereford.						
Start (hours)	0642	1112	1600			
SE	±20m	±21m	±24m			
Range	4 hrs	6 hrs	8 hrs			
Teat changes	142±9	119±1	128±10			NS
Udder butts	38±5	23±5	20±4			P<0,01
Suckled four times/14 hours.						
6 Afrikaner, 3 Mashona, 2 Hereford.						
Start (hours)	0548	0954	1406	1718 hours		
SE	±12m	±36m	±30m	±15m		
Range	2 hrs	6 hrs	5 hrs	3 hrs		
Teat changes	137±16	127±16	121±11	140±12		NS
Udder butts	47±9	31±5	20±3	104±23		P<0,05
Suckled five times/14 hours.						
1 Afrikaner, 3 Hereford.						
Start (hours)	0518	0900	1112	1506	1748	
SE	±12m	±36m	±68m	±24m	13m	
Range	1 hr	3 hrs	3 hrs	2 hrs	1 hr	
Teat changes	122±11	167±21	130±30	134±30	143±27	NS
Udder butts	35±13	25±7	17±4	20±7	24±2	NS

SE Standard error of the mean. n Size of sample. m Minutes
NS Group means on this line do not differ significantly.

Table 29 illustrates the most significant finding in this study. Regardless of the number of times a calf suckled during a 14 hour period, virtually the same amount of effort was put into each suckling bout by the calf. For this reason the intensity of suckling stimulus to which the post-partum cow is exposed is more dependent upon suckling frequency (bouts/24 hour period) than patterns of suckling behaviour at each suckling event.

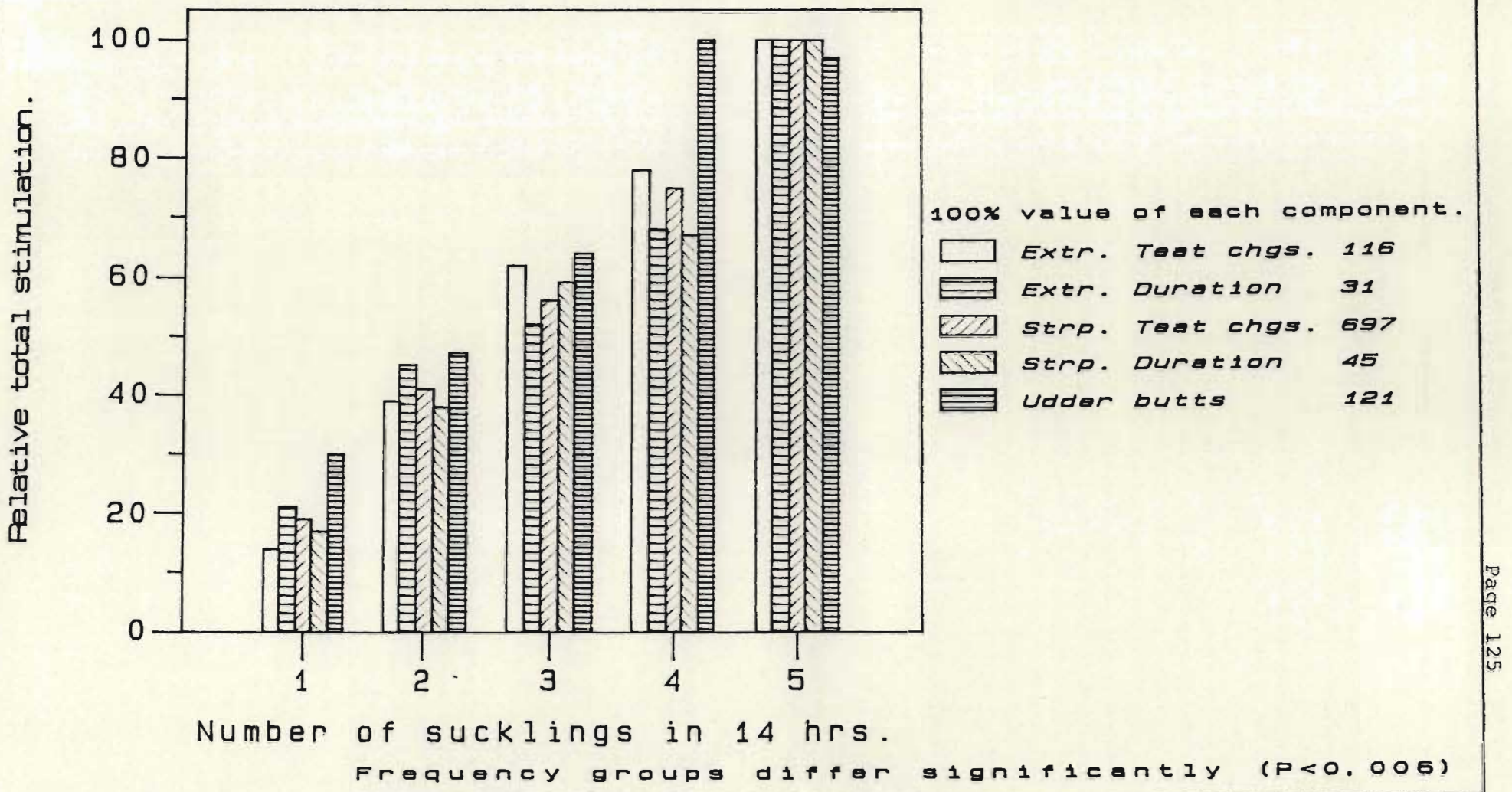
Mean total number of teat changes at each suckling did not differ significantly between calves which suckled twice per day or four times per day. Teat changes were similar for all suckling events within a day. Only the total number of udder butts per suckling session appeared to change significantly in cows suckled three ($P<0,01$) or four ($P<0,05$) times per day. In these two sub-groups udder butting was highest at the first suckling session of the day (38-47) and declined thereafter to 19-29 at the last suckling of the day.

Total diurnal suckling activity

Following the findings summarised in Table 5, Figure 11 illustrates the total stimulation experienced by cows suckled from one to six times per day. The total udder stimulation experienced by cows over the period from 0500 hours to 1900 hours differed significantly ($P<0,01$) according to the number of times cows suckled during the observation period. Cows suckled twice a day, experienced a total suckling time of 31 minutes during the 14 hour observation period, whereas in cows which were suckled four times a day this figure rose to 51 minutes.

Within these respective periods mean total teat changes ranged from 330 (suckled twice a day) to 615 (suckled four times a day)

Figure 11. Total suckling stimulation between 0500 and 1900 hrs. as a % of highest frequency.



Frequency groups differ significantly (P<0.006)

total udder butts increased from 59 to 125. For cows suckled more than four times a day, the data indicate that the amount of time spent suckling and total udder stimulation will rise linearly, yielding figures in excess of 1100 for teat changes over a total suckling period of more than 100 minutes within the hours of 0500 and 1900. Over 24 hours these figures are likely to be in the region of 60% higher again.

Suckling activity in relation to milk yield

Suckling behaviour data was classified into four categories on the basis of estimated gain in live mass of calves following suckling. These four groups covered the range from 0 to 4,2 kg of estimated milk intake (Table 30). As milk intake increased the duration of each suckling event increased significantly ($P<0,01$) from 8,7±0,34 mins (up 1 kg of milk) to 10,4±0,36 mins (from 3 to 4,2 kg of milk). Differences in amounts of milk removed from the udder at any one suckling were more readily detected by changes in suckling behaviour during the extraction phase than during the stripping phase. This was indicated by the fact that characterisitics of suckling behaviour during the extraction phase differed to a greater extent between the four categories of milk intake than during the stripping phase (Table 7).

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 3,5 seconds to 7±1 seconds. At the same time the total duration of this extraction phase increased significantly ($P<0,01$) by 1,1 minutes to 4,3±0,24 minutes. During this phase the number of udder butts did not differ

significantly 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 only 1 kg or less at a suckling.

In contrast to the extraction phase, the number of teat changes and udder butts during, or total duration of the stripping phase did not differ significantly between the four milk intake groups. Elapsed time between teat changes and udder butts differed significantly between the four levels of milk intake ($P<0,05$) but there was no trend consistent with either increasing or decreasing milk intake.

As a general rule it would appear that the calf with a good appetite suckling a high yielding dam (+3 kg per suckling) will 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. For this reason the extraction phase is longer in the higher 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 during the stripping phase than her lower yielding contemporary. The net effect appears to be similar amount of udder stimulation in high and low yielding cows.

Table 30. Patterns of suckling behaviour associated with four levels of milk intake during a single suckling bout in the Mashona cow.

Suckling component	Estimated milk production in kg 0-0,9	1,0-1,9	2,0-2,9	3,0-4,2	Signif. of diff.
Estimated mean milk prod.	0,54±0,05	1,31±0,03	2,26±0,03	3,40±0,11	P<0,0001
Extraction phase.					
Teat changes	55±5,6	64±3,1	54±2,7	44±3,9	P<0,01
Udder butts	4,5±0,72	3,1±0,34	3,1±0,48	2,9±0,84	NS
Phase duration in mins.	3,2±0,21	3,4±0,12	3,8±0,13	4,3±0,24	P<0,01
Time in seconds between teat changes	4,0±0,5	3,3±0,1	4,8±0,3	6,8±0,3	P<0,01
Time in seconds between udder butts	46,8±6,7	90,0±9,0	105,0±10,1	102,6±15,9	P<0,01
Stripping Phase					
Teat changes	131±11,5	161±7,7	161±8,7	141±20,5	NS
Udder butts	9,3±1,5	13,8±1,3	13,8±1,4	14,7±2,1	NS
Phase duration in mins.	5,56±0,40	6,52±0,19	6,31±0,27	6,08±0,47	NS
Time in seconds between teat changes	2,8±0,03	2,6±0,1	2,6±0,1	3,3±0,4	P<0,05
Time in seconds between udder butts	73,8±18,4	41,4±4,2	46,2±6,8	32,4±4,7	P<0,05
Duration in minutes of one suckling	8,7±0,34	9,8±0,16	10,1±0,16	10,4±0,36	P<0,01

NS Group means do not differ significantly.

Calf growth and the resumption of ovarian activity post-partum

Drewry *et al.* (1959) established that total suckling time and suckling frequency per 24 hours were significantly ($P<0,05$ and $P<0,01$ respectively) positively correlated to calf live mass up to 60 days *post partum*. On the strength of this finding Figures 12 and 13 were compiled to illustrate the relationship between calf growth in early lactation with post-partum interval to the resumption of ovarian activity.

The data for these two figures (from Chapters 3 and 4) clearly demonstrate the absence of any delay in the post-partum interval to first ovulation due to cows having to rear heavier calves. This finding was consistent in both Afrikaner and Mashona cows (Figure 12). In the case of the Mashona there was a very slight indication that heavier calves may have lengthened the post-partum interval to first ovulation and this could well have approached significance with a larger number of animals. Figure 13 is particularly interesting in that it indicates that in both normally suckled Afrikaner cows (suckled 4 to 6 times per 24 hours) and Afrikaner cows suckled once per 24 hours *post partum*, interval to first ovulation was still totally unaffected by calf size. This is even more surprising knowing that heavier calves suckle more frequently per 24 hours up to 30 days *post partum* than their lighter contemporaries (Drewry *et al.*, 1959). By 90 days *post partum* the situation is reversed and heavier calves suckle their dams less frequently than lighter calves (Drewry *et al.*, 1969).

Thus, it is apparent that differences in patterns of suckling frequency are not responsible for the large variation in post-partum interval to first ovulation recorded in nursing cows.

Figure 12. The influence of calf growth to 75 days of age on post-partum interval to first ovulation.

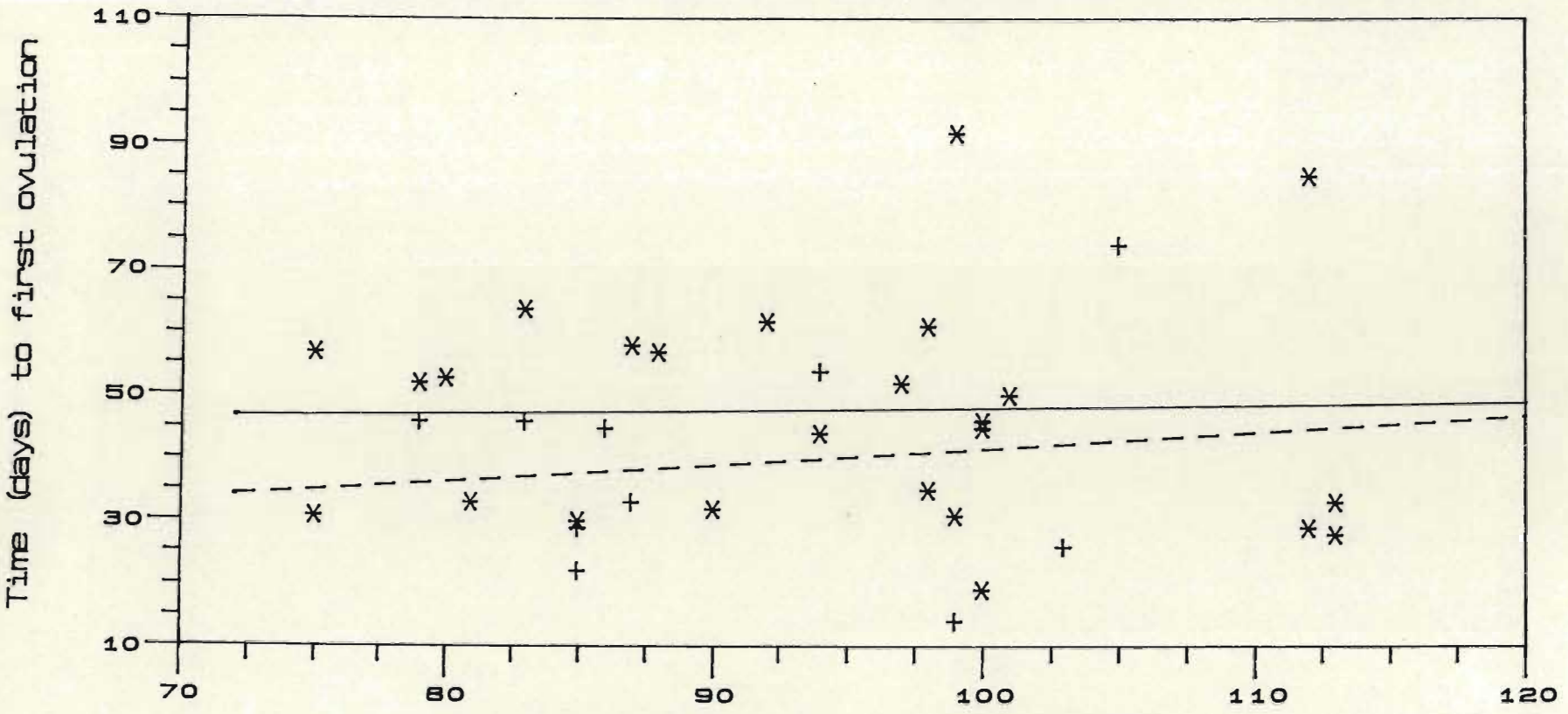
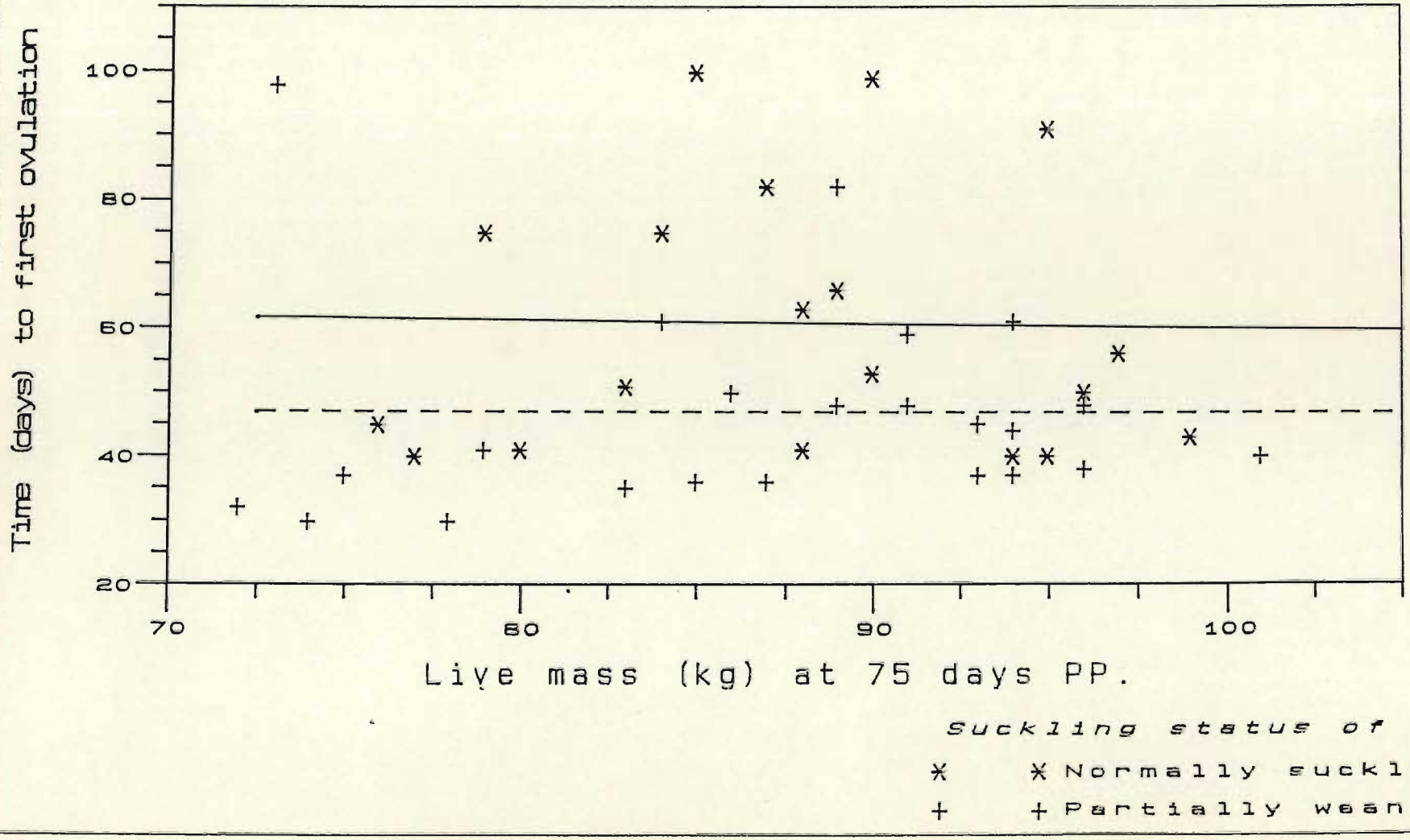


Figure 13. The influence of calf growth to 75 days of age on post-partum interval to first ovulation.



DISCUSSION

No references have been found describing in detail, patterns of behaviour within a single suckling event. The two distinct phases which have been identified in this study have been described as phase one or the extraction phase (mean duration in this study of 5,9±0,3 minutes with a total of 24±2 teat changes) and phase two or the stripping phase 8,5±0,3 minutes during which a total of 135±4 teat changes were recorded.

Calves changed teats and butted the udder less often and more slowly whilst milk was removed easily from the udder during the extraction phase. With a change in pattern of behaviour marking the transition from the extraction to the stripping phase the rate of removal of milk from the udder is presumed to have decreased markedly. Thus, during the stripping phase when the milk was not being quickly removed, calves butted the udder more often and more quickly with increasing yield in the dam.

The terms extraction phase and stripping phase were supported by the finding that milk yield differences modifed suckling behaviour during the extraction phase to a greater degree (P 0,01) than during the stripping phase ($P < 0,05$). This is probably a consequence of greater vigour and persistence of calves of higher milk producing dams. Since the intensity of any 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, the trends observed across different levels of milk production within any suckling event are of no significance in reproductive function *per se*. The only point of interest will be the correlation between these patterns

of behaviour and either total suckling time or frequency of suckling bouts per 24 hours.

Extending this argument further one might hypothesise that any depression of reproductive function attributable to suckling is more likely to be observed in cows weaning lighter calves provided that cows suckling faster growing calves are not under any nutritional stress. In instances where cows are in a negative energy balance due to high lactation demands then prolonged post-partum anoestrous is more likely to be observed in cows weaning heavier weaners.

Figures 12 and 13 showed conclusively that no significant correlation existed between live mass of calf at 75 days post partum (a function of milk yield and calf appetite) and post-partum interval to first ovulation. This is somewhat surprising in the light of the substantial improvements in post-partum reproduction following partial and complete weaning. The literature clearly shows that heavier calves suckle less frequently than their slower growing contemporaries (Drewry et al., 1959; Odde et al., 1985). However this benefit of reduced total suckling stimulation might be balanced by the increased metabolic demands of higher milk production. Partial weaning (suckling once a day) represents at least a 70% to 80% decrease in total suckling stimulus whereas differences in total suckling stimulus in relation to milk production and calf growth are unlikely to exceed 20%. Nicol and Sharafeldin (1975) reported that each additional minute of total suckling time per 24 hours would increase daily gain by 0,05 kg/day. However it would appear (Figures 12 and 13) that this increased daily gain will have no effect on post-partum interval to the resumption of

ovarian activity.

The mean duration of a suckling bout of 14,4 minutes in the present study was longer than that reported elsewhere (8,4 to 10,2 minutes, Drewry *et al.*, 1959; 10,4 minutes, Wagnon, 1963; 11 minutes, Nicol and Sharafeldin, 1975; 9 minutes, Odde *et al.*, 1985). Other work in *Bos indicus* by Reinhardt and Reinhardt (1981) estimated the mean duration of suckling to be from 8 to 10 minutes long and in conjunction with the results for Mashona cows suckled twice a day (Table 30), would indicate that the longer duration of suckling in Afrikaner and Mashona cows is not a consistent trait of *Bos indicus* cattle.

Cole and Johansson (1933) reported that milk production in Aberdeen-Angus cows reached a maximum 28 days after calving whilst the duration of a suckling event reaches a maximum 40 to 60 days *post partum* and remains constant to 120 days after calving (Nicol and Sharafeldin, 1975). The Afrikaner cows in this study reached a peak ($P<0,05$) in the number of teat changes (182) 53 days after calving, at a level which was significantly higher ($P<0,05$) than either Mashona (134) or Hereford cows (142, Table 28). After this time suckling activity within a suckling event declined in all three breeds. No such peak was recorded by Reinhardt and Reinhardt (1981) working with East African Zebu. Since duration of suckling is closely related to milk yield (Table 30) the absence of any significant changes in duration of suckling might be a consequence of the absence of a peak in milk yield in the East African Zebu.

Butting of the udder by calves during suckling appeared to vary widely between calves, as the ranges for this parameter in Table 26 illustrate. There was some evidence to indicate that udder butting was most prevalent amongst calves of Afrikaner cows and least prevalent amongst calves of Mashona cows. The results from Table 30 would indicate that this is a result of higher milk production in the Afrikaner than either the Mashona or the Hereford. But since calf genotype is confounded with breed of dam, this hypothesis cannot be verified. Genotype has been shown to influence suckling behaviour and therefore this question will remain unanswered until calves of the same genotype can be fostered onto different breeds of cow.

At 29-36 days post partum suckling activity did not differ significantly between Afrikaner and Mashona cows. As lactation progressed suckling activity, particularly second phase teat changes in Afrikaner cows was highest 53±1 days post partum. If this finding is a feature of the breed in general, then this increase in suckling activity coincides with the mean post-partum interval to first full ovulation in suckled Afrikaner cows (Tables 1 and 6).

Although total suckling stimulus does not appear to be correlated to the resumption of ovarian activity, this does not preclude the possibility that the suckling stimulus may alter conception rates via its effects on gamete transport (Vandemark and Hays, 1952) and possibly uterine environment following coitus. Results from Chapters 4 and 5 indicate that conception rates in the Afrikaner are highest after 50 days post partum, irrespective of post-partum interval to the onset of ovarian activity. The fact that this finding is consistent in suckled and non-suckled cows

minimises the possible role of suckling at the time of conception.

The limited data available in this study indicate that a large variation exists in the number of times cows will allow their calves to suckle in one day. Wagnon (1963) reported that 83% of suckling periods were initiated by the calf with the remainder being initiated by the mother calling its calf. In this study the range was one to six, between the hours of 0500 and 1900. Interestingly there were no significant breed differences in the proportions of cows which suckled their calves from one to six times on any one observation day. Considering the total suckling activity as presented in Figure 11, it is apparent that total udder stimulation (in terms of percentage change in each of the components of suckling) in one day is more a function of number of sucklings per day than variations in patterns of suckling activity at single sucklings. It was interesting to note the change in the relationship between suckling frequency and calf mass. Up to 30 days of age the two are positively correlated with the larger calf suckling more often than the smaller one whilst at 90 days of age a negative correlation exists with the heavier calf suckling less frequently but removing more milk from the udder (Drewery et al., 1959).

From this study 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 side whilst calf appetite (a function of growth potential) and suckling persistence are driving forces on the calf's side contributing to differences in suckling

behaviour. With 85% of suckling events being initiated by the calf (Wagnon, 1963) and milk production being limited by calf appetite (Gifford, 1949 and 1953), suckling behaviour appears to be more a function of calf genotype than milking potential of the dam.

CONCLUSIONS

Since the majority of Afrikaner and Mashona cows suckled their calves two, three or four times during daylight hours, the number of suckling bouts per 24 hours was estimated to be in the region of four to eight. Reducing suckling access to once each day then represents a reduction in total udder stimulation of approximately 80%. By comparison the variation between cows and between breeds of cow in total udder stimulation within a single suckling event is somewhere in the region of 20%. Afrikaner cows and Mashona cows appear to differ very little in their patterns of suckling activity. Yet the reproductive performance between these two breeds is very different. These findings and the lack of covariance in calf mass at 75 days post partum and post-partum interval to conception confirm that either suckling activity with unrestricted levels of suckling intensity is not related to ovarian function after calving in the Afrikaner and Mashona, or the effects of suckling activity on endocrine events operate on different thresholds of sensitivity in the Mashona and Afrikaner breeds.

Statistically valid estimates of differences between cows in sensitivity to the suckling stimulus could only be investigated by the observation of calves of a similar genotype which were totally independent of the dam's genotype. This could only be achieved with fostering of calves or the use of embryo transfer.

Chapter 8

Characteristics of oestrous behaviour in Afrikaner and Mashona cows which were either normally suckled for the duration of the trial or weaned three days post partum.

INTRODUCTION

For conception to occur, essential cow - bull interactions must precede fertilisation. Cows and bulls should pair prior to ovulation and initiate behaviour which will culminate in insemination of the cow by the bull.

A survey of the literature indicates that studies in bovine reproduction have been primarily aimed at physiological mechanisms in the cow. Because successful natural insemination is of such fundamental importance in reproduction, the first aim of this study was to characterise the sequence of events associated with oestrus in the Afrikaner.

Although endocrine and behavioural components of reproduction are closely linked, very few published studies have attempted to present both the endocrine aspects of the resumption of ovarian activity (monitored by means of plasma progesterone changes) as well as the details of all oestrus-related behaviour in the post-partum cow. In this series of trials this was done and a discussion of the ovarian component of the behaviour data to be reported in this chapter has already been presented in Chapter 3.

Complete weaning of Afrikaner cows significantly ($P < 0,001$) reduced the post-partum interval to first full ovulation by 27 days (Table 2). Although mean post-partum interval to conception was shortened ($P<0,001$) to 47 ± 3 days (Table 3), it would appear

that the probability of conception to these early ovulations was lower than that associated with ovulations occurring after 50 days post partum. The possibility exists that the lower incidence of successful conception to these early ovulations was caused by an absence of service prior to these early ovulations. For this reason the second objective of this study was to establish if significant differences in oestrous behaviour existed between suckled and non-suckled Afrikaner cows prior to primary and first full ovulations. The incidence of natural inseminations at these ovulations was clearly of fundamental importance.

Weaning calves from anoestrous cows has been reported to induce oestrus in 76% of cows within 10 days of treatment being applied (Odde et al., 1980; Ramirez-Godinez et al., 1981). The incidence of conception failure to these first ovulations is in the region of 84% (Ward et al., 1979). This evidence would indicate that conception failure to primary ovulations is limited by post-insemination problems rather than any behavioural deficiencies. The present study would confirm whether or not this also applied to Afrikaner and Mashona cows.

The approach of oestrus is signalled by a decline in progesterone levels (one to three days prior to oestrus), the release of oestrus-specific odours (beginning three days prior to oestrus) and an increase in oestradiol-17-beta concentrations (24 hours prior to the start of oestrus) (Henricks et al., 1971; Kiddy et al., 1978; Kiddy and Mitchell 1981). Since none of these changes are readily detected by man, changes in animal behaviour offer the only real hope of accurately identifying the approach of oestrus. From this view the question arises as to how much

information about the reproductive status of the cow exists in subtle changes in behaviour.

The classic definition of oestrus as being the period when cows were observed to be in standing heat was inadequate in this study for three reasons. Firstly, with treatment groups of 30 to 40 cows and given the limited duration of the study (3 to 100 days after calving on an individual cow basis, with the possibilty of protracted post-partum anoestrus) the probability of two or more cows being in standing heat at the same time was low. Secondly, earlier observations in Afrikaner cows (not reported in this thesis) indicated that homosexual activity occurred in less than 20% of ovulations, and thirdly, the number of services per oestrus was usually two or less.

MATERIALS AND METHOD

Experimental animals

The data reported in this chapter was compiled from observations of experimental cows used in the study reported in Chapter 3. All 80 experimental cows were run in one of two groups. In the normally suckled control group 30 Afrikaner cows and 10 Mashona cows comprised one herd and 30 Afrikaner cows and 10 Mashona cows whose calves had been weaned three days post partum comprised the weaned herd.

Observation of oestrous behaviour

All cows were monitored continuously between 0500 hours and 1900 hours each day. For each treatment group, two observers recorded all oestrus related behaviour including: cows forming associations with the bull prior to oestrus (cow-bull pairing); cows mounting other cows (homosexual mounting); cows standing to

be ridden by other cows (homosexual standing); cows mounting the bull (heterosexual mounting) and service.

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-squared test and Fisher's Exact Test were used to assess treatment effects on the incidence of specific components of oestrous behaviour (Rayner, 1967).

RESULTS

Ovarian acitivity

The number of anovulatory cows in each treatment was not altered by breed or suckling status and therefore the total number of full ovulations was similar between treatments within each breed (102 ovulations in 60 Afrikaner cows and 36 ovulations in 20 Mashona cows). Despite a similar number of opportunities (ovulations) for conception in each treatment group, removing the suckling stimulus improved conception rates by 30% (Table 31). The lower conception rates in suckled cows was due almost entirely to the failure of conception in cycling cows (11/30, Table 31). A comparison of the number of ovulations in relation to the number of conceptions indicates that conception failure to early ovulations was common in both suckled and non-suckled cows. This situation provided ample opportunity to assess whether this was a behaviour problem or an endocrine one.

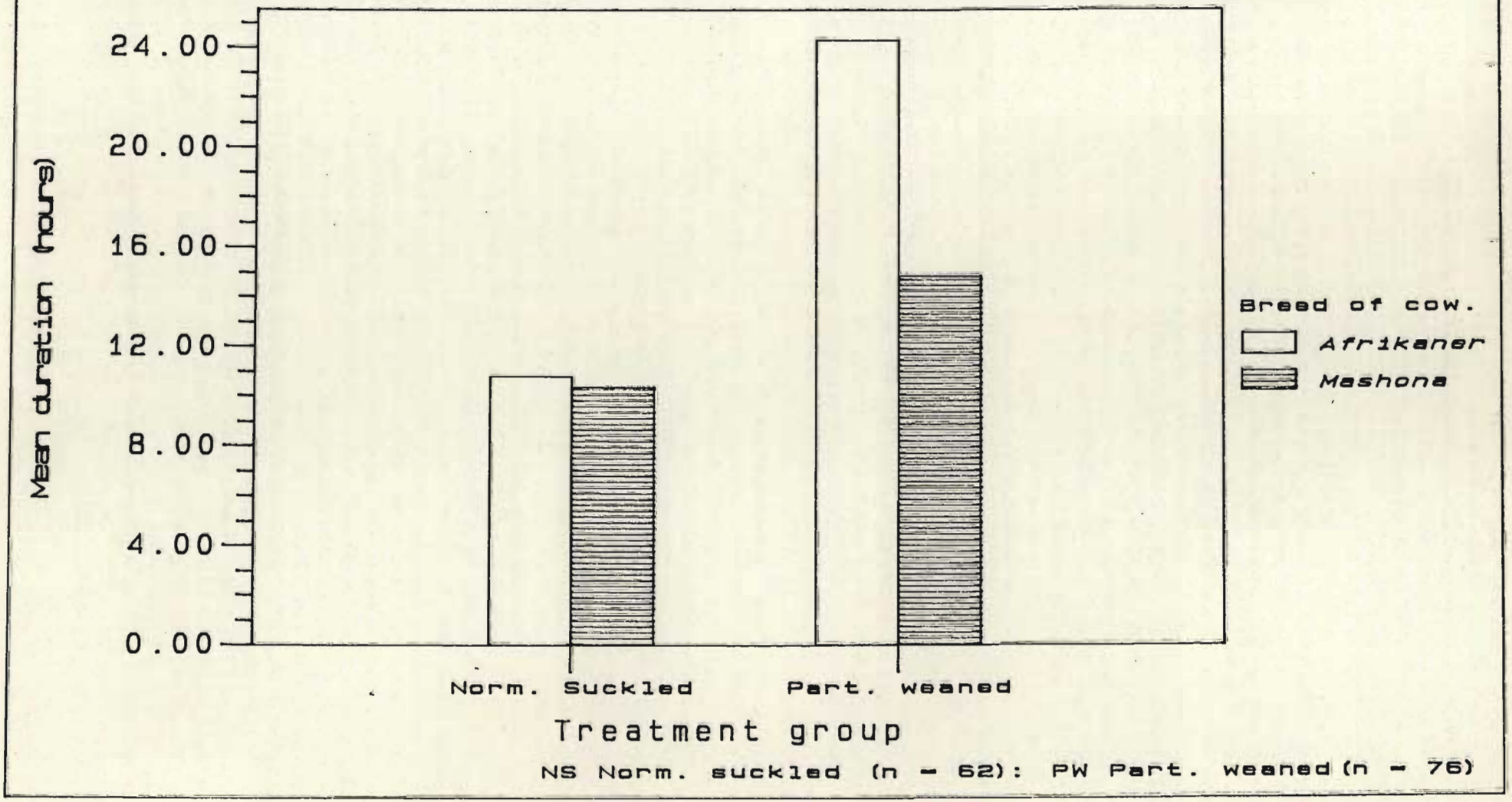
Table 31. The incidence of full ovulations within 100 days of calving in Afrikaner and Mashona cows which were either normally suckled or weaned three days after calving.

Breed	Afrikaner		Mashona	
Suckling status	Control	Weaned	Control	Weaned
Number of cows	30	30	10	10
Cows anovulatory for 100 days	4	1	1	0
Total number of full ovulations	46	56	16	20
Number of first full ovulations	25	28	9	10
Number of conceptions	15 P<0,01	24	9	10

Duration of pro-oestrus and oestrus

Non-suckled Afrikaner cows spent more more than twice as much time associating with a sexually active group (with or without the bull) during oestrus than their normally suckled contemporaries (Figure 14). Although a similar trend was apparent in the Mashona cows the difference was not as marked. The durations illustrated in Figure 14 do not include any nocturnal observations and for this reason the non-suckled cow clearly shows some evidence of oestrus for as long as 36 hours.

Figure 14. Mean total time (hours) during which cows in oestrus were with a sexually active group.



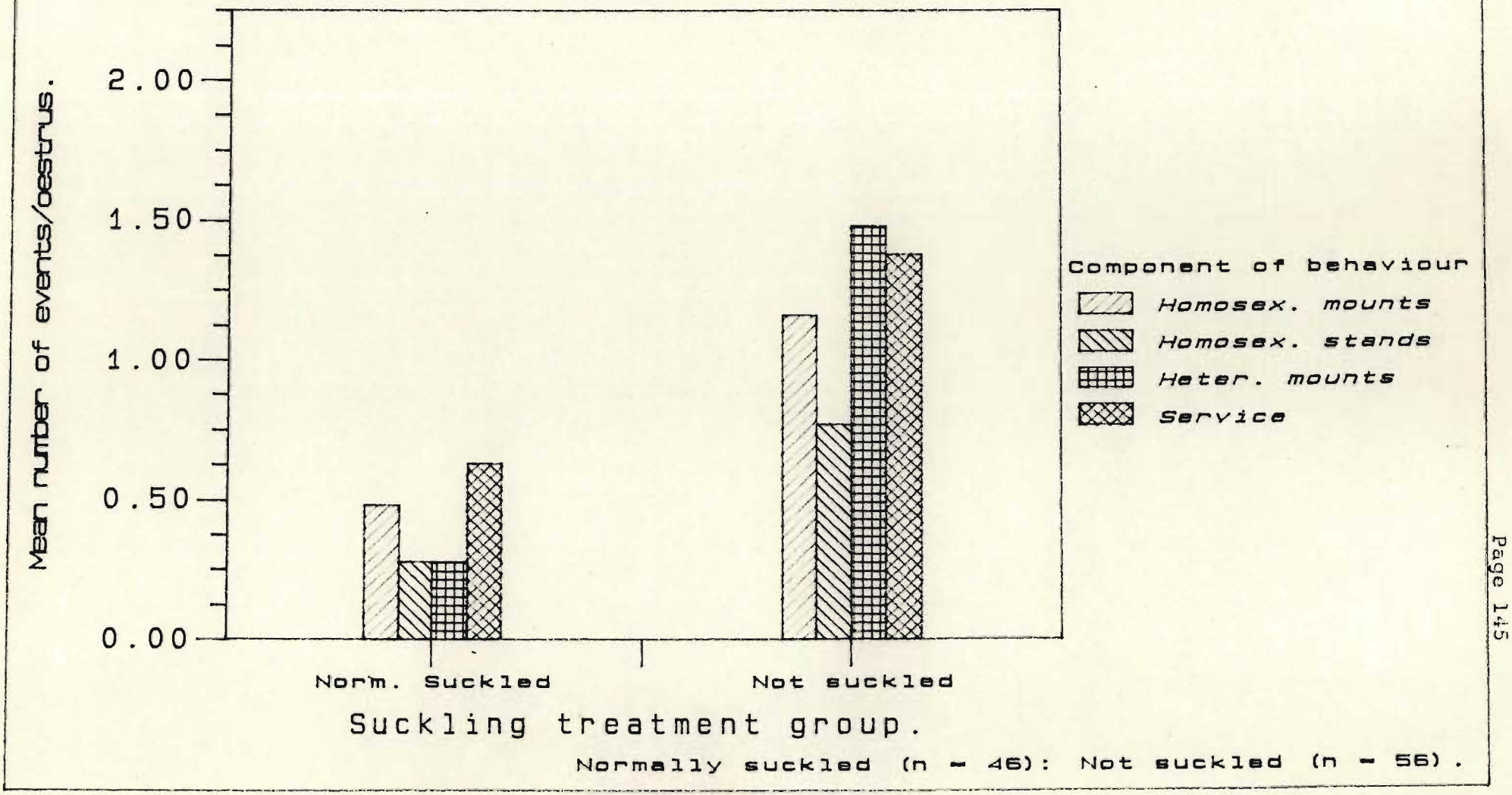
Intensity of expression of components of oestrus behaviour

Using the ratio of the number of events of each component of oestrus per ovulation as an indicator of the intensity of expression of oestrus, Figures 15 and 16 illustrate how the removal of the suckling stimulus increased the mean activity during oestrus in both Afrikaner and Mashona cows. The most striking feature of these results was the low intensity of oestrus in suckled Afrikaner cows. These data (Figure 15) indicated that on average less than half of all oestrous periods have one homosexual mount or stand or one heterosexual mount by a cow. In the absence of suckling, Afrikaner cows mounted the bull more frequently during oestrus ($P<0,01$) compared to normally suckled cows.

Non-suckled Mashona cows in oestrus may have been mounted more frequently by herd mates who were not in oestrus than normally suckled cows (Figure 16). Since Afrikaner and Mashona cows in the same treatment group were run as a single herd the results indicate that Afrikaner cows in oestrus mounted Mashona cows in oestrus in preference to other Afrikaner cows.

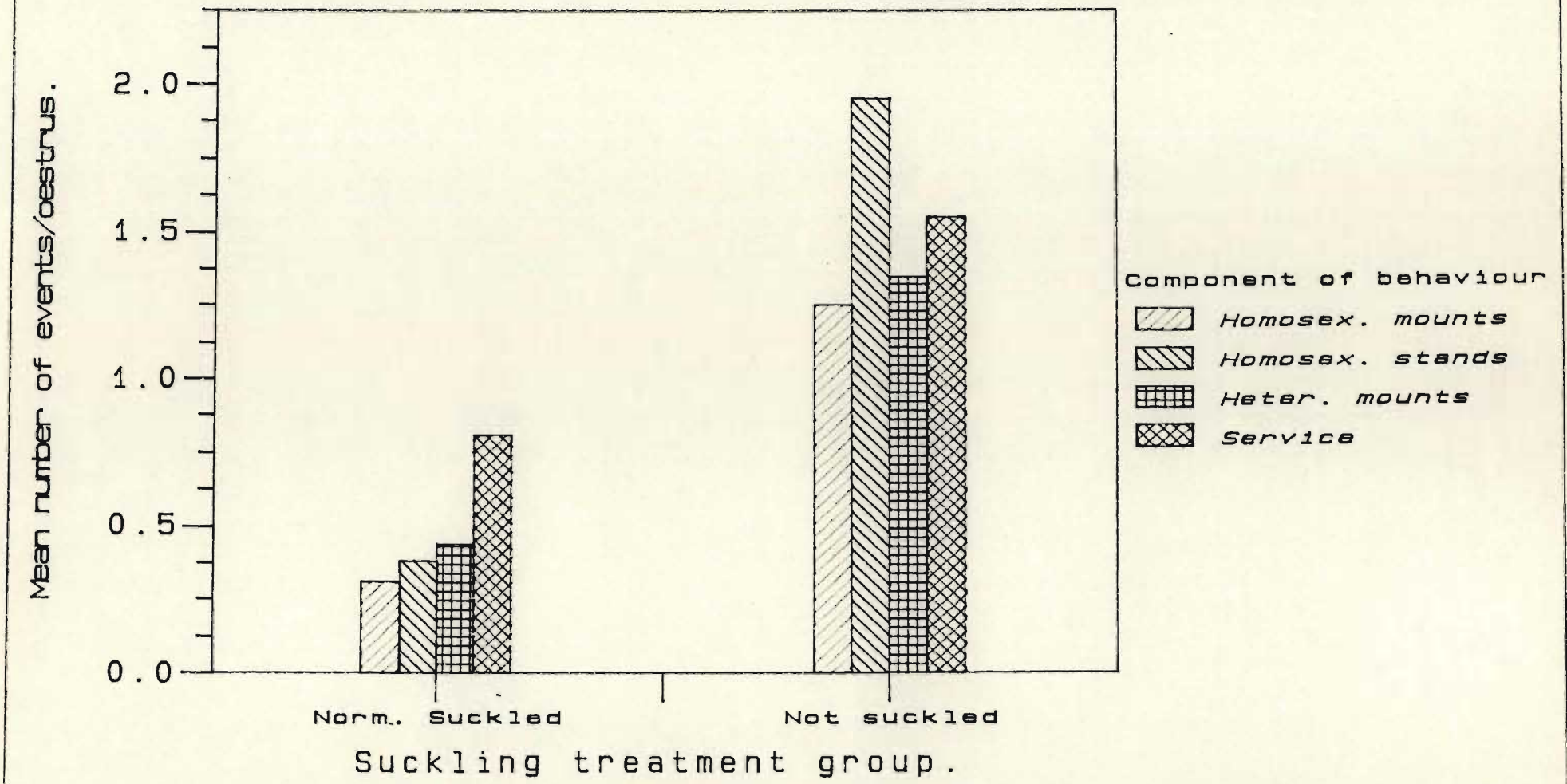
Despite the notable differences in the reproductive performances of Afrikaner and Mashona cows, the removal of the suckling stimulus enhanced the intensity of oestrus in both breeds.

Figure 15. The mean incidence/oestrus of each type of behaviour in control and weaned Afrikaner cows.



Normally suckled (n = 46): Not suckled (n = 56).

Figure 16. The mean incidence/oestrus of each type of behaviour in control and weaned Mashona cows.



Normally suckled (n = 16): Not suckled (n = 20).

Incidence of observed service prior to ovulation

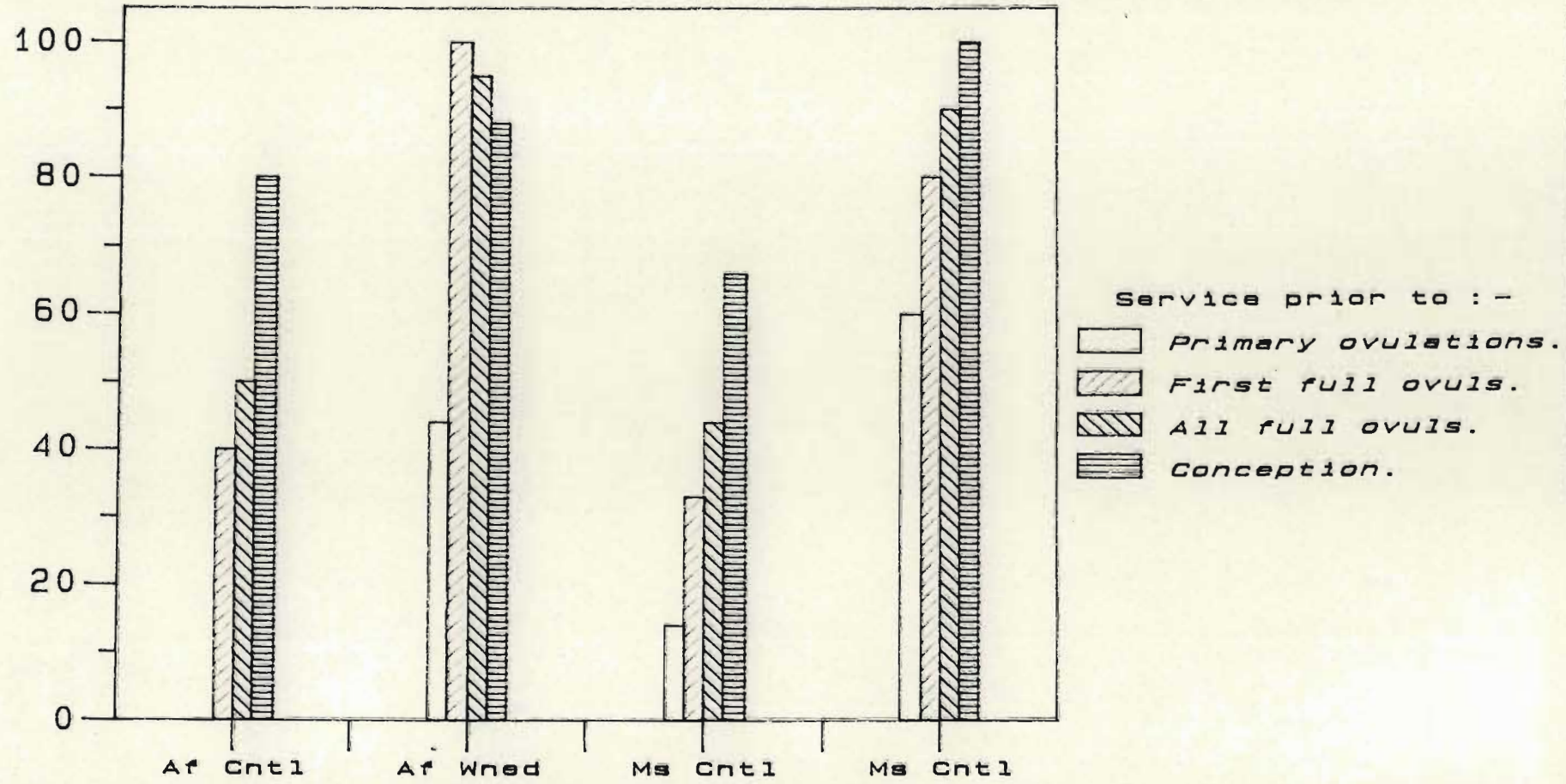
Of 498 recorded periods including homosexual and heterosexual behaviour, 150 services were recorded in this study. With surveillance of herds being confined to 0500 to 1900 hours the percentage of observed services which corresponded to conception was unexpectedly high (better than 80%). This indicated that 15% of conceptions in this study occurred during the night between the hours of 1900 and 0500 hours (Figure 17).

In the absence of suckling a significantly ($P<0,001$) higher proportion of primary ovulations were accompanied by observed service in both Afrikaner and Mashona cows (Figure 17). This higher ($P<0,01$) incidence of observed service with ovulations in weaned cows was also observed with all subsequent full ovulations. However, the lower incidence of observed service in suckled cows might have been caused, in part, by a higher proportion of lactating cows being served at night. In normally suckled Afrikaner and Mashona cows only 50% of full ovulations were accompanied by observed service which would mean that a percentage of conception failures in suckled cows in these groups might be attributed to a behavioural deficiency.

Principal characteristics of components of oestrus behaviour

A total of 115 records (one record pertaining to one day of behaviour) were available which included observed service. From this data base the next three tables (Table 32 to 34) were compiled to illustrate the temporal relationships between homosexual behaviour and heterosexual mounting to first service as well as the number of events and duration of each type of behaviour during a single oestrus.

Figure 17. The incidence of observed service prior to primary, first and all full ovuls. and concept.



Cntl - Control Group: Wned - Weaned 3 days post p.

Table 32. The incidence, duration and interval between homosexual mounting and first service in Afrikaner and Mashona cows which were either normally suckled or weaned three days post partum.

Breed	Afrikaner		Mashona	
Treatment	Control	Weaned	Control	Weaned
Group number	30	30	10	10
Full ovulations	46	56	16	20
Incidence	7/23	9/61	2/10	5/21
Number mounts per oestrus	2,1±0,67	1,8±0,36	1,5±0,5	1,8±0,2
Range	1-6	1-4	1-2	1-2
Duration of multiple mounts	2,4±0,90 (4)*	2,1±0,52 (4)	(1)	5,0±2,28 (4)
Range	1,0-5,0	1,0-3,5		1,0-10,5
Interval from first homosexual mount to first service	1,3±1,20	1,8±0,67	1,0±0,67	1,0±0,5
Range	7,0 to +2,0	5,5 to +1,5	1,5 to +0,5	12,5 to 1,0

* The number of oestrus periods during which two or more mounts were recorded.

Homosexual mounting

The number of homosexual mounts did not differ significantly between breeds or treatments and only 20% of services were accompanied by this type of oestrous behaviour. Neither duration of homosexual mounting (range 1,0 to 10,0 hours) nor interval from first mount to first service (range 1,5 hours before first service to 2,0 hours after first service) differed significantly between breed of cow or suckling status.

Table 33. The incidence, duration and interval between homosexual standing and first service in Afrikaner and Mashona cows which were either normally suckled or weaned three days post partum.

Breed	Afrikaner		Mashona	
Treatment	Control	Weaned	Control	Weaned
Group number	30	30	10	10
Full ovulations	46	56	16	20
Incidence	7/23	14/61	3/10	7/21
Number of stands per oestrus	1,6±0,30	2,0±0,42	2,0±,58	2,5±0,65
Range	1-3	1-5	1-3	1-6
Duration of multiple mounts	2,5±1,32 (3)*	4,5±1,69 (5)	1,5±0,50 (2)	3,5±1,5 (4)
Range	5,0-10,5	2,0-10,5	1,0-2,0	1,0-7,0
Interval from first homosexual stand to first service	0,2±0,32	0,4±0,83	+0,3±1,92	2,7±1,45
Range	1,0 to +1,0	10,0 to +4,0	2,5 to +4,0	12,5 to +0,5

* The number of oestrus periods during which two or more stands were recorded.

Homosexual stands

Cows were observed to stand for cows in only 27% of oestrus periods. This very low incidence of this component of behaviour was similar in Afrikaner and Mashona cows and remained unaffected by suckling status of cow. Although cows standing for other cows was observed from 1 to 6 times per oestrus a mean of 2 mounts per oestrus was recorded over the whole study. Neither the duration (range 1,0 to 10,0 hours) nor the interval from first stand to first service (range from 10,0 hours before to 4,0 hours after) differed between treatment groups.

Table 34. The incidence, duration and interval between heterosexual mounting and first service in Afrikaner and Mashona cows which were either normally suckled or weaned three days post partum.

Breed	Afrikaner		Mashona	
Treatment	Control	Weaned	Control	Weaned
Group number	30	30	10	10
Full ovulations	46	56	16	20
Incidence	7/23	25/61	4/10	12/21
Number of mounts per oestrus	1,3±0,18	1,8±0,26	1,8±0,25	1,7±0,41
Range	1-2	1-7	1-2	1-6
Duration of multiple mounts	5,8±4,8 (2)*	2,8±0,41 (12)	1,3±0,44 (3)	5,1±2,25 (4)
Range	1,0-10,5	2,0-7,0	0,5-2,0	0,5-10,5
Interval from first heterosexual mount to first service	4,5±2,16	2,9±0,52	2,1±1,0	3,3±1,17
Range	14,0 to 0,5	10,5 to 1,5	5,0 to 1,0	13,0 to +1,0

* The number of oestrous periods during which more than one mount was observed per oestrus.

Heterosexual mounts

The incidence of heterosexual mounts in conjunction with observed service was at 42% (48/115) the most common component of oestrous behaviour recorded apart from service. As with the previous components of oestrus, neither breed nor treatment had any significant effect on characteristics of heterosexual mounting recorded in this study. Cows mounted the bull from 1 to 7 times per oestrus over a period of from 0,5 to 10,5 hours with the interval from first mount by the cow to first service varying widely from 14,0 hours prior to first service to 1,0 hour after first service.

Table 35. The incidence and duration of multiple services in in Afrikaner and Mashona cows which were either normally suckled or weaned three days post partum.

Breed	Afrikaner		Mashona	
Treatment	Control	Weaned	Control	Weaned
Group number	30	30	10	10
Full ovulations	46	56	16	20
Conceptions	15	24	9	10
Incidence of single and multiple services				
Incidences of one service per oestrus	13	37	5	5
Primary ovulation	0	6	1	2
First full ovulation	7	16	2	6
Conception	7	13	3	8
Incidences of two services per oestrus	8	23	5	4
Primary ovulation	0	2	2	1
First full ovulation	3	7	3	2
Conception	4	7	3	2
Duration of double service period in hours				
Mean	2,06±0,42	2,43±0,52	2,0±1,04	0,88±0,24
Range	1,0-4,0	0,5-12,5	0,5-6,0	

Incidence of multiple services during oestrus

Suckled cows were seen to mate less often than non-suckled cows ($P<0,05$, Table 32). This supports the results in Figure 14, which illustrated the enhanced general oestrous activity in non-suckled cows. In the absence of suckling the proportion of oestrous

periods during which cows were served more than once was similar in suckled 38% (8/21) and non-suckled 38% (23/60) Afrikaner cows (Table 35). In Mashona cows the incidence of double service during oestrus was slightly higher at 50% although this difference was not significant. Again there was an indication that non-suckled Mashona cows were served more often during oestrus than suckled Mashona cows.

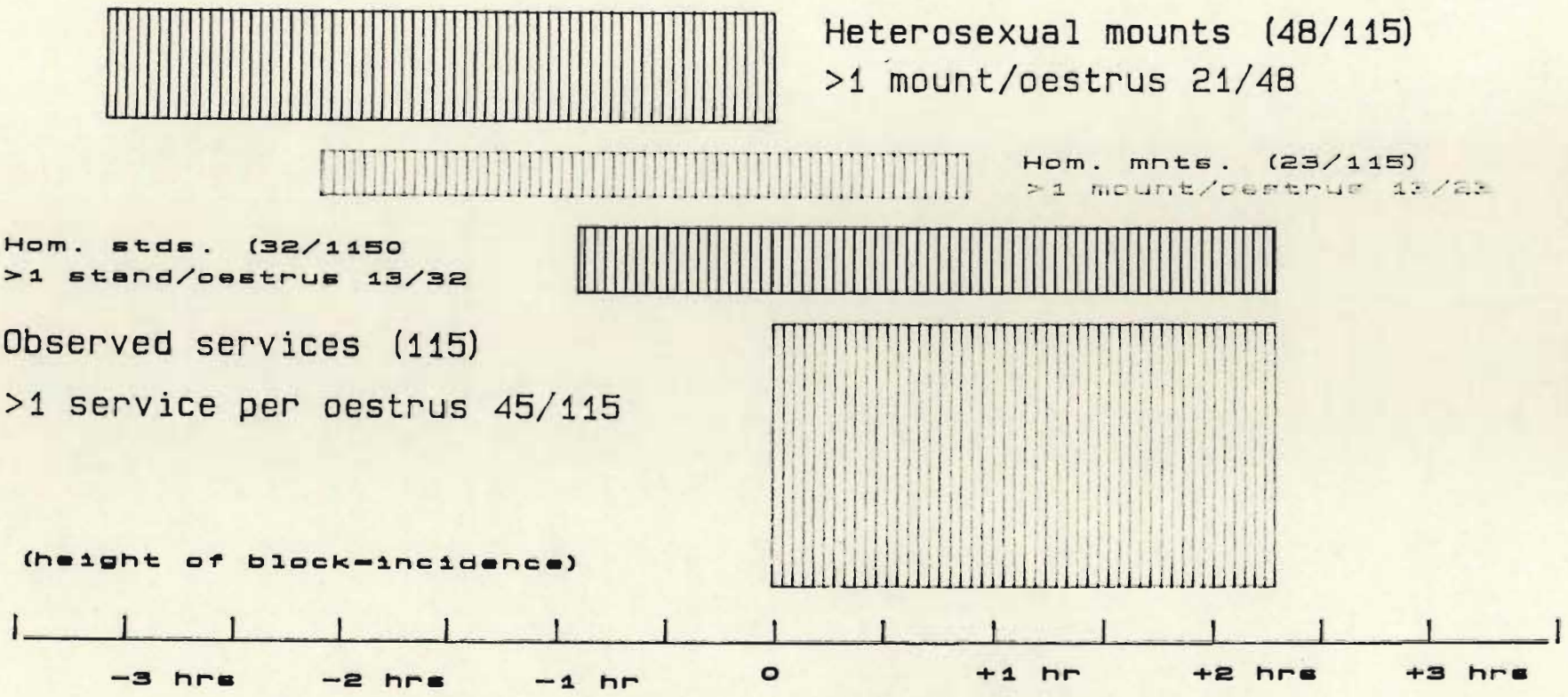
Where more than one service was observed the interval from first to last service was generally 2 hours with a range of from 30 minutes to 12 hours (Table 35).

A general pattern of oestrus

Cows mounted the bull in conjunction with observed service more often (42%) than any other component of oestrus (Figure 18). The first mount occurred 3,2±0,5 hours before the first service and the bull was mounted 1,7±0,2 times. Cows stopped mounting the bull when they were served for the first time. Homosexual mounting was generally observed before homosexual standing. In all of these components of mounting behaviour, activity was very low with only one to two examples of each type of behaviour being observed during an oestrus. The final notable point was the cessation of all oestrous activity with the last service. The summary in Figure 18 indicates that oestrus as defined by standing heat lasted about 4 hours with cows mounting the bull for an hour before oestrus.

With the low incidence of homosexual behaviour recorded in this study, the classical concept of "standing oestrus" is inadequate in some cases and a less specific definition is required.

Figure 18. A summary of the incidence , time of onset and duration of each component of oestrous behaviour recorded with observed service.



Pro-oestrus behaviour

From the behaviour records compiled during this study, it was clear that certain cows displayed evidence of an impending oestrus up to 2 days before ovulation. Tables 36 and 37 show that cow-bull pairing will commence as many as two days prior to oestrus in up to 50% of cows. In general, no interactive behaviour (mounting and standing) within these bull-pairing groups occurred on the days preceding ovulation and oestrus.

Table 36 and 37 differ from those presented above in that they have included all observations of oestrous behaviour. To some extent this will have included incidental activity exhibited by cows in di-oestrus . The findings derived from this larger data base support earlier results which showed that in both Afrikaner and Mashona cows, significantly more interactive behaviour was recorded in non-suckled post-partum cows prior to primary ovulation than in their normally suckled contemporaries. However, prior to first full ovulation, homosexual and heterosexual activity did not differ significantly in suckled and non-suckled cows. This contrast follows a trend in luteal function recorded in Chapter 3 when, following primary ovulation non-suckled cows had a significantly higher luteal output than suckled cows. However following their first full ovulation there were no apparent differences in luteal function between the two groups. Reduced suckling appears to exert its most noticeable effects on behaviour and endocrine function at the resumption of ovarian activity.

Table 36. A summary of all oestrus-related behaviour on the day of ovulation and for two days preceding ovulation in Afrikaner cows which were either normally suckled or weaned three days after calving.

	Primary ovulation		First full ovulation		Conception	
	Control	Weaned	Control	Weaned	Control	Weaned
Day of ovulation						
Sample number	1	13	17	27	11	20
Homosexual						
Mounts	0	1	3	4	1	2
Stands	0	4	0	6	2	1
Heterosexual						
Cow+bull	5,0	4,4±0,7	6,3±1,0	5,0±0,8	6,1±1,0	7,6±1,0
Mounts	0	6	6	8	1	3
Services	0	7	8	19	4	14
Day before ovulation						
Sample number	0	5	8	8	5	12
Homosexual						
Mounts	0	0	0	0	0	0
Stands	0	0	0	1	0	0
Heterosexual						
Cow+bull	0	1,8±0,6	5,9±1,6	6,4±1,4	8,6±2,1	6,5±1,3
Mounts	0	0	1	3	0	1
Services	0	0	0	0	0	0
2 days before ovulation						
Sample number	0	5	1	4	3	9
Homosexual						
Mounts	0	2	0	0	0	0
Stands	0	0	0	0	0	1
Heterosexual						
Cow+bull	0	2,3±0,8	0	5,2±2,7	10,1±3,8	6,2±1,5
Mounts	0	2	0	0	0	0
Services	0	0	0	0	0	0

Table 37. A summary of all oestrus-related behaviour recorded on the day of ovulation and on the two days preceding ovulation in Mashona cows which were either normally suckled or weaned three days after calving.

	Primary ovulation		First full ovulation		Conception	
	Control	Weaned	Control	Weaned	Control	Weaned
Day of ovulation						
Sample number	1	8	7	10	5	6
Homosexual						
Mounts	0	2	1	3	1	2
Stands	0	3	2	4	1	2
Heterosexual						
Cow+bull	3,0	4,3±0,9	4,7±1,1	5,6±1,4	3,9±0,8	7,6±1,6
Mounts	0	5	1	5	1	2
Services	0	5	4	8	4	4
Day before ovulation						
Sample number	1	3	5	4	1	3
Homosexual						
Mounts	0	0	0	0	0	1
Stands	0	2	0	0	0	1
Heterosexual						
Cow+bull	8,0	1,2±0,4	6,0±2,2	6,4±1,8	14,0	8,3±3,0
Mounts	0	0	0	0	0	0
Services	0	0	0	0	0	1*
2 days before ovulation						
Sample number	1	1	2	4	1	2
Homosexual						
Mounts	0	0	0	0	0	0
Stands	0	0	0	0	0	0
Heterosexual						
Cow+bull	10,0	2,5	8,3±3,8	1,0±0,3	1,5	8,9±3,4
Mounts	0	0	0	0	0	0

* This service was recorded at 1820 hours on the day preceding ovulation.

DISCUSSION

The two most striking features of oestrus in the Afrikaner cow are firstly the low incidence of interactive behaviour between cows and between cows and bulls prior to service and secondly the short duration of heterosexual activity (three to four hours, Figure 18). Duration of oestrus is shorter (4 hours) in Bos indicus cattle than in Bos indicus*Bos taurus crosses (7,5 hours) managed under the same conditions (Anderson, 1944).

Of 84 oestrous periods in this study culminating in observed service, 41% of these had no other form of interactive behaviour during that oestrus. Of the remaining instances, 64% involved one other form of interactive behaviour in conjunction with service. This low incidence of interactive behaviour other than observed service was characteristic of both suckled and non-suckled cows (Figures 15 and 16). Although the number of Mashona cows in this study was small (two groups of ten each) oestrus tended to be a low intensity affair with observed service in conjunction with only one other component, being the most frequently recorded pattern of behaviour. These findings contrast sharply with reports on dairy cows where oestrus lasted 14 hours and cows were mounted 56±35 times (Esslemont and Bryant, 1976).

The expression of oestrus is extremely variable and is affected by temperature (Bond and MacDowell, 1972; Gangwar, Branton and Evans, 1965) and the number of animals in oestrus (Hurnik et al., 1975; Esslemont et al., 1980; Glencross et al., 1981; Hurley et al., 1982). Hurnik et al. (1975) reported that the number of mounts/cow increased from 11 with one cow in oestrus to 53 with

three cows in oestrus. Bos indicus cows may differ from Bos taurus in their expression of oestrus. Certainly in Buffalo the incidence of homosexual mounting in animals in oestrus was less than 50% in the study by Putu and Fletcher (1986) and less than 20% in a study by Singh et al., 1984. This change in bovine behaviour might be related to heat tolerance in tropically and sub-tropically adapted breeds where all unessential activity needs to be kept to a minimum.

How then is oestrus defined in the Afrikaner cow in terms of behaviour? With 40% (34/84) of oestrous periods in this study comprising one or more services and no other interactive behaviour, a universal and valid definition is unlikely. Of these 34 oestrus periods 47% comprised one single observed service and no other behaviour and oestrus in these instances must be described as an event rather than a period. Classical definitions usually have involved a test of the receptivity of the cow in response to an applied challenge in the form of a recently introduced bull. In this way Anderson (1944) was able to demonstrate that zebu cows remained in a receptive condition for 4 hours during a single oestrous period. However such a period of receptivity has little bearing on the natural situation if cows are mated once in the absence of any other activity.

With such 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 data reported in this study generally concur with those of Kilgour and Dalton (1983) who reported that cows will mount other cows prior to standing to be mounted themsleves. The mean intervals from the onset of

mounting and standing to first service in this study were similar (Figure 18). There was no fixed progression of one form of behaviour to the next with the approach of service. Rather service is most likely to be the last form of interactive behaviour, but any other form of interactive behaviour may precede it and in certain cases follow it.

The second objective in this study was to establish whether conception failure at first full ovulations which were 27 days earlier in weaned cows than in control cows was due to any behavioural deficiency, specifically the absence of service during oestrus.

Since 33 out 39 conceptions in the Afrikaner and 16 out of 19 conceptions in the Mashona were supported by recorded services, the incidence of conceptions during the night outside of the surveillance period was fortunately low.

Removal of the suckling stimulus significantly ($P<0,01$) improved the incidence of observed service with primary ovulations in the Afrikaner cows from 0/21 to 8/18 (Figure 17).

Removal of, or a reduction in the suckling stimulus increases gonadotropin activity earlier post partum than in the normally suckled cow (Short et al., 1972; Randel et al., 1976; Radford et al., 1978; Hinshelwood et al., 1985). This enhanced gonadotropin activity is probably involved with the higher incidence of observed service at primary ovulations as a result of either an increase in plasma oestrogens and/or an enhanced responsiveness of the behaviour centres to steroid stimulation. Similarly, the incidence of observed service at first full

ovulations was significantly increased from 10/25 in normally suckled cows to 28/28 in weaned cows. Clearly behavioural deficiencies were not the cause of conception failure to first full ovulations induced by a removal of the suckling stimulus.

If the increase in oestrous behaviour in weaned cows has followed changes in gonadotropin activity and plasma oestrogen concentrations, then the increased luteal output in non-suckled cows would be expected. If, on the other hand, the enhanced expression of oestrus is in response to changes in the behaviour centre receptor-activity then there should be no evidence of any change in subsequent luteal output.

Removal of the suckling stimulus probably had its effect in two ways. The first was an increase in GnRH activity following a reduction of the suckling suppression of hypothalamic activity early post partum. The second was as a result of the increased oestrus-related activity which might well have stimulated both an earlier and higher level of hypothalamic activity in anovulatory herd mates. Evidence for such an effect is provided by the fact that chemical substances secreted by oestrous females have been shown to improve oestrous synchrony (Izaard and Vandenbergh, (1982b) and that housing cows with vasectomised bulls may shorten the interval to first oestrus (MacMillan et al., 1979; Zalesky et al., 1984)).

CONCLUSIONS

Homosexual activity in post-partum Afrikaner and Mashona cows seems to occur in less than 30% of oestrous periods accompanied by observed service. Heterosexual mounts by cows occurred more frequently (42% of oestrous periods) than any other component of oestrus, other than service. Only 15% of conceptions occurred between 1900 hours and 0500 hours. Removal of the suckling stimulus increased the incidence of all components of oestrus related activity and increased the intensity of oestrus prior to primary and first full ovulations to a point where cows would stand to be mounted.

The poor conception rates to first full ovulations in non-suckled cows were not due to the failure of cows to stand to be served. Thus, these early ovulations appear to be deficient in some aspect related to the post-insemination phase of oestrus. Two possible mechanisms have been proposed which might account for the higher incidence of observed service in non-suckled cows compared to suckled cows, prior to primary and first full ovulations. The first is based on an increase in gonadotropin concentrations and their effect on oestradial concentrations and luteal function and the second is based on a change in the responsiveness of those behaviour centres in the brain responsible for the expression of oestrus.

Chapter 9

Characteristics of oestrous behaviour in Afrikaner cows which were either normally suckled or partially weaned between 28 days and 100 days post partum.

INTRODUCTION

The findings in Chapter 8 illustrated two important features of oestrous behaviour in suckled and non-suckled Afrikaner cows.

Firstly, although the number of primary ovulations (21 in suckled cows and 18 in non-suckled cows) and first full ovulations (25 in suckled cows and 28 in non-suckled cows) were similar in the two treatment groups, the incidence of observed service in conjunction with these ovulations was significantly ($P < 0,01$) higher in cows whose calves had been removed three days after calving (from 0/21 to 8/18 with primary ovulations and from 10/25 to 28/28 with first full ovulations). Removal of the suckling stimulus either markedly increased the behavioural response to endocrine signals (a purely neural effect) or the strength of these signals was increased (increased concentrations of oestradiol-17-beta, a steroid mediated effect) thereby enhancing the intensity of oestrus expression. Following on from this, the question arose as to whether once-a-day suckling would have the same positive effects on oestrus behaviour as removal of the suckling stimulus had done in the previous study. This was particularly interesting given that once-a-day suckling was as effective as complete weaning in increasing post-partum fertility in the Afrikaner.

The second feature of note from the last chapter was the very low intensity of oestrus in all Afrikaner and Mashona cows in terms

of homosexual and heterosexual activity. Of a total of 84 ovulations amongst Afrikaner cows, 34 of these were not accompanied by any form of interactive behaviour other than service and a further 32 of the remaining 50 ovulations were accompanied by one other form of interactive behaviour with observed service.

In the previous chapter only four types of interactive behaviour were recorded: homosexual mounting and standing, heterosexual mounting and service. Pairing of cows and bulls was also recorded but there appeared to be a high incidence of companionship or investigatory cow-bull pairing which could not be related to any plasma progesterone changes indicative of oestrus and was therefore assumed to be unrelated to the approach of oestrus. Accurate records of further components of behaviour associated with oestrus were needed if a more meaningful picture of oestrus in the Afrikaner and possibly most Bos indicus breeds was to be compiled. To this end, chin-resting by the bull and nosing of a cow's perineum were also recorded for the present study.

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 recorded as an oestrus-related component. This was necessary to eliminate non-oestrus-related surveillance of cows by bulls. 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 himself from the ground.

The transition from the pro-oestrous phase marking the beginning of oestrus prior to ovulation was assumed to be accompanied by a measurable increase in oestrus-related behaviour. An increase in mounting activity associated with the transition from pro-oestrus to oestrus in dairy cows has been reported in the literature (Esslemont et al., 1980; Glencross et al., 1981; Helmer and Britt, (1985). However from the data reported in Chapter 8 there was little consistent evidence indicative of the beginning of oestrus in the Afrikaner cow. Gustafsson et al., (1986) has described the transition from pro-oestrus to oestrus (in a controlled challenge situation) as the time when cows spontaneously lowered their backs in response to the advances of a teaser bull without standing to be mounted. In all of the above references there were clearly more than 2 homosexual mounts per oestrus and for this reason a marked change in mounting rate probably was a reasonable estimate of the onset of oestrus. In the Afrikaner and Mashona cow however, this is not the case and homosexual behaviour is clearly a very unreliable indicator of oestrus in these breeds.

The following study was designed to provide more meat to the "bones of oestrus" in the Afrikaner and to assess the effect of once a day suckling on patterns of oestrous behaviour.

MATERIALS AND METHOD

Experimental animals

Details of treatment methods have been provided in Chapter 4. A total of 66 mature multiparous Afrikaner cows were used in this study. Calves in the partially weaned treatment group suckled their dams twice a day from 28 days post partum to 42 days post partum and once-a-day thereafter until 100 days post partum.

Bulls (two per treatment group) were run with the cows throughout the study. All bulls were rotated between groups on a weekly basis.

Observation of oestrous behaviour

Cows were observed in two groups comprising normally suckled and partially weaned cows. All cows were observed from 0500 hours in the morning until 1900 hours in the evening. Seven components of oestrus-related behaviour were recorded and included homosexual mounting and standing, heterosexual mounting by the cow, chin-resting by the bull and nosing of the perineum by the bull. Cows were not removed from the treatment groups until the last cow to calve had completed 100 days in the trial. This was essential to avoid any bias in the opportunity for interactive behaviour in those cows calving late. However for the purpose of analysis only data up to 100 days post partum were used for each cow.

Statistical analysis

Continuous data such as duration of cow-bull pairing and intervals from the onset of one component of behaviour to the onset of another were examined using one-way analysis of variance. Non-parametric data was analysed using Chi-squared methods and Fisher's Exact Test (Rayner, 1967). Each behaviour record for a single day was classified on the basis of type of ovulation, success or failure of conception and the interval to day of ovulation (serial). This serial was assigned to consecutive records relating to a single ovulation. Any data relating to more than two days prior to an estimated ovulation were not included in any analysis.

Estimation of day of ovulation

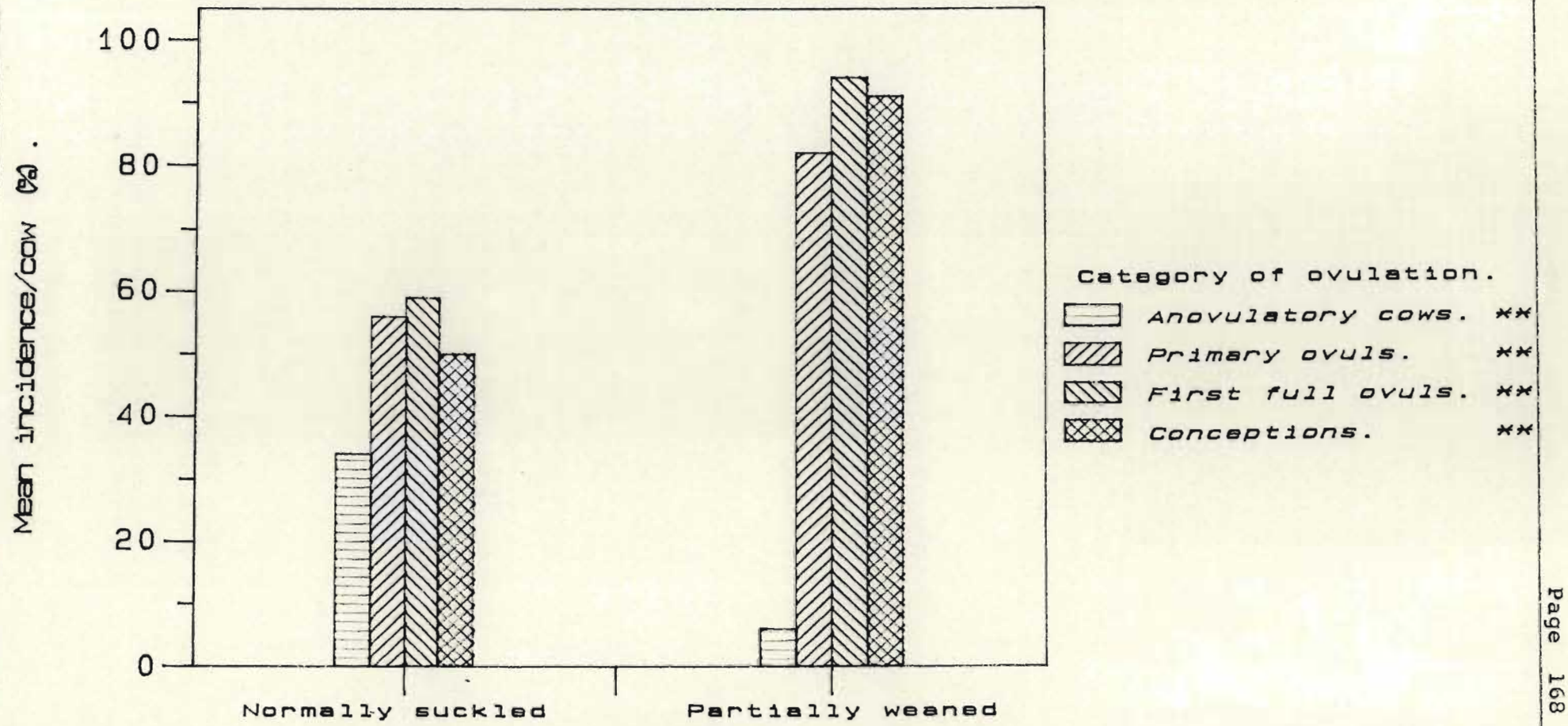
In the absence of any suitable reference point from the literature, the estimation of the day of ovulation required an *a priori* definition. If service was observed during a nadir in plasma progesterone concentrations, then that day was designated the day of ovulation. On the very few occasions (< 3) where service was recorded in an afternoon and again the following morning, day of ovulation was designated the second day. Where service was not in evidence, the day of greatest activity and longest cow-bull pairing was designated the day of ovulation. In most cases the designated day of ovulation was supported by preceding days of cow-bull pairing and marked the end of any oestrus-related behaviour. Behaviour records which related to luteal phase or anovulatory periods were not included in the analysis of oestrous behaviour.

RESULTS

Ovarian activity

Partial weaning from 28 days *post partum* significantly ($P<0,01$) reduced the number of anovulatory Afrikaner cows by 28% (Figure 19). The opportunity to assess oestrus-related behaviour was improved in partially weaned cows where a total of 42 full ovulations occurred as against 30 in the normally suckled cows. The proportion of first full ovulations preceded by primary ovulations did not differ between normally suckled cows (90%) and partially weaned cows (84%, Figure 19). Ultimate conception rates were significantly ($P<0,001$) improved by once a day suckling from 48% in normally suckled cows to 91% in partially weaned cows.

Figure 19. Ovarian activity in normally suckled and partially weaned post-partum Afrikaner cows.



Significant differences (** P=0.01; * P=0.05)

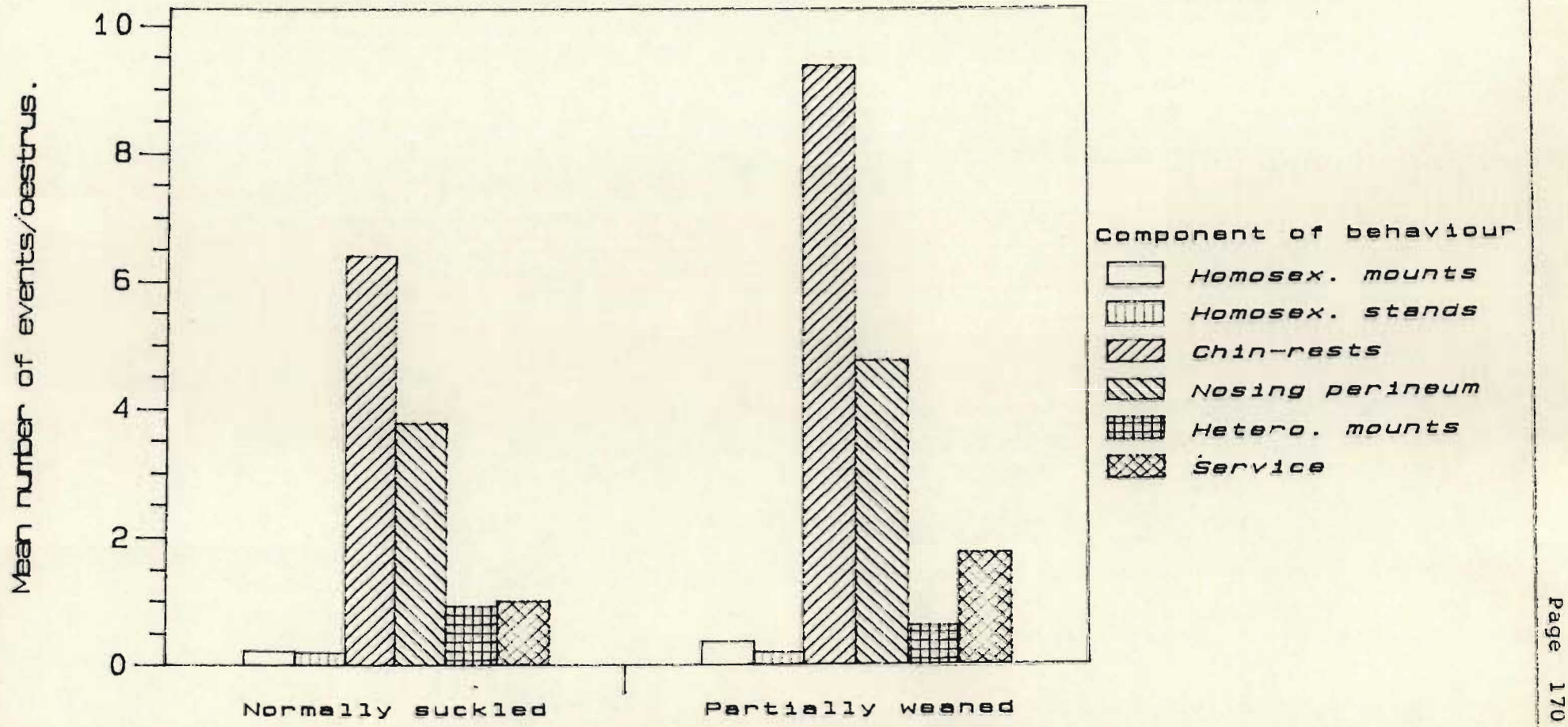
Occurrence of oestrus-related activity

The incidence of homosexual activity was extremely low in this study with a total of 13 homosexual mounts and stands being observed in normally suckled cows and 23 homosexual mounts and stands being recorded in the partially weaned group (Table 38). In contrast to this low level of homosexual activity, cow by bull interactions were much more frequent. A total of 363 heterosexual events were recorded in the normally suckled group along with 692 events in the partially weaned group. Total heterosexual activity was comprised largely of behaviour initiated by the bull, including nosing of the perineum and chin-resting (Table 38).

As with homosexual activity, instances of cow/bull mounting activity were very low and the only trend worthy of mention was that bulls appeared to mount partially weaned cows more often than they mounted normally suckled cows. Whether this was due to more mounts per oestrus in partially weaned cows or a higher incidence of nocturnal matings in the case of normally suckled cows could not be determined.

Since total oestrous activity was confounded with the total number of full ovulations, Figure 20 illustrates the components of oestrus as a mean per full ovulation recorded in each group. Chin-resting and nosing of the perineum feature prominently in oestrus in the Afrikaner and are the only two components of oestrus which have a high probability of being observed in every oestrus (Figure 20). Unfortunately this fact is likely to mean that these two components will also feature prominently in pro-oestrus and thus detract from their usefulness as definitive evidence of oestrus in the Afrikaner.

Figure 20. Mean incidence/ovulation of each type of oestrous behaviour in post-partum Afr. cows.



NS Norm. suckled (n = 30): PW Part. weaned (n = 42)

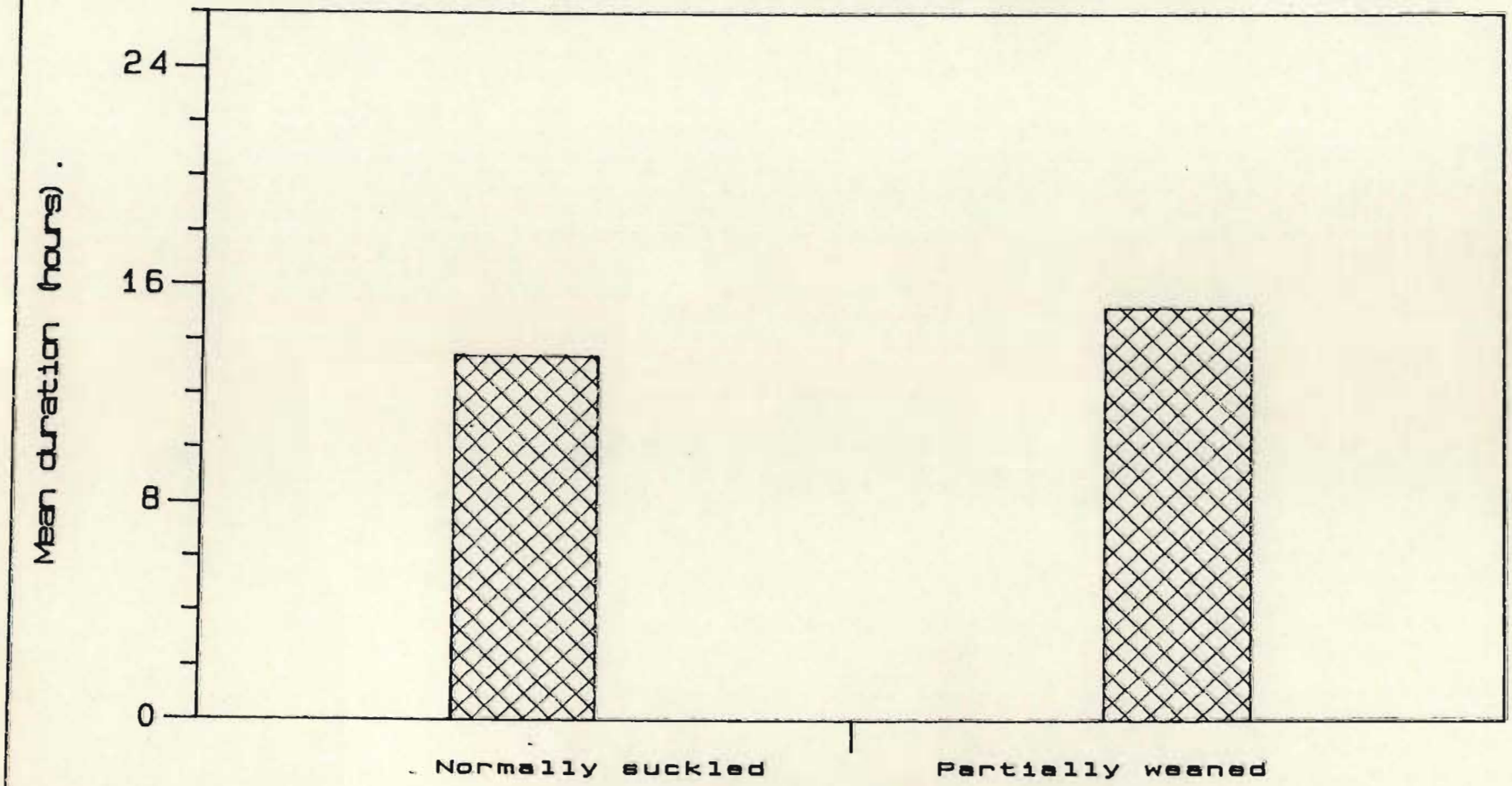
Table 38. Estimates of total activity within each of seven components of oestrus-related behaviour in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

Treatment group	Normally suckled	Partially weaned
Group number	32	33
Full ovulations	30	42
Behaviour component		
Homosexual mounts	7	15
Homosexual stands	6	8
Total homosexual events	13	23
Chin-resting	192	393
Nosing of perineum	113	199
Heterosexual mounts	28	26
Services	30	74
Total heterosexual events	363	692

Cow-bull pairing

The increased activity of bulls amongst partially weaned cows particularly in chin-resting is interesting. Considered in the light of the findings in Figure 21, normally suckled and partially weaned cows spent an equal amount of time with the bull, however, partially weaned cows appear to have stimulated more interest from the bull in the form of chin-resting. The similarity in the incidence of nosing of the perineum in suckled and non-suckled cows indicates that perhaps the increased bull interest in partially weaned cows was not due to any increase in pheromones. An alternative, is that the behaviour of partially weaned cows was more stimulating (immobility of cow on being solicited) than that of normally suckled cows.

Figure 21. Mean total time (hours) during which cows in oestrus were with a sexually active group.



Observation of service during oestrus

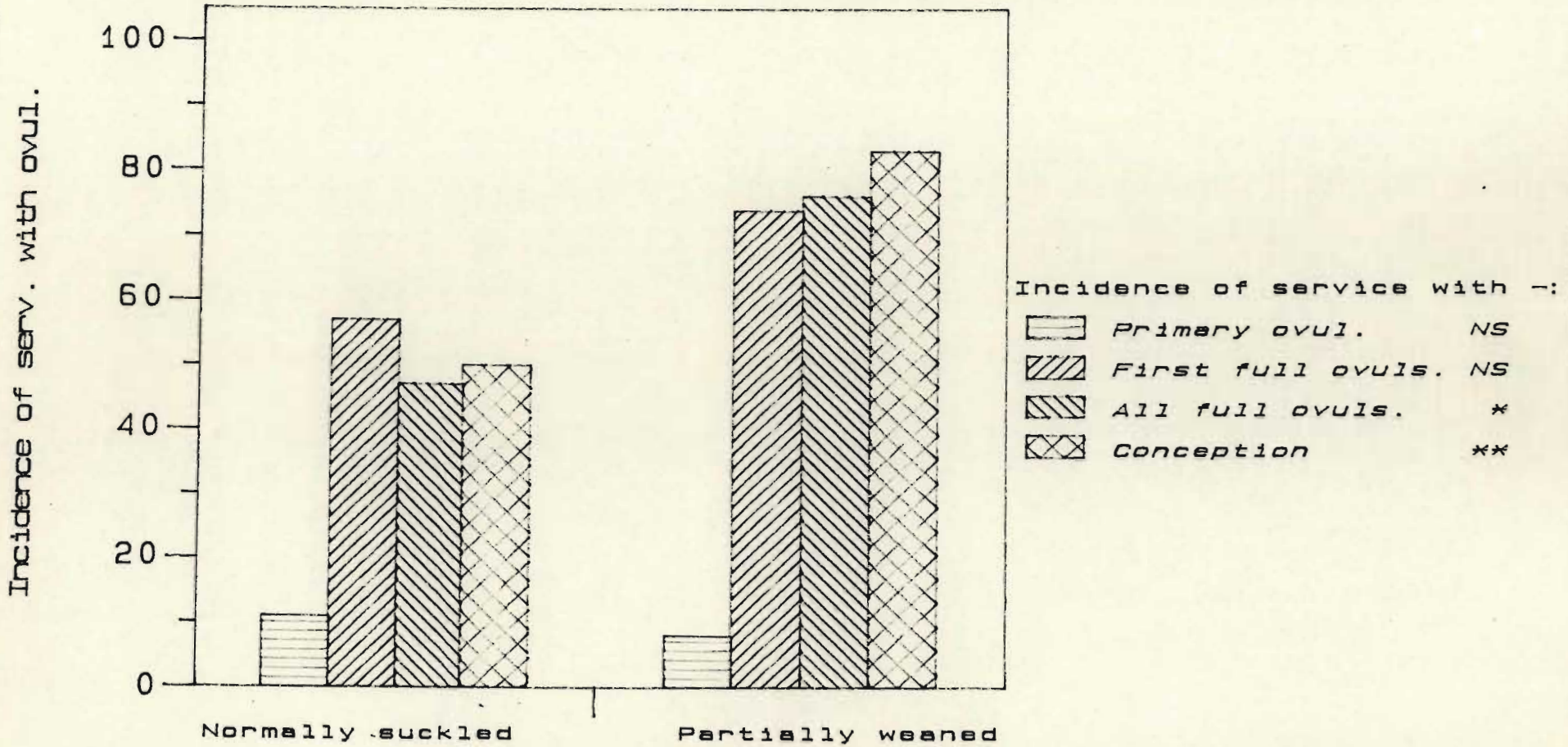
Partial weaning did not significantly increase the incidence of observed service with either primary or first full ovulation (Figure 22). However over all full ovulations, partial weaning significantly ($P<0,01$) increased the incidence of observed service during oestrus. Similarly, significantly ($P<0,052$) more observed services were recorded at the time of conception in partially weaned cows (25/30) than in normally suckled cows (8/16, Figure 22) .

This last finding is in contrast to the results of the last chapter where the observation of service was as successful in suckled cows as it was in non-suckled cows. This lower incidence of observed service in normally suckled cows than in weaned cows in the present study might provide evidence of a shift in the pattern of oestrous behaviour in the suckled Afrikaner cow. With a calf at foot 50% of Afrikaner cows in this study have been served between 1900 hours and 0500 hours compared to 17% of partially weaned cows.

Details of heterosexual behaviour

With the very low incidence of homosexual behaviour only heterosexual interactive behaviour was analysed in detail and is presented in Tables 39 to 42. In an effort to stabilise the variation in patterns of behaviour recorded in this and earlier studies, only records of oestrus including at least one observed service were extracted and analysed.

Figure 22. The incidence of observed service prior to primary, first, all ovulations and conception.



* - P=0.011; ** - P=0.017; NS - Not sig. diff.

Chin-resting

Chin-resting was the component of oestrous behaviour most frequently recorded in association with observed service (82% of oestrous periods). Partial weaning did not modify this component of oestrus-related behaviour in any way (Table 39). These findings may appear to contradict the trends in Figure 20. However this is not the case since the present data is a sub-sample of all the behaviour data, whereas Figure 20 was compiled from the whole data set. This apparent contradiction does however indicate that in partially weaned cows there is a high incidence of chin-resting on days preceding oestrus and on days where oestrus is not observed, a trend which is not consistent in normally suckled cows. This finding does confirm that partial weaning facilitates the stimulus which induces the bull to chin-rest. Furthermore this stimulus is evident up to 2 days prior to ovulation.

Table 39. The incidence, duration and interval from first chin resting by the bull on a cow to first observed service in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

Treatment group	Normally suckled	Partially weaned
Group number	32	33
Full ovulations	30	42
Incidence	14/16	27/34
Mean number of chin-rests per oestrus	6,5±1,3	5,5±0,9
Range	1-16	1-17
Time in hours from first to last chin-rest	8,6±1,3 (10)	6,8±0,9 (22)
Range	1,0 - 13,5	1,0 - 13,5
Interval in hours from first chin-rest to first service	4,2±1,2	3,2±0,7
Range	13,0 to +2,0	11,5 to 1,0

Nosing of the perineum

Nosing of the cow's perineum was at 70% the second most frequently observed component of oestrus (Table 40). In 31% of cases this type of behaviour occurred only once during an oestrus. Again, partial weaning did not significantly modify any characteristic of this component of oestrus. Figure 20 would indicate that this activity should have occurred more often during oestrus. The discrepancy is due to a high incidence of this behaviour in cows which were not in oestrus. Thus, nosing of the perineum is an investigatory activity which is not specifically related to oestrus, although it does occur at this time. Unlike chin-resting, there is a similar incidence of this behaviour in suckled and partially weaned cows.

Table 40. The incidence, duration and interval from first nosing of the perineum by the bull to first service in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

Treatment group	Normally suckled	Partially weaned
Group number	32	33
Full ovulations	30	42
Incidence	10/16	25/34
Events per oestrus	3,1±0,5	2,7±0,3
Range	1 - 7	1 - 7
Time in hours from first to last event	8,3±1,2 (9)	7,0±0,9 (15)
Range	2,5 - 14,0	1,0 - 11,0
Interval in hours from first event to first service	5,4±1,2	2,4±1,0
Range	10,5 to +0,5	12,0 to + 10,0

Heterosexual mounting by cows

Whereas chin-resting and nosing of the perineum were recorded in more than 70% of oestrous periods, cows mounted the bull in less than 40% of oestrous periods. Partial weaning did not increase the incidence of heterosexual mounting over that observed in normally suckled cows. When heterosexual mounting occurred, two mounts were observed, on average, with a range of one to four over a period of three to five hours. Since this activity began just under two hours before the first service, heterosexual mounting and service often occurred simultaneously.

Table 41. The incidence, duration and interval from first heterosexual mount to first service in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

Treatment group	Normally suckled	Partially weaned
Group number	32	33
Full ovulations	30	42
Incidence	8/16	11/34
Number of heterosexual mounts per oestrus	1,9±0,5	1,8±0,4
Range	1 - 4	1 - 4
Time in hours from first to last heterosexual mount	5,8±2,9	3,4±1,2
Range	2,0 - 11,5	0,5 - 5,5
Interval in hours from first mount to first service	1,9±1,5	1,7±0,9
Range	10,5 to +4,5	6,5 to +3,5

Cow-bull pairing

Observed service very seldom occurred without prior evidence of cow-bull pairing. In normally suckled cows pairing was recorded in 94% (15/16) of oestrous periods which was not significantly different from the 88% (30/34) of oestrous periods in partially weaned cows (Table 42). Overall, cow-bull pairing commenced 4,2±0,6 hours before the first service and lasted for 6,9±0,6 hours.

Table 42. The incidence, duration and interval from first cow-bull pairing to first service in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

Treatment group	Normally suckled	Partially weaned
Group number	32	33
Full ovulations	30	42
Incidence	15/16	30/34
Interval in hours from first to last cow-bull pairing	7,4±1,3	6,7±0,9
Range	1,5 - 13,5	0,5 - 14,0
Interval from start of pairing to first service	4,7±1,2	3,9±0,7
Range	13,5 to 0	13,0 to 0

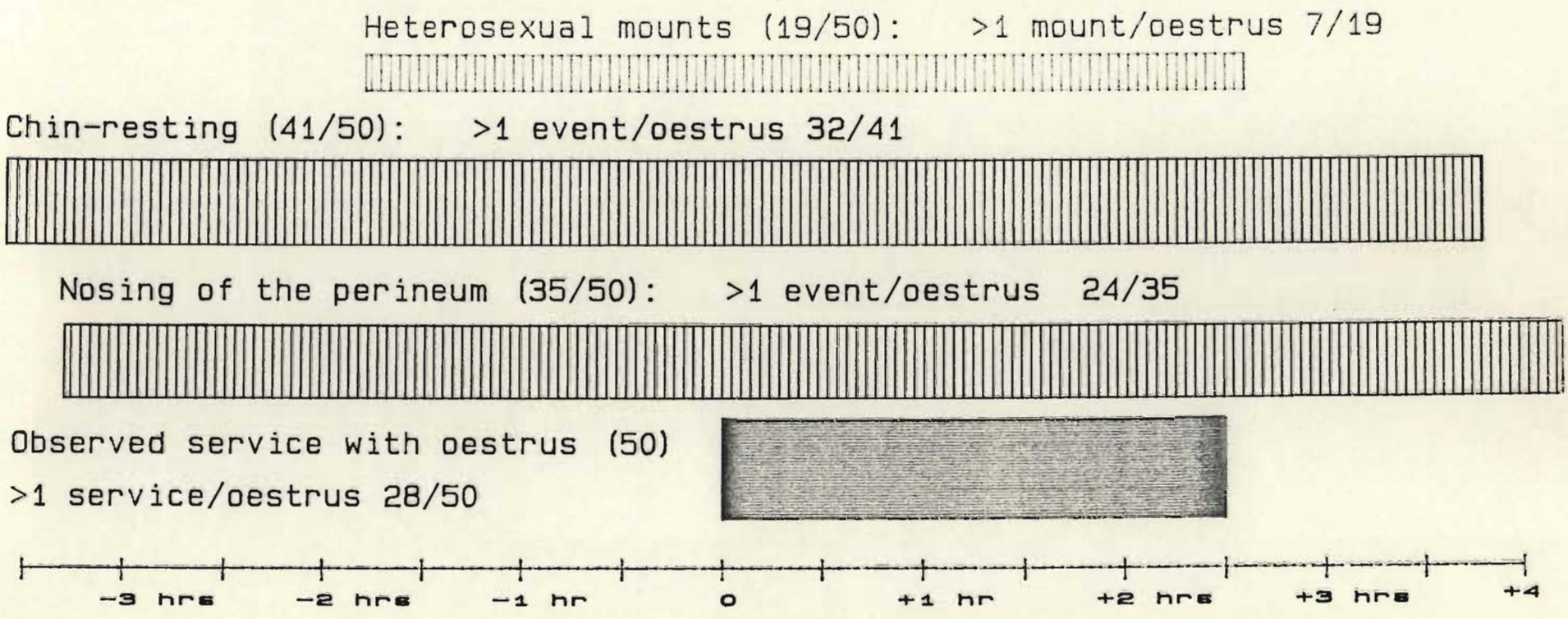
A general pattern of oestrus (including observed service)

In the absence of any significant treatment effects on heterosexual components of oestrous-related behaviour, Figure 23 illustrates the temporal relationship between all the components reported in the preceding four tables except for cow-bull pairing.

Oestrus commenced with cow-bull pairing 4,2±0,6 hours prior to first service and lasted for 6,9±0,6 hours. Following pairing, the first of 5,9±0,7 instances of chin-resting were recorded 3,6±0,6 hours before the first service. Almost at the same time (3,3±0,8 hours before first service) as the chin-resting commenced, bulls began investigating the perineum of cows in oestrus indicating that perhaps pheromones are closely involved with chin-resting. Cows mounted the bull for the first of 1,8±0,3 times, starting 1,8±0,8 hours before the first service. Multiple services during a single oestrus were recorded in 56% (28/50) of oestrous periods and in general, cows were served 2,0±0,2 times over an interval lasting 2,5±0,4 hours.

Following the last service heterosexual mounts by cows ceased immediately whilst bulls continued to display interest in oestrous cows for up to one to two hours after the last service (Figure 23). From this data, chin-resting and nosing of the perineum are not closely related to the peak of oestrus (service) and therefore have limited value as indicators of oestrus. Heterosexual mounting by cows is more reliable but its low incidence (38%) severely limits its reliability. Observed service remains the only consistently reliable indication of oestrus in the Afrikaner cow.

Figure 23. A summary of the incidence, time of onset and duration of four components of heterosexual behaviour in the Afrikaner cow during oestrus.



Multiple services with primary and full ovulations

Unlike complete weaning, once-a-day suckling did not increase the incidence of observed service prior to primary ovulation compared to normally suckled cows (Table 43). Single, double and multiple services were observed with similar frequencies prior to both first full ovulations and with conception (Table 43). However there may have been a trend for conceptions in partially weaned cows to be accompanied by more multiple services than in normally suckled cows. These observations are not unexpected given the trends observed with chin-resting by which bulls might test the receptivity of cows.

Table 43. The incidence and duration of multiple services with primary and full ovulations and conception in Afrikaner cows which were either normally suckled or partially weaned from 28 days post partum.

Treatment group	Normally suckled	Partially weaned	
One service per oestrus			
Primary	2/19	2/26	
First full	5/21	8/31	
Conception	4/8	10/14	
Two services per oestrus			
Primary	0	0	
First full	3/21	7/31	
Mean duration	1,3±0,1	1,6±0,5	
Range	1,0 - 1,5	0,5 - 6,0	
Conception	2/4	6/10	
Three or more services per oestrus			
Primary	0	0	
First full	4/21	8/31	NS
Mean duration	3,3±0,9	3,6±0,8	
Range	1,5 - 5,0	1,5 - 8,5	
Conception	2/4	9/10	NS

A summary of general oestrous behaviour (including oestrous periods not accompanied by observed service)

From the data compiled in this behaviour study, there were many instances of substantial oestrus-related behaviour in the absence of any observed service. There were also instances of oestrus-related behaviour occurring one, two and three days before the day of ovulation.

Partial weaning did not alter any of the behaviour parameters recorded in Table 44. Primary ovulations were accompanied by very little interactive behaviour between cows and bulls on the day prior to ovulation. With first full ovulations at which conception did not occur 66% of ovulations had some evidence of interactive behaviour one day before ovulation. This behaviour included cow-bull pairing, chin-resting and nosing of the perineum by the bull (non-specific investigatory behaviour). Although only eight of the sixteen conceptions were observed in the control cows, four of the remaining eight conceptions occurred in the absence of any oestrus-related behaviour at all, indicating that the entire oestrous period ocurred outside of the observation period. Although 25/30 conceptions were accompanied by observed service in partially weaned cows, all five of the remaining conceptions were associated with some evidence of oestrus-related behaviour.

On the strength of these last two findings, partial weaning would appear to have increased the intensity of oestrous behaviour slightly but not to the same degree as was observed when the suckling stimulus was removed. Thus suckling appears to suppress both gonadotropins and oestrous activity proportionally with intensity of the suckling stimulus.

The bull was accurately detecting the approach of oestrus up to 48 hours prior to oestrus. No component of behaviour studied to date will provide any accurate indication of the approach of oestrus in the Afrikaner cow in the absence of the bull.

Table 44. Characteristics of oestrus-related behaviour prior to and in conjunction with primary and full ovulations and conception in Afrikaner cows which had been either normally suckled or partially weaned from 28 days post partum.

Category of ovulation	Primary ovulation		First full ovulation		Conception	
Treatment grp.	Control	Weaned	Control	Weaned	Control	Weaned
Day of ovulation						
Evidence of oestrus	6/19	7/26	10/21	8/31	12/16	30/30
Homosexual						
Mounts	0	1	1	0	1	5
Stands	0	2	2	0	2	5
Heterosexual						
Cow+bull	2,9±0,9	3,3±0,6	6,3±1,7	5,9±1,7	2,2±2,5	7,1±2,5
Chin-rests	4	4	7	5	9	26
Nosing perineum	5	6	5	5	11	22
Mounts	1	1	4	3	5	9
Services	1	2	5	6	7	25
Day before ovulation						
Incidence	2	0	7	5	5	19
Homosexual behaviour						
Mounts	0		0	0	1	0
Stands	0		0	0	0	0
Heterosexual behaviour						
Cow+bull	6,8±5,8		2,7±1,1	4,3±1,4	7,2±5,0	8,8±1,1
Chin rests	1		3	3	4	18
Nosing perineum	2		3	4	4	15
Mounts	2		1	0	0	2

DISCUSSION

One of the objectives in this study was to record and analyse additional data which might more clearly indicate the transition from pro-oestrus to oestrus. Although high levels of chin-resting and nosing of the perineum were recorded up to two days prior to oestrus these activities were probably stimulated by odours which emerge in vaginal fluids during the three days before oestrus and are virtually absent the day following oestrus (Kiddy and Mitchell, 1981). The timing of the appearance of these odours corresponds to the rise in oestradiol-17-beta which reaches a peak two days prior to oestrus (Glencross et al., 1973) and drops sharply with the conclusion of oestrus. Of interest was the finding that chin-resting or nosing of the perineum ceased within two hours of the last service. Thus, chin-resting and nosing of the perineum are too imprecise to be used as confirmation of the transition from pro-oestrus to oestrus.

Homosexual and heterosexual mounting is still the only conclusive evidence of oestrus in the Afrikaner. However the very low incidence (±0.3 mounts per oestrus, Figure 19) of these components of behaviour makes the consistent detection of oestrus using these paramters in the absence of males impossible. Furthermore a precise definition of oestrus under free mating conditions is unlikely to be described considering the variation in patterns of behaviour between animals which conceived. This statement is founded on the considerable variation in patterns of behaviour between animals which were successfully mated within the same treatment group in this study.

Oestrous behaviour may not be observed because certain ovulations may not be accompanied by behavioural events at all (Moller, 1970). Some oestrous periods are very short in duration (Hurnik et al., 1975; Esslemont and Bryant, 1976; O'Farrel, 1975,1980) or confined to the hours of darkness (Hurnik et al., 1975). Artificially and naturally hot climatic conditions have been shown to reduce the length of oestrus (by up to 50%, modal value 6 hours) and increase the incidence of anoestrus (up to 33%) in Holstein heifers (Gangwar, Branton and Evans, 1965).

Oestrus in the Afrikaner and Mashona cow and possibly many Bos indicus breeds appears to be a subtle affair with a minimum of unnecessary activity during oestrus. Evidence for this is the short (±4 hours) duration of oestrus in Bos indicus breeds (Anderson, 1944 and 1936; DeAlba et al., 1961; Plasse et al., 1970 and this study) and the lower intensity of either natural or induced oestrus in Bos indicus (Brahman) compared to Bos taurus (Randel, 1983).

The diurnal distribution of service has an important bearing on the interpretation of results in this study. The ratio of observed services at time of conception to the number of conceptions indicate that 50% of services in suckled cows have occurred at night whilst only 17% of conceptions in partially weaned cows occurred at night. Results from Chapter 8 indicated that about 15% of conceptions in both suckled and non-suckled cows occurred at night. All partially weaned cows suckled their calves in the afternoon, a fact which may have a bearing on the time of onset of oestrus. A tight temporal relationship exists between episodic LH secretion and oestradiol secretion rate from the ovary (Baird, Swanston and Scaramuzzi, 1976). Since

suckling is known to depress gonadotropin activity (Saiduddin et al., 1968; Carruthers et al., 1980) it is possible that once-a-day suckling at the same time each day might influence the timing of onset of oestrus by altering LH and oestradiol activity at specific times during the day. Such a mechanism might account for the 50% versus 83% incidence of observed service between 0500 and 1900 hours in normally suckled and partially weaned cows respectively.

Complete weaning stimulated a significant increase in heterosexual mounting behaviour during oestrus (Chapter 8) whereas partial weaning in the present trial had no significant effect. Since calves of Afrikaner cows suckled their mothers from three to eight times per 24 hours period (Chapter 7) reducing the suckling frequency to once each day represents a substantial reduction in the total suckling stimulus. Yet, once-a-day suckling was sufficient to almost eliminate the incidence of observed service prior to primary ovulation (2/26) compared with the non-suckled cow (8/18, Chapter 7). Nevertheless reducing the suckling frequency to once a day was sufficient to improve post-partum conception rates by more than 30% compared with the normally suckled cow (Chapter 4).

Without a higher proportion of observed services with first full ovulation (presently 12/19 in normally suckled cows and 21/30 in partially weaned cows) the failure of early conceptions in this present study really rests on whether or not these first full ovulations were accompanied by natural service. On the strength of conception data, service rates would appear to have been 20% higher than the observed service rate would indicate. The data

in the present study indicate no significant effect of treatment on the proportion of observed services at first full ovulation. Bearing in mind that the post-partum intervals to first ovulation (64±5 and 53±4 days post partum, Chapter 3) did not differ with suckling status in this study and occurred after the critical 50 days post-partum interval, the question of conception at first full ovulation was not as critical as in the previous study (Chapter 8) where first full ovulations were 27 days earlier (30 days post partum) in non-suckled cows than in suckled cows. The present findings do not detract from the proposal that conception rates to ovulations around 30 days post partum might be limited by uterine and endocrine factors rather than behavioural deficiencies.

In the previous study (Chapter 8) low conception rates were a result of a high incidence of conception failure in cows which were known to be cycling. In the present study 11/32 normally suckled cows were anovulatory against 2/31 in the partially weaned group. The increased ovarian activity in partially weaned cows may have been a consequence of both a reduction in the suckling stimulus and an increased level of overt oestrous activity in herd mates. A psychic stimulation (Lindsay, 1966) of ovulation by the introduction of rams (Schinckel, 1954; Watson and Radford,1960; Tervit et al., 1977) or the presence of other mating sheep (Lindsay, 1966) prompts the hypothesis that increased oestrous activity in post-partum cows hastens the resumption of ovarian activity in anovulatory cows. Early post-partum exposure of cows to bulls has been shown to reduce the interval to post-partum resumption of ovarian activity (Zalesky et al., 1984) whilst the general activity of cows in oestrus is increased in the presence of either other cows in oestrus or a

vasectomised bull (Sipilov, 1966; Ebert *et al.*, 1972; MacMillan *et al.*, 1979).

Since the Afrikaner has proved to be very responsive to the effects of reduced suckling intensities on reconception *post partum* perhaps the post-partum Afrikaner cow would respond to increased bull to cow ratios and teasing with vasectomised bulls prior to the breeding season.

CONCLUSIONS

In the absence of suckling, the incidence of observed service prior to primary and first full ovulations in the post-partum Afrikaner cow was significantly increased. In contrast, reducing the suckling frequency to once a day, did not increase the incidence of observed service prior to primary and first full ovulation over that observed in the normally suckled cow. The absence of service to these early ovulations was probably not due to insufficient steroid concentrations. Following primary and first full ovulations, luteal activity in partially weaned cows was at least equal to, if not higher than in normally suckled cows. The enhanced plasma progesterone concentrations in partially weaned cows after primary ovulation might have been associated with a degree of asynchrony in the endocrine control of oestrus and ovulation. Unlike the primary and first full ovulations in non-suckled cows these same ovulations in partially weaned cows appear to have been accompanied by very little or no oestrous behaviour.

The two additional components of oestrus in the observation data (chin-resting and nosing of the perineum) substantially increased the oestrus-data-base but did not resolve the question of a definitive description of oestrus or enable pro-oestrus and oestrus to be distinguished. Oestrus in the Afrikaner remains highly variable, in terms of both duration and behaviour. All mounting activity was concluded simultaneously, whilst chin-resting and nosing of the perineum were concluded two to three hours after the last observed service.

Chapter 10

Characteristics of oestrous behaviour in Afrikaner and Mashona cows which were either partially weaned before the breeding period, partially weaned during the breeding period or normally suckled throughout the study.

INTRODUCTION

In both of the previous studies on behaviour reported in this thesis (Chapters 8 and 9), variability in individual expression of oestrus between cows was high. The duration of oestrus was no more than four hours which is shorter than has been reported for Bos taurus (beef, 9 to 14 hours, Quinlan, Bisschop and Adelaar, 1941; dairy, 7 to 10 hours , Esslemont et al., 1980 and Hurnik, King and Robertson, 1975) and Bos indicus (beef, 6 hours , Anderson 1936; 8 hours 7/8 Afrikaner, Quinlan, Bisschop and Adelaar, 1941) and Bos indicus*Bos taurus (dairy, 13 hours, Baker, 1967). This short duration in conjunction with low intensity of oestrus in the Afrikaner makes the detection of oestrus more difficult.

Although homosexual mounting activity was low, the formation of homosexual groups amongst cows had not been monitored before in this series of studies. This was included in the present study since the formation of these groups may precede oestrus in the absence of any mounting activity. In addition, the flehmen response was also recorded to support data on male initiated behaviour during oestrus.

The findings reported in Chapter 5 indicated that a reduction in the suckling stimulus (partial weaning) 35 days prior to the breeding period was insufficient stimulus to significantly reduce the number of anovulatory Afrikaners in the group (Table

12, Chapter 5).

Plasma progesterone profiles of all cows in this study indicated that significantly fewer Afrikaner cows (22/62) than Mashona cows (36/39) were cycling prior to the introduction of bulls. Clearly, all oestrus-related activity in the absence of herd sires would have been homosexual. Knowing the low incidence of homosexual behaviour in both Afrikaner and Mashona cows in the presence of the bull, the first objective in the present study was to assess the amount and characteristics of oestrus-related behaviour in Afrikaner and Mashona cows in the absence of the male. In dairy cows the degree of expression of homosexual behaviour is correlated to the number of cows in oestrus at the same time (Esslemont et al., 1980; Hurnik et al., 1975).

Since endocrine factors, notably the increase in oestradiol-17-beta following the decline in progesterone are responsible for the onset of oestrus (Asdell et al., 1945; Carrick and Shelton, 1969; Glencross et al., 1973), an increase in homosexual activity might be expected in the absence of bulls prior to ovulation as a form of displacement behaviour.

The results from preceding chapters prompted the question as to whether or not the association of anovulatory cows with cows in oestrus in the same herd was any stimulus to the reproductive system of anovulatory cows. Should this situation exist, then the intensity of overt oestrous behaviour in the absence of the bull is important. The question also arises as to whether the lack of response to partial weaning prior to breeding could have been correlated with the absence of low intensity of homosexual behaviour in cows not exposed to the bull.

The second objective in this study was to investigate the possibility of an interaction between suckling status (normally suckled or partially weaned) and a male/bull effect (presence or absence of the bull). This possibility had to be considered since this was the first time that cows were observed whilst exhibiting oestrus in the absence of the bull. Previous results in Chapters 8 and 9 indicated that suckling intensity had no significant effect on characteristics of oestrus analysed. For although total activity (both time and number of events) was increased with partial weaning, detailed analysis of components did not reveal any significant changes with reductions in suckling intensity.

MATERIALS AND METHODS

Experimental animals and method

Details of treatments for this study have been described in Chapter 5. Afrikaner and Mashona cows were blocked according to calving date and randomly allocated to one of three treatment groups. Two out of the three treatment groups were partially weaned at different times in relation to the beginning of the breeding season. One group of 25 Afrikaner cows and 13 Mashona cows was partially weaned for 35 days (21 Dec to 25 Jan) prior to the onset of the breeding season. The other partially weaned group of 22 Afrikaner cows and 16 Mashona cows was partially weaned during the 39 day breeding period (24 Jan to 5 Mar). The third group was a control group of 15 Afrikaner cows and 10 Mashona cows all of which were normally suckled for the duration of the trial and bred from 25 Jan to 5 Mar.

Observation of oestrous behaviour

Due to a shortage of skilled observers and the requirement for three observation groups, behaviour was monitored for a limited period each day beginning on 21 December and continuing through until completion of the trial on 5 March. All cows were observed from 0500 hours until 0900 hours and from 1500 hours until 1900 hours. In this study a total of nine components of oestrus-related behaviour were recorded.

Homosexual behaviour records

Homosexual behaviour including mounting and standing to be mounted by cows was recorded as before. The periods between instances of homosexual mounting when cows were associated with other cows in synchronous oestrus were recorded as homosexual pairing.

Heterosexual pairing

All components of heterosexual activity which had been previously recorded including; chin resting; nosing of the perineum; heterosexual pairing; heterosexual mounting by cows and service were compiled for the present data set. In addition to the above all instances of flehmen were recorded.

Analysis of behaviour data

Primary, full ovulations and conceptions were identified from plasma progesterone profiles analysed in Chapter 5. Continuous data pertaining to intervals and durations were analysed using One-way Analysis of Variance methods whilst all frequency data were analysed using Chi-squared methods and Fisher's Exact Test (Rayner, 1967). In order to avoid the inclusion of any behaviour not specifically related to oestrus, the bulk of the analysis to follow deals with behaviour records sub-sampled from only those

occasions when observed service accompanied oestrus. Table 51 presents all behaviour data compiled regardless of its relation to specific ovulations and includes data recorded up to four days prior to an ovulation.

RESULTS

Resumption of ovarian activity

During the pre-breeding period partial weaning did not reduce the number of anovulatory Afrikaner cows compared with the normally suckled cows in the remaining two groups (Table 45). However, from Figure 24 it appears that partial weaning per se may have increased the mean ovulation rate per cow over the 35 day pre-breeding period amongst the cows which did ovulate. This trend may have followed the earlier resumption of ovarian activity within this pre-breeding period in partially weaned cows compared with their contemporaries. This could only be confirmed with larger sample numbers.

During the breeding period partial weaning significantly (P 0,05) reduced the number of anovulatory cows in the group and tended to increase the mean ovulation rate per cow (Figure 24).

Most Mashona cows had ovulated prior to the start of the pre-breeding period and therefore their mean ovulation rate was higher than in Afrikaner cows (Figure 25). Partial weaning during this pre-breeding period tended to induce a higher mean ovulation rate, although the differences in ovulation rate between normally suckled cows and partially weaned cows were small. During the breeding period partial weaning appeared to reduce the mean ovulation rate. In fact this was due to

partially weaned cows conceiving significantly earlier (107±5 days post partum) than their normally suckled contemporaries (123±7 days post partum, Table 15).

Table 45. Characteristics of the resumption of ovarian activity in Afrikaner and Mashona cows in three treatment groups. The first group was partially weaned during the breeding season and the second group was weaned for 35 days prior to the breeding season. A control group was normally suckled for the duration of the trial.

Breed	Afrikaner			Mashona		
Partial weaning and breeding	With breeding	Prior to breeding	None	With breeding	Prior to breeding	None
Group no.	22	25	15	16	13	10
Cows anovulatory for the duration of the trial	4 P 0,05	12	8	1	0	0
21 December to 25 January						
Full ovulations within this period	8	15	4	20	23	16
Cycling cows	7	10	5	13	13	10
25 January to 5 March						
Full ovulations within this period	23	15	7	17	17	13
Cycling cows	18	13	7	16	13	10

Figure 24. Mean number of ovuls. per cow prior to and during the breeding period in Afrikaner cows.

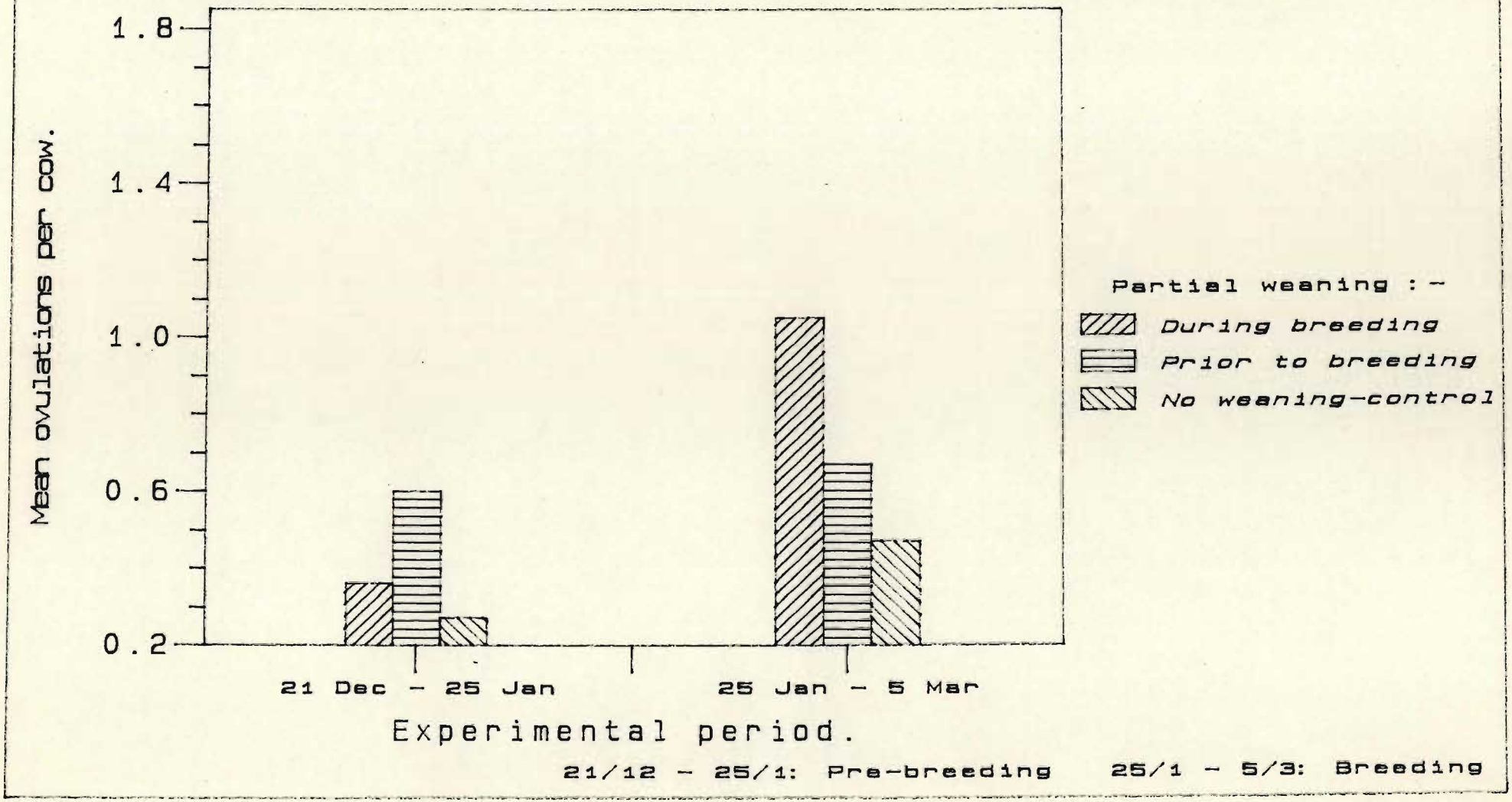
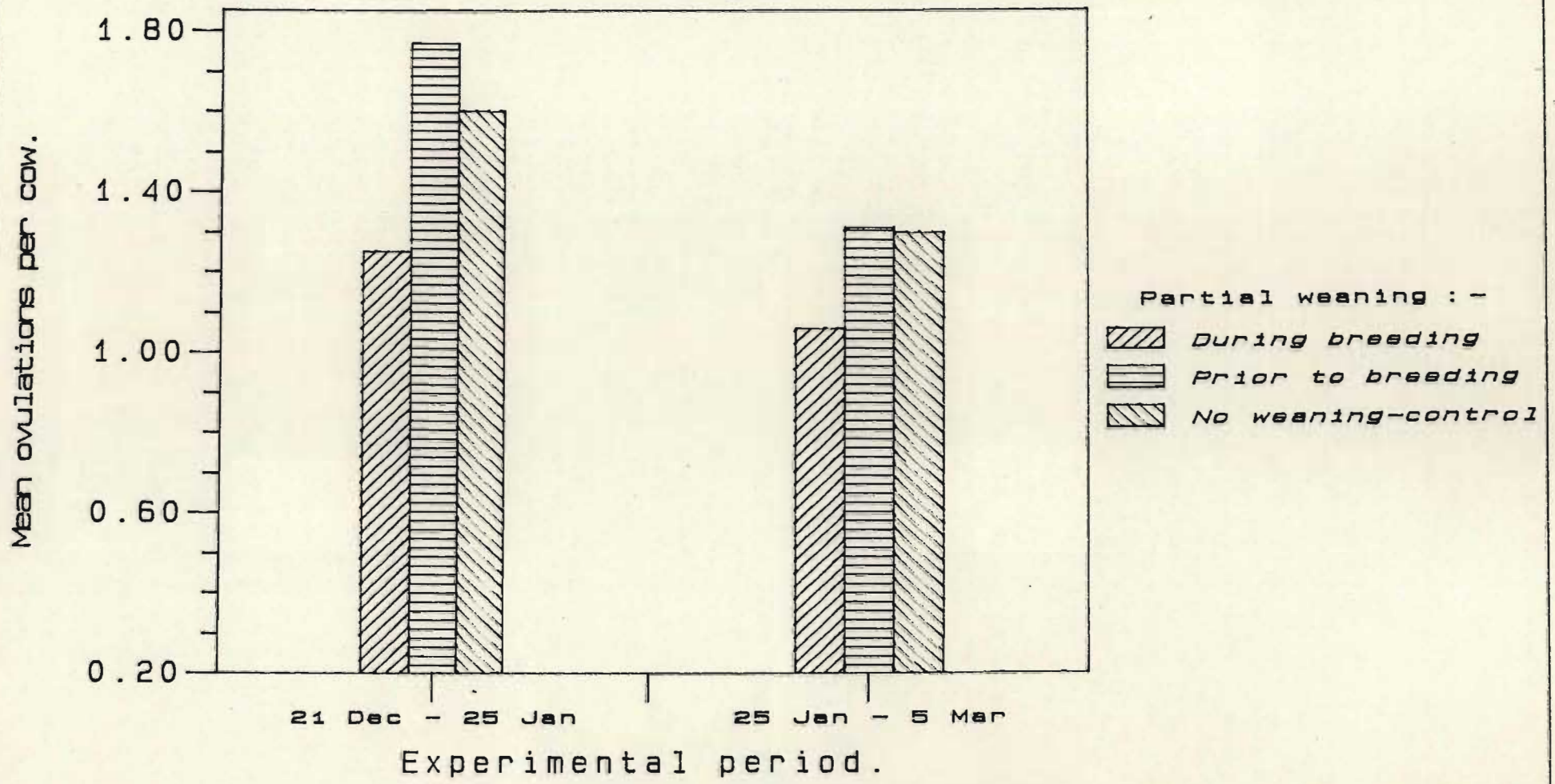


Figure 25. Mean number of ovuls. per cow prior to and during the breeding period in Mashona cows.



Homosexual activity during the pre-breeding period

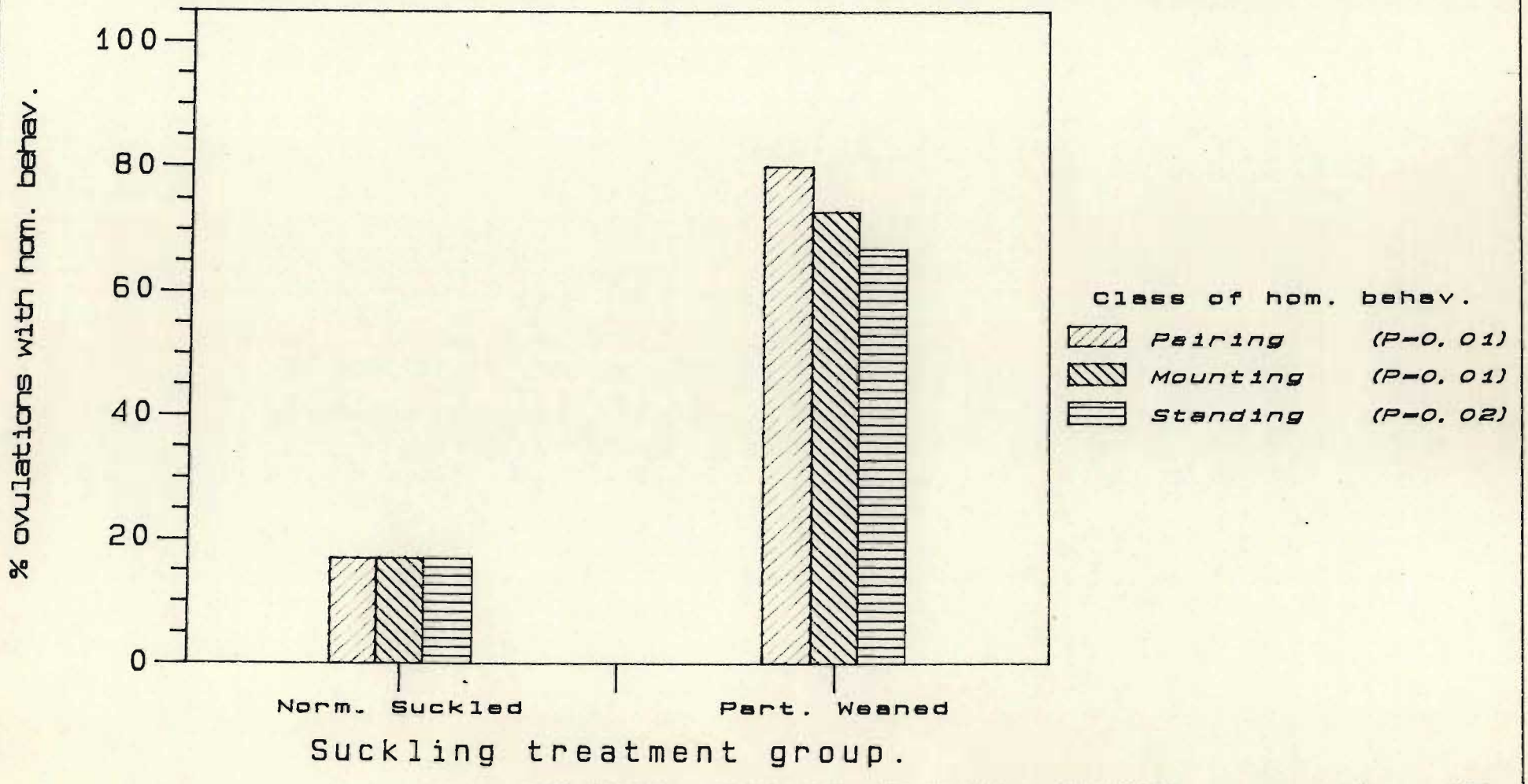
Only behaviour which occurred on the day cows were estimated to have ovulated has been included in the analysis for Table 46 and Figures 26 and 27. Partial weaning in the absence of any male effect for 35 days prior to the breeding period significantly (range $P<0,01$ to $P<0,05$) increased the incidence of homosexual pairing amongst partially weaned Afrikaner and Mashona cows (Tables 26 and 27). Although highly variable between groups, the duration of homosexual pairing tended to be shorter in Mashona cows than in Afrikaner cows (Table 46).

Partial weaning in the absence of the bull significantly increased the incidence of homosexual mounting and standing prior to full ovulations in Afrikaner cows (range $<P$ 0,01 to $P<0,05$ and P 0,01 to P 0,05 respectively, Figure 26). The mean number of mounts per oestrus varied markedly and no consistent differences were found between any of the treatment groups in the range $4,0\pm1,5$ to $10,8\pm1,4$.

The mean number of stands per oestrus was also highly variable and ranged from $4,0\pm1,5$ to $11,8\pm2,0$. The discrepancy in the total number of stands and the total number of mounts during this period indicates that cows in oestrus were often mounted by cows not in oestrus.

The homosexual activity of partially weaned Mashona and Afrikaner cows during this pre-breeding period was almost identical (Figures 26 and 27). However, normally suckled Mashona cows in oestrus spent more time in homosexually active groups, mounted more often and were mounted more often than Afrikaner cows during the same period.

Figure 26. The incidence of homosexual behaviour in NS and PW Afrikaner cows prior to breeding.



NS Norm. suckled (n = 12): PW Part. weaned (n = 15)

Figure 27. The incidence of homosexual behaviour in NS and PW Mashona cows prior to breeding.

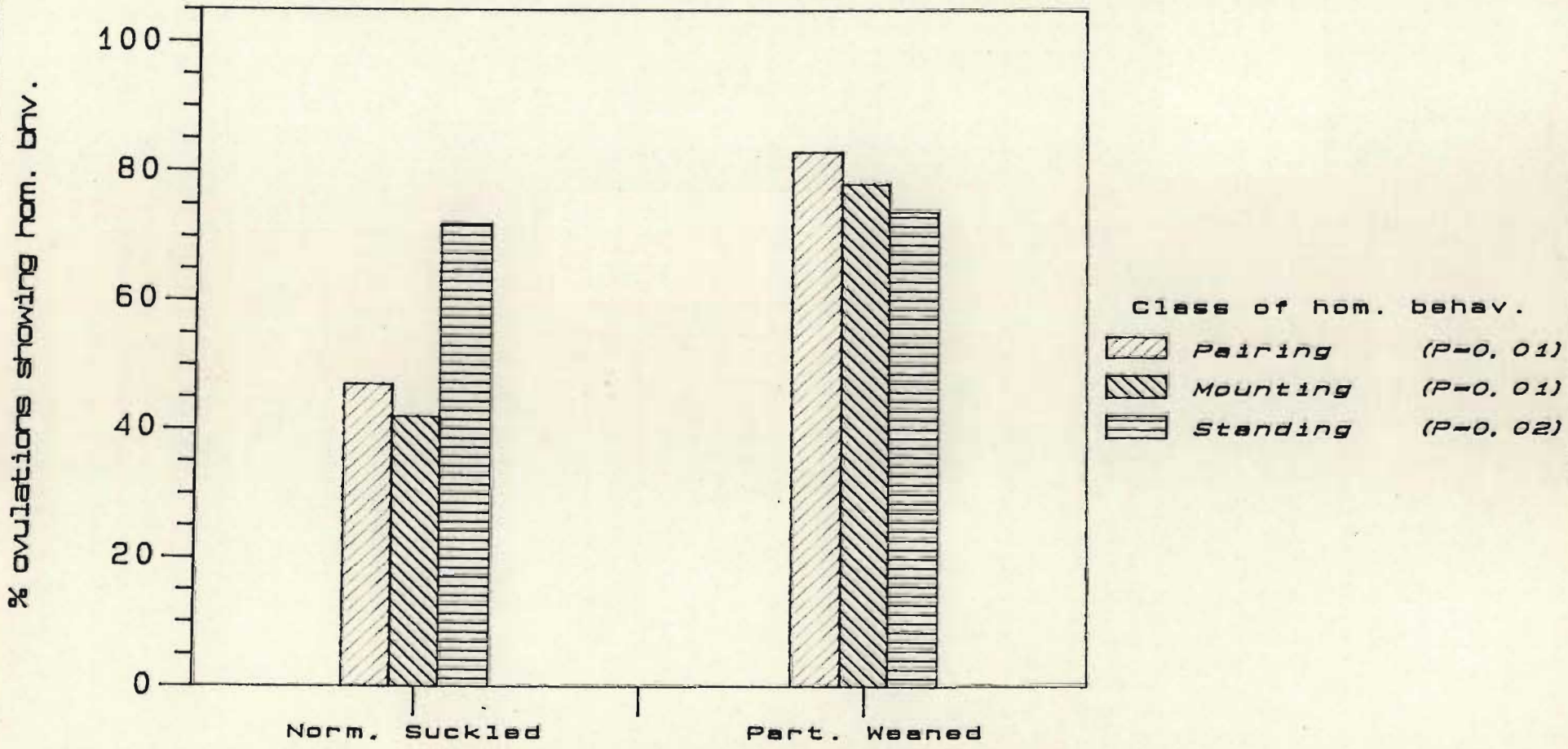


Table 46. Homosexual behaviour during 35 days prior to the breeding period in Afrikaner and Mashons cows which were either normally suckled or partially weaned.

Breed	Afrikaner			Mashona		
Partial weaning	With breeding	Prior to breeding	None	With breeding	Prior to breeding	None
Group number	22	25	15	16	13	10
Full ovulations	8	15	4	20	23	16
Homosexual pairing						
Incidence	1 P<0,01	12 P<0,10	1	11 P<0,05	19 P<0,01	6
Mean duration	2,25	1,9±0,4	2,25	1,1±0,3	1,3±0,2	2,2±1,0
Mounting						
Incidence	1 P<0,01	11 P<0,10	1	10 P<0,05	18 P<0,01	5
Mean number of mounts per oestrus	10	4,7±0,5	15	4,0±1,5	4,0±1,4	10,8±1,4
Standing						
Incidence	1 P<0,05	10	1	10 P<0,10	17 P<0,01	5
Mean number of stands per oestrus	9	9,7±2,4	7	4,0±1,5	6,7±1,0	11,8±2,0

Homosexual activity during the breeding period

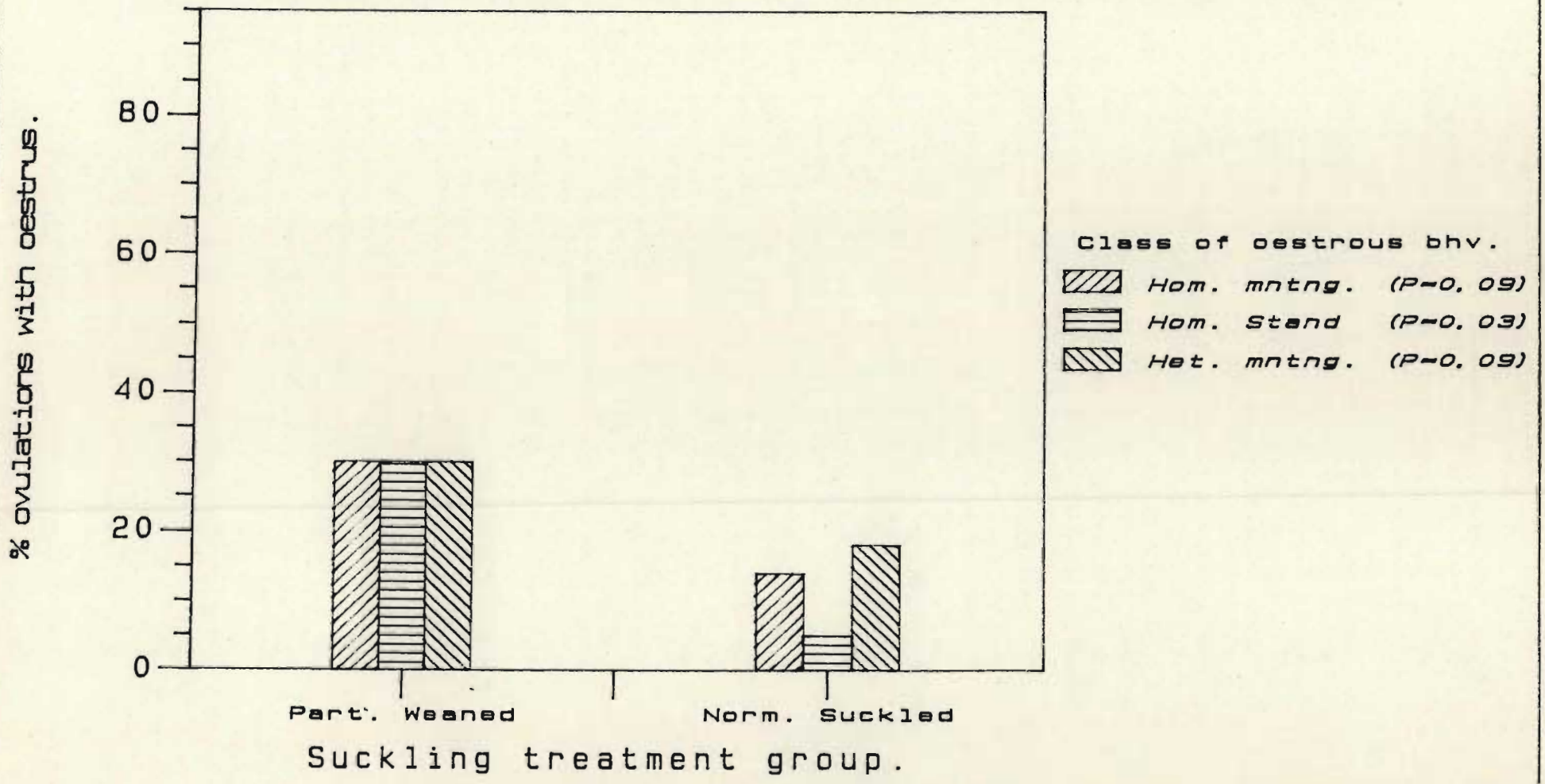
With the very low incidence of observed service in the control group (three out of seven ovulations) most comparisons in this section will be concerned with cows partially weaned during the breeding period and cows weaned prior to the breeding period.

Of the oestrous periods during the breeding period when service was observed, less than 15% were accompanied by homosexual mounting activity in normally suckled Afrikaner cows (Figure 28). Partial weaning of Afrikaner cows during the breeding period tended to increase ($P < 0,05$ to $P < 0,10$) the incidence of homosexual mounting activity to 30% of oestrous periods compared to normally suckled cows.

A similar trend ($P<0,10$) was observed in Mashona cows where the incidence of homosexual activity increased from around 25% in normally suckled cows to approximately 40% in partially weaned cows (Figure 29). In the control Mashona group, nine cows were seen to be served and in none of these oestrous periods was there any evidence of homosexual activity. There appears to have been a small increase in the expression of both components of homosexual mounting behaviour in cows partially weaned prior to the breeding period compared to cows normally suckled throughout the trial. However sample numbers are small and this trend requires confirmation.

In both Afrikaner and Mashona cows partial weaning during the breeding period significantly ($P<0,05$) increased the proportion of oestrous periods during which more than one homosexual event was recorded (Table 47). Instances where more than one mount per oestrus were recorded indicated that these took place over a

Figure 28. The incidence of oestrous behaviour in NS and PW Afrikaner cows during breeding. 25/1-5/3



NS Norm. suckled (n = 23): PW Part. weaned (n = 15)

Figure 29. The incidence of oestrous behaviour in NS and PW Mashona cows during breeding: 25/1 - 5/3

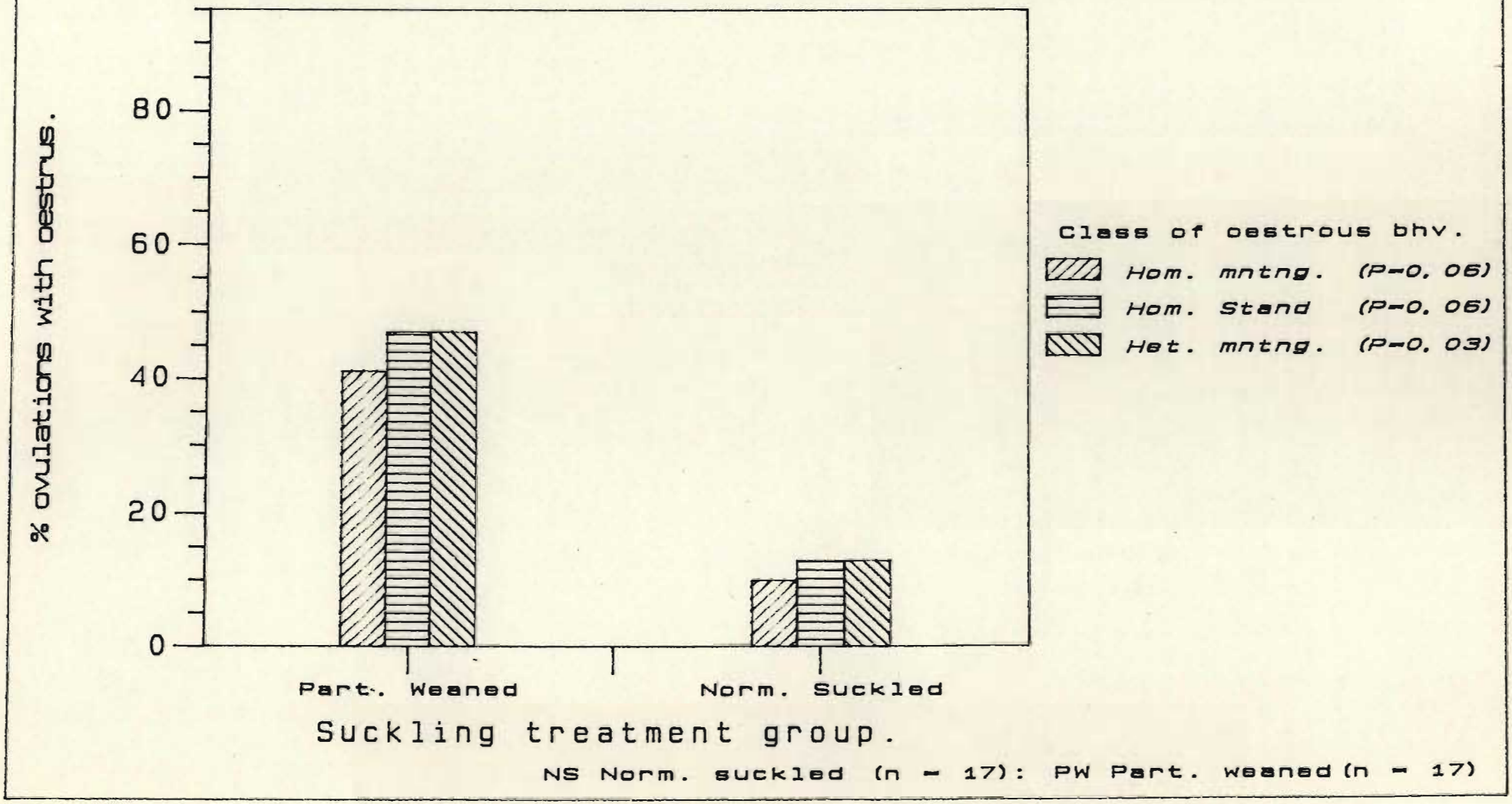


Table 47. Homosexual activity in conjunction with observed service during the breeding period from 25 January to 5 March in Afrikaner and Mashona cows which were either normally suckled, partially weaned for 35 days prior to breeding or partially weaned during the breeding period.

Breed	Afrikaner			Mashona		
Partial weaning	With breeding	Prior to breeing	None	With breeding	Prior to breeding	None
Full ovuls.	23	15	7	17	17	3
Observed services	17	14	3	13	15	9
Mounting other cows						
Incidence	7 P<0,01	2	1	7 P<0,10	3	0
Mean number of mounts per 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 P<0,05	0	0	6 P<0,05	0	0
Mean duration of multiple mounts	2,0±0,2a	-	-	0,8±0,2b	-	-
Mean interval first mount to first service	2,0±0,3c	0,5±0,04	7,0	0,7±0,3d	2,9±1,7	-
Standing for other cows						
Incidence	7 P<0,05	1	0	8 P<0,10	4	0
Mean number of stands per oestrus	1,7±0,4	2,0±	-	1,9±0,4	3,8±2,4	-
Incidence of multiple stands	3	0	0	5	2	0
Mean duration of multiple stands	1,1±0,3	-	-	3,9±2,0	-	-
Mean interval from first stand to first mount	1,2±0,4	0,7	-	3,6±1,8	0,2±0,7	-

a--b P<0,01 c--d P<0,01

significantly ($P < 0,01$) shorter period (by 1,2 hours) in the Mashona cow than in the Afrikaner cow (Table 47). In cows partially weaned with the introduction of bulls, the interval from first homosexual mount to first observed service was significantly ($P < 0,01$) shorter period (by 1,3 hours) in Mashona cows than in Afrikaner cows.

Heterosexual activity

Heterosexual mounting (by cow)

Partially weaning cows at the start of the breeding period significantly ($P<0,05$) increased the incidence of cows mounting the bull by 25% in Mashona cows and 20% in Afrikaner cows (Figure 29).

The mean number of mounts per oestrus ranged from one to three with no significant differences between treatments. However there was a consistent trend in both breeds for partially weaned cows to have a higher mean number of mounts per oestrus (Table 48). These data, although not conclusive, indicate an increase in the intensity of expression of oestrus amongst partially weaned cows compared to their normally suckled contemporaries.

Table 48. Heterosexual activity with observed service over the breeding period in Afrikaner and Mashona cows which were either normally suckled, partially weaned for 35 days prior to breeding or partially weaned in conjunction with breeding.

Breed	Afrikaner			Mashona		
Partial weaning	With breeding	Prior to breeding	None	With breeding	Prior to breeding	None
Full ovuls.	23	15	7	20	23	16
Observed services	17	14	3	13	15	9
Mounting the bull						
Incidence	7 P<0,10	2	2	8 P<0,05	3	1
Mean number of mounts/oestrus	3,0±1,2	1,0±0	1,5±0,5	2,6±0,7	1,3±0,3	1,0
Incidence of multiple mounts	5 P<0,01	0	1	7	1	0
Mean duration of multiple mounts	1,7±0,5	-	10,7	0,8±0,3	0,08	-
Mean interval first mount to first service	1,4±0,5	0,4±0,3	8,8±2,0	0,5±0,4	5,8±2,4	0,8
Chin-resting by the bull						
Incidence	15	13	2	13	14	7
Mean number of rests/oestrus	8,0±1,4	5,9±1,5	10,0±5,7	8,1±1,9	8,2±1,6	5,3±1,3
Incidence of multiple chin-rests	14	11	2	11	13	7
Mean duration of multiple rests	3,5±1,0	5,5±1,5	10,6±1,3	5,5±1,3	5,2±1,3	2,5±1,3
Mean interval from first rest to first service	3,2±1,0	3,1±1,3	10,0±1,4	3,9±1,4	4,1±1,2	3,8±4,0

Chin-resting

In all of the above components of oestrus there has been a consistent trend for cows partially weaned with the introduction of bulls to exhibit more overt oestrous periods characterised by higher levels of activity than normally suckled cows. In contrast to this observation, chin-resting by the bull was the only component of heterosexual activity which appears to have been totally immune to the effects of partial weaning on behaviour during breeding (Figures 28 and 29).

During oestrus, chin-resting was the most frequently observed component of oestrus, closely followed by nosing of the perineum (Figures 28 and 29). The number of events per oestrus period did not differ significantly between groups and ranged from 5,5±1,5 to 8,2±1,6 (Table 48). The duration of chin-resting during oestrus was similar across all treatment groups and ranged from 2,5±1,3 hours to 5,5±1,5 hours. Similarly, the interval from first event to first service was not modified by partial weaning and ranged from 3,1±1,0 hours to 4,1±1,2 hours.

Table 49. The incidence and characteristics of nosing of the perineum by the bull and flehmen with observed service, over the breeding period in Afrikaner and Mashona cows, which had been normally suckled throughout or partially weaned for 35 days prior to breeding or partially weaned in conjunction with breeding.

Breed	Afrikaner			Mashona		
Partial weaning	With breeding	Prior to breeding	None	With breeding	Prior to breeding	None
Full ovuls.	23	15	7	17	17	13
Observed services	17	14	3	13	15	9
Nosing of perineum						
Incidence	7 P<0,05	11	2	13 P<0,01	8	6
Mean number of sniffs/oestrus	2,9±0,8	3,4±1,0	5,0±3,0	2,6±0,4	6,5±1,1	5,7±0,7
Incidence of multiple sniffs	5	7	2	9	8	6
Mean duration of multiple sniffs	5,3±2,5	7,8±1,7	10,8±1,8	4,5±1,3	6,3±3,9	4,7±1,5
Mean interval first sniff to first service	4,5±2,0	4,0±1,7	9,9±1,4	3,8±1,4	7,0±1,7	4,4±1,6
Flehmen by the bull						
Incidence	5	9	2	6	5	4
Mean number of flehmen/oestrus	1,2±0,2	1,7±0,3	1,5±0,5	1,3±0,2	2,8±0,5	2,3±0,8
Incidence of multiple flehmen	1	3	1	2	3	2
Mean duration of multiple flehmen	1,8	3,9±3,6	11,4	1,2±0,6	3,8±2,1	1,6±0,5
Mean interval from first flehmen to first service	5,1±2,7	2,2±2,5	9,8±1,4	3,7±2,3	6,8±2,0	1,2±0,8

Nosing of the perineum

With this component of oestrus, there was a significant breed by suckling status interaction. Afrikaner cows, which had been partially weaned prior to the breeding period stimulated significantly ($P<0,05$) more (75% of oestrous periods) sniffing by the bull than cows which had been partially weaned when the bulls were introduced (30% of oestrous periods, Figure 28). In Mashona cows however the situation was reversed. Bulls nosed partially weaned cows in significantly ($P<0,01$) more oestrous periods (75%) than in the case of normally suckled cows (50%, Figure 29) weaned prior to breeding.

The incidence of multiple events in this component of oestrus did not differ between any of the treatment groups. Mean duration and mean interval from first event to first service showed considerable variation and no significant treatment effects were recorded (Table 49). Mean number of events ranged from $2,6\pm0,4$ to $6,5\pm1,1$ hours with a range in mean duration of $5,4\pm1,3$ to $7,8\pm1,7$ hours. Mean interval from first event to first service varied greatly between the treatment groups over the range $3,8\pm1,4$ to $7,0\pm1,7$ hours.

Flehmen

In keeping with all components of male initiated behaviour in the Afrikaner, cows which had been partially weaned prior to the breeding period tended to stimulate a bull response (in this case flehmen) in a greater number of oestrous periods than cows partially weaned with breeding (Table 49). In Mashona cows the incidence of flehmen was almost identical in the two treatment groups. Of the three male components of oestrus excluding service, chin-resting, nosing of the perineum and flehmen, the

latter was observed in fewer oestrous periods than the other two.

Of the periods when flehmen was observed only 5/16 in the Afrikaner and 7/15 instances in the Mashona involved more than one occurrence per oestrus (Table 49). The mean number of events per oestrus was surprisingly low and did not differ significantly between any of the groups in the range 1,2±0,2 to 2,8±0,5. Large variations in the mean interval from first occurrence to first service were recorded and with no significant treatment effects. This interval ranged from 1,2±0,8 hours to 6,8±2,0 hours.

Observed service

During the breeding period of 39 days 45 and 47 full ovulations were estimated to have occurred in the Afrikaner and Mashona cows respectively (Table 50). Of these full ovulations during the breeding period, 69% and 79% were accompanied by observed service amongst Afrikaner and Mashona cows respectively.

During the study 29 and 35 conceptions (confirmed by the birth of a calf) were recorded in the Afrikaner and Mashona cows respectively and more than 70% conceptions in each breed were accompanied by observed service . This ratio is comparable to the figure of 74% of full ovulations which were accompanied by observed service and would indicate that more than 90% of the ovulations in this study were accompanied by natural insemination.

A significantly ($P<0,05$) higher proportion of cows weaned prior to the breeding period (100%) compared with the control group

(43%) were served during the surveillance period than at any other time. Cows weaned during the breeding period tended to have a lower incidence of observed service than cows weaned prior to breeding but the difference was not significant.

This trend was also observed in Mashona cows although once again the difference was not significant. Across the two breeds, partial weaning prior to breeding significantly ($P < 0,01$) increased the incidence of service during the hours of 0500 to 0900 and 1500 to 1900 from 40% in control cows to 91% in weaned cows. Within each breed, partial weaning during the breeding period did not improve the observation of service in conjunction with oestrus, compared with either of the remaining two groups.

Partial weaning in conjunction with breeding significantly ($P<0,01$) increased the incidence of multiple (two or more) services from 24% in cows which had been partially weaned prior to breeding to 63% in cows partially weaned during breeding (derived from Table 50). Within each breed, this difference approached significance in Afrikaner cows ($P<0,10$) and was significant in Mashona cows ($P<0,05$, Table 50).

The interval from the first to last service, where multiple services were observed, did not differ significantly between any of the treatment groups (Table 50).

Table 50. Characteristics of observed service in Afrikaner and Mashona cows in three treatment groups. The first group was partially weaned during the breeding season and the second group was weaned for 35 days prior to the breeding season. A control group was normally suckled for the duration of the trial.

Breed	Afrikaner			Mashona		
Partial weaning	With breeding	Prior to breeding	None	With breeding	Prior to breeding	None
Group no.	22	25	15	16	13	10
25 January to 5 March						
Full ovulations within this period	23	15	7	17	17	13
The incidence of observed service with oestrus	17	14 P<0,05	3	13	15	9
Mean number of services per oestrus	1,5±0,2	1,3±0,2	2,0±0,6	2,1±0,2	1,3±0,2	1,2±0,2
Incidence of multiple observed services (>1)	10 P<0,10	3	2	9 P<0,05	4	1
Mean duration of multiple services	0,8±0,2	0,6±0,3	1,1±0,6	1,2±0,4	1,2±0,6	2,2
Confirmed conceptions	13	9 P<0,10	7	15 P<0,10	11	9
Observed conceptions	10	9 P<0,05	3	10	10 P<0,10	5
Mean PPI to observed conception	117±9	119±3	111±13	99±7	123±5	126±8

PPI Post-partum interval.

Conception rates

Although partial weaning prior to and during breeding increased the total number of full ovulations within this period, a proportion of these induced ovulations must have been sub-fertile since there was a significantly ($P<0,10$ and $P<0,05$ respectively) higher incidence of full ovulations without conception in the Afrikaner cows in these two groups compared with the control group. Amongst Mashona cows the number of conceptions in relation to the number of full ovulations did not differ between the three treatment groups.

Significantly more observed services ($P<0,05$ in Afrikaner cows and $P<0,10$ in Mashona cows) corresponding to conception were recorded amongst cows which had been weaned prior to the breeding period than in the control cows in this study (Table 50). This was due to the high incidence of service during the day in cows which had been weaned prior to the breeding period. This shift in time of oestrus was not marked in cows partially weaned during breeding. In support of the findings from Chapter 9, the results in Table 50 indicate that the normally suckled Afrikaner and Mashona cow may have had as high as a 60% incidence of service during the night in this study. It is difficult to explain this apparent change in preferred mating times. In cows weaned during breeding, the onset of oestrus may have been delayed due to the afternoon suckling programme. However this does not account for the high incidence of observed service in cows which had been weaned before breeding commenced. In these cows, suckling was on a free access basis during breeding and there seems to be little possibility that the timing of oestrus could have been inadvertently linked to management factors.

A summary of all oestrus related behaviour during breeding

Homosexual

All oestrus-related behaviour observed during the breeding period including ovulations where service was not observed has been summarised in Table 51.

With partial weaning, the incidence of homosexual mounting was significantly ($P<0,05$) increased in Afrikaner cows whereas in the Mashona this increase only approached significance ($P<0,10$). The incidence of homosexual standing was not affected by breed or suckling status to the same degree. Thus, reduced suckling intensity during breeding would appear to have increased the incidence of the proceptive components of oestrus (mounting) without increasing the receptive components (standing).

Within an oestrous period the finer characteristics of homosexual activity were not significantly altered by suckling status.

Heterosexual

Bull initiated activity including nosing of the perineum, chin-resting and flehmen were observed significantly ($P<0,05$, $P<0,05$ and $P<0,05$, respectively) more frequently in Afrikaner cows which had been partially weaned prior to the breeding period than in Afrikaner cows normally suckled throughout the trial, supporting earlier findings (Table 51). Cows partially weaned during the breeding period tended to have an incidence of these traits which was intermediate and did not differ significantly from either of the other two groups.

Table 51. A summary of all oestrus-related behaviour in Afrikaner and Mashona cows, partially weaned during the breeding period (With), partially weaned for 35 days before the breeding period (Prior) or normally suckled throughout the trial (None).

Breed	Afrikaner			Mashona		
Partial weaning	With breeding	Prior to breeding	None	With breeding	Prior to breeding	None
Group number	22	25	15	16	13	10
Full ovulations	23	15	7	17	17	13
Homosexual activity						
Mounting						
Incidence	9 P<0,05	2	2	6 P<0,10	2	1
Mean number of mounts/oestrus	3,2±0,6	2,0±1,0	2,5±0,5	2,8±0,5	1,5±0,5	1
Standing						
Incidence	7 P<0,08	1	0	6	3	1
Mean number of stands/oestrus	1,7±0,4	2	0	2,5±0,5	2,0±0,6	1
Heterosexual activity						
Nosing of the perineum						
Incidence	9	16 P<0,05	4	10	10	8
Mean number of events/oestrus	1,8±0,4	3,0±0,5	3,8±1,4	2,2±0,5	5,8±1,2	4,3±0,8
Chin-resting						
Incidence	21	18 P<0,05	4	11	15 P<0,10	8
Mean number of events/oestrus	6,0±1.0	5,2±1,2	8,5±2,0	6,6±2,3	6,9±1,5	4,1±1,2
Flehmen						
Incidence	6 P<0,01	13 P<0,05	3	7	7	3
Mean number of events/oestrus	1,0±0	1,7±0,3	1,3±0,3	1,4±0,3	2,3±0,4	2,7±0,9
Cow mounting the bull						
Incidence	8	2	2	8 P<0,10	3	1
Mean number of mounts/oestrus	2,8±1,1	1,0±0,0	2,5±0,5	1,8±0,3	1,3±0,3	1

Amongst Mashona cows, bull initiated activity did not differ between any of the three treatment groups, Table 51.

From the pooled results of Afrikaner and Mashona cows within treatments, it appears that cows partially weaned during breeding (16/40) are more likely ($P<0,05$) to mount the bull prior to ovulation than normally suckled cows (3/20). The incidence of heterosexual mounting by cows which had been partially weaned prior to breeding did not differ from that recorded for cows in the control group.

DISCUSSION

The first significant finding in this study has been the marked increase in homosexual activity observed in cows suckling their calves once a day prior to the introduction of bulls. Yet when these cows had their calves returned to them and their contemporaries were partially weaned the situation was immediately and significantly reversed. Thus, partial weaning per se will enhance the expression of homosexual behaviour during oestrus. Perhaps the clue to this intensified expression of oestrus lies in the fact that there appears to have been a slight increase in ovulation rate in partially weaned cows. In the absence of a decrease in the number of anovulatory cows in the group this increased mean ovulation rate has followed an earlier resumption of ovarian activity in certain partially weaned cows, possibly these cows were within 20 to 30 days of spontaneous ovulation.

Partial weaning in the absence of any male effect appears to have increased gonadotropin activity including baseline concentrations

of LH. However this increase was insufficient to induce ovulation in all anovulatory cows without being preceded by a spontaneous endogenous increase in gonadotropin activity. Partial weaning and the introduction of bulls in unison presumably raised gonadotropin concentrations to a level, which in anovulatory cows, was able to induce persistent cyclic ovarian activity. Admittedly, treatment application in the present study was confounded with post-partum interval but earlier studies (Chapter 4) demonstrated that partial weaning in the presence of bulls 28 days post partum induced ovulation by 53 days post partum (Table 7).

Reduced suckling intensity is known to induce higher LH concentrations in anovulatory post-partum cows within 48 hours (Carruthers and Hafs, 1980; Walters et al., 1982b; Edwards, 1985). The magnitude of this increase might depend on whether males are present or not. The present study has shown that some cows will ovulate earlier with reduced suckling in the absence of the bull and that the intensity of oestrus expression will be increased per se. This increased intensity of oestrus takes the form of a higher number of oestrous periods (+50% in Afrikaners and +30% in Mashonas) being accompanied by homosexual behaviour as distinct from an increase in activity within each oestrous period.

Within either the pre-breeding period or the breeding period, the number of mounts and stands per oestrus did not differ between treatments. In the presence of bulls however, there was a decline in homosexual activity from a mean of 7,3 mounts during pre-breeding to 3,3 during the breeding period (derived from Table 47). This concurs with work by Kilgour et al. (1977) and

Mattner et al. (1974) who reported that riding activity in sexually active groups was inhibited by the near presence of a bull.

Oestrous behaviour is driven by oestrogens in cattle (Short et al., 1973; Rhodes and Randel, 1978) and in sheep (Scaramuzzi et al., 1971) but the ultimate expression of these stimuli appears to be modified by higher brain function. Lindsay (1966) makes this observation in sheep where social factors (size of paddock, ram densities, proximity of mating sheep) altered the expression of oestrus.

The increased incidence of homosexual activity per ovulation in partially weaned cows in the absence of males was interesting. This finding indicates that either oestrogen levels were higher or the responsiveness of behaviour centres to similar steroid concentrations was enhanced when suckling intensity was reduced. This enhanced activity might have hastened the resumption of ovarian activity in herd mates in this group. Such an effect has been proposed in sheep (Riches and Watson, 1954; Schinkel, 1954; Radford and Watson, 1957). Such a situation could have occurred in the light of the following: running vasectomised bulls with cows after calving has shortened the mean post-partum interval to oestrus (MacMillan et al., 1979; Zalesky et al., 1984); oronasal applications of cervical mucous have improved the synchronisation of oestrus following induced luteolysis (Izard and Vandenbergh, 1982b); inclement weather, proximity of observers and slippery floors may inhibit the expression of oestrous behaviour (Houpt and Wolski, 1982). These references testify to the fact that the endocrine system and therefore the

expression of oestrus is closely linked to many components of the cow's environment.

Partial weaning during the breeding period enhanced the expression of all components of homosexual behaviour in Afrikaner and Mashona cows. The male component of oestrus in Afrikaner and Mashona cows was not as simple. Bulls were more stimulated apparently by cows which had been partially weaned prior to the breeding period (service observed in 91%, of ovulations) than in cows partially weaned during the breeding period (more active homosexually, yet service was observed in only 70% of ovulations, $P<0,05$).

In Afrikaner cows this heightened interest may have been prompted by stronger odours (increased nosing of the perineum) in cows which had been partially weaned prior to the breeding period (Figure 28). Odours specific to oestrus are excreted in the urine (Ladewig and Hart, 1981) and are in evidence up to three days prior to ovulation (Kiddy and Mitchell, 1981). They appear to be essential during oestrus for partner seeking (Fletcher and Lindsay, 1968). Why partial weaning prior to breeding should enhance (apparently) these odours during the breeding period is not readily apparent. The effect may be related to post-partum interval to first ovulation and might be partly responsible for the low incidence of observed service with first ovulation *post partum* reported by King *et al.* (1976).

The situation is not simplified by the fact that Mashona cows, weaned during the breeding period, had a higher incidence of nosing of the perineum by bulls during oestrus even though their observed service rate was lower than in cows weaned prior to

breeding. Possibly these cows released pheromones which attracted the bull but at the same time were not receptive to being mounted by the bull.

The Mashona cows in this study were predictably immune from treatment effects on either the resumption of ovarian activity or conception rate (Table 45). The only effect restricted suckling had in the Mashona, was to shorten the mean post-partum interval to conception in cows weaned with the introduction of bulls. Despite no changes in luteal function and reproductive performance being recorded, changes in patterns of oestrous behaviour did occur in the Mashona and the Afrikaner with changes in suckling status. From the above it would appear that modifications of behaviour as a consequence of reduced suckling intensity are unlikely to be mediated by the endocrine system. If this is the case, then changes in suckling intensity are altering the responsiveness of higher brain centres governing the expression of oestrus.

CONCLUSIONS

Partial weaning *per se* in the absence of bulls appears to stimulate ovulation in cows in shallow anoestrous and induce a more intense expression of homosexual activity during oestrus than in normally suckled cows. Of great interest was the finding that partial weaning prior to the breeding period significantly increased the incidence of observed service during breeding when bulls were introduced, compared to cows partially weaned with the introduction of bulls. In Afrikaner cows this effect may have been a result of bulls more easily finding cows weaned prior to breeding. Partner seeking may have been facilitated in cows

weaned prior to breeding because they excreted more potent odours at the approach of oestrus than their contemporaries.

Partial weaning applied with the introduction of bulls will stimulate ovulation in the majority of anovulatory Afrikaner cows. In the Afrikaner, this synergistic effect of reduced suckling and the presence of bulls induced higher levels of homosexual and heterosexual mounting activity and nosing of the perineum by the bull. Although in both Afrikaner and Mashona cows partial weaning during breeding increases the incidence of multiple service prior to ovulation, more ovulations were seen to be accompanied by service amongst cows which had been partially weaned prior to breeding. This apparent contradiction is not important since ultimately partial weaning prior to breeding did not improve conception rates whereas partial weaning during breeding did. Thus, cows weaned before breeding appear to have released stronger odours at the approach of oestrus to which the bull was more attracted initially. In contrast, cows weaned during breeding were more receptive to the bull as indicated by increased mounting activity.

In the presence of increased attention from bulls, homosexual and heterosexual activity initiated by cows was depressed in Afrikaner cows and to a lesser extent in Mashona cows. Cows partially weaned prior to the breeding period stimulated more interest in bulls than cows weaned with breeding. Thus once partial weaning has been applied it must be maintained until the end of the breeding season, there was no evidence to indicate that temporary partial weaning before breeding is useful in the Afrikaner.

Partial weaning prior to the breeding period had three effects: firstly it was able to induce a higher number of full ovulations in the Afrikaner cow; secondly it caused a higher incidence of overt homosexual activity in both Afrikaner and Mashona cows; and thirdly it significantly increased the incidence of service being observed prior to full ovulation in Afrikaner cows.

In the Mashona cows partial weaning at any time had no significant effect on the incidence of observed service prior to full ovulation. This finding is emphasised by the fact that in all other components of oestrus-related behaviour, cows which had been partially weaned prior to the breeding season behaved similarly to their contemporaries in the control groups.

The significant reduction in the number of cows weaned during the breeding period which were anovulatory for the duration of this trial may have been related to the increased oestrous activity during breeding (hypothalamotropic stimulation) or an increase in hypothalamic responsiveness (up-regulation of receptors) to higher brain stimulation.

Chapter 11

The effect of exogenous progesterone for nine days on oestrus-related behaviour in Afrikaner and Mashona cows which were either normally suckled or partially weaned from 25 days post partum.

INTRODUCTION

All of the behaviour data presented in this chapter was compiled during the study described in detail in Chapter 6. From the results presented in that chapter it was apparent that partial weaning applied 25 days post partum and the insertion of a progesterone releasing intra-vaginal device (PRID) for nine days from 35 days post partum significantly increased the incidence of ovulation 45 to 55 days post partum. In normally suckled cows treated with a PRID 10% of ovulations occurred between 45 and 55 days post partum whilst in partially weaned cows 50% ovulations occurred during this same period. Not one cow conceived to the ovulations which occurred within this period.

Findings to date, indicate that the failure of conception to first ovulations induced by a reduction in the suckling stimulus are almost certainly not as a result of any behaviour deficiency and in particular the absence of natural insemination. Furthermore, Ramirez-Godinez et al. (1981) has reported that the administration of a progestogen before the first post-partum oestrus increased conception rates and reduced the percentage of previously anoestrous cows exhibiting short cycles after their calves were removed. Thus, the lack of response to progesterone treatment reported in Chapter 6 was surprising.

However, the conditions prevailing at the time of this study were

less than optimal due to poor rainfall. Nevertheless, a primary objective in the present study was to establish whether the provision of progesterone prior to early ovulation induced by partial weaning, had any effect on oestrous behaviour prior to this ovulation in weaned compared to normally suckled cows, treated with progesterone.

The role of progesterone in behavioural oestrus in the cow is not clear. Melampy et al. (1957) suggested that small doses of progesterone had a synergistic effect when administered around the time of an exogenous oestrogen injection. Shelton and Carrick (1969) reported that progesterone restored sensitivity to exogenous oestradiol in heifers which had become refractory to its effects at the time of calving. These latter researchers proposed an interesting hypothesis which stated that following the high levels of oestrogen secreted during late pregnancy (Gorski and Erb, 1959) cows become refractory to endogenous oestrogen until being exposed to progesterone after primary ovulation, a theory which accommodates the high incidence of "silent oestrus" with the first ovulation post partum. Following the first short luteal phase sensitivity to oestrogen is restored and the full expression of oestrus occurs at first full ovulation.

MATERIALS AND METHODS

Experimental animals and method

A total of 22 Afrikaner and 23 Mashona cows were used in this study. Of these cows, 10 Afrikaner cows and 11 Mashona cows were suckled once a day from 25 days until 100 days post partum when the trial ended and calves were returned to their dams. The balance of the cows and calves, 12 Afrikaners and 12 Mashonas

comprised the control groups and were normally suckled for the duration of the trial. A Progesterone Releasing Intra-vaginal Device was inserted into all cows at exactly 35 days post partum and removed nine days later.

All cows were run in two groups one of normally suckled cows (control) and the other group of partially weaned cows. Partially weaned cows suckled their calves once each day at 1600 hours. Although cows were only in this trial from day 25 to 100 post partum the two treatment groups were left intact until the last cow had completed 100 days in the trial. For this reason calves were only returned to their dams when all cows had completed the trial.

Observation of oestrous behaviour

Observers monitored nine components of oestrus-related behaviour between 0500 hours and 1900 hours each day. Data was compiled on the basis of a five minute observation interval. Two observers were assigned to each group and constant monitoring of herds was possible within the hours indicated.

Experimental area

In the year that this trial was conducted, rainfall was below normal and paddocks had to be used that were not ideal. The main problems were large paddocks which involved excessive movement of cows during grazing, walking to and from water points and during mustering for bleeding and suckling of calves. The effects of this stress were reflected in the longer (+15 days) than usual post-partum intervals to conception, particularly in the Mashona cows.

RESULTS

Although there were fewer anovulatory cows amongst partially weaned Afrikaner cows 10% versus 25% of normally suckled cows the difference was not statistically significant. All Mashona cows ovulated within 100 days of calving (Table 52).

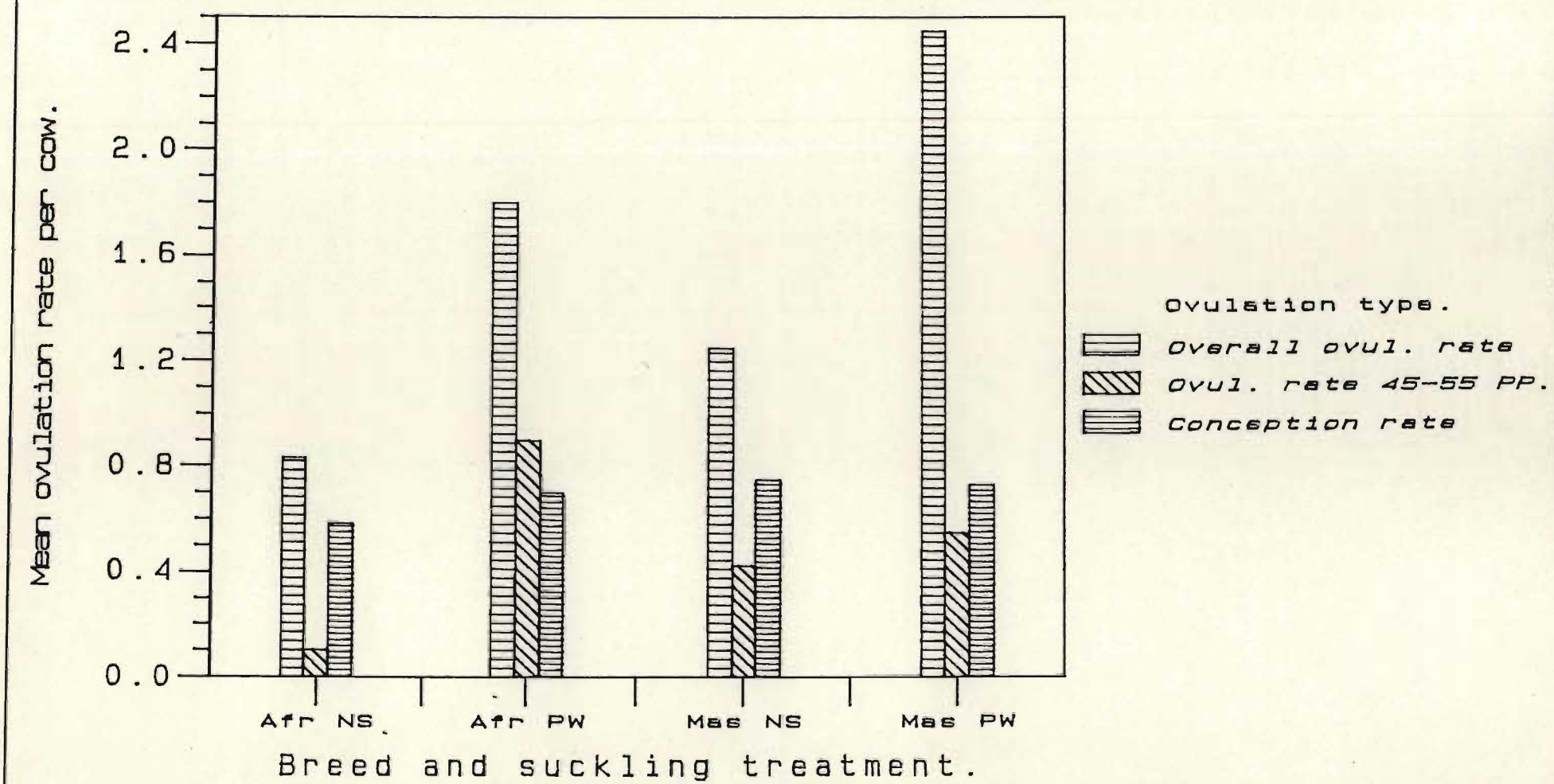
Table 52. Characteristics of the resumption of ovarian activity in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to exposure to exogenous progesterone from a PRID inserted 35 days post partum.

Breed	Afrikaner		Mashona	
Treatment	Normally suckled	Partially weaned	Normally suckled	Partially weaned
Group no.	12	10	12	11
Cows anovulatory for the duration of the trial	3	1	0	0
Incidence of two or more ovulations	1 P<0,05	6	5 P<0,05	10
Total number of full ovulations	10	18	15	27

Ovulation rate

As expected, partial weaning enabled both Afrikaner and Mashona cows to escape from the suckling suppression of the early resumption of ovarian activity. The first evidence of luteal function was at 49±3 days and 37±4 days post partum in the Afrikaner and Mashona respectively, some 20 days earlier than their normally suckled contemporaries (Table 19, Chapter 6). This had the effect of significantly (P<0,05) increasing the mean ovulation rate per cow over the experimental period in partially weaned cows of both breeds (Figure 30).

Figure 30. The mean number of ovulations per cow in NS and PW Afr. and Mas. cows up to 100 days PP.



The period of greatest interest in this study was 45 to 55 days post partum. Partial weaning and PRID treatments in the Afrikaner cows were successful in that a significantly ($P<0,001$) higher proportion of cows in this group ovulated within the target period compared to normally suckled Afrikaner cows (Figure 30). Partial weaning in the Mashona cows 25 days post partum did not significantly increase the incidence of ovulation during this target period to the same extent. The mean ovulation rate indicates that most of the Mashona cows had ovulated just prior to the insertion of the PRID and did not ovulate until 21 days later (Figure 30). This is confirmed by the results presented in Table 17 (Chapter 6).

Homosexual activity

For the first time in this series of studies, partial weaning failed to increase the incidence of homosexual activity. In general there was very little homosexual activity during those oestrous periods during which service was observed and follows the pattern observed in the last study. The homosexual activity which was recorded amounted to less the 10% of oestrous periods (Table 53).

Equally surprising was the finding that all homosexual activity recorded in this trial occurred after cows were served (Table 53). This interval ranged from five to eleven hours after first service was observed.

Table 53. Homosexual activity during oestrus with observed service in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to the insertion of a PRID for nine days, beginning 35 days post partum.

Breed	Afrikaner		Mashona	
Treatment	Normally suckled	Partially weaned	Normally suckled	Partially weaned
Full ovuls.	10	18	15	27
Observed services	10 P<0,001	3	15 P<0,001	14
Mounting other cows				
Incidence	0	1	2	2
Mean number of mounts/oestrus		2,0	1,0±0,0	3,0±2,0
Incidence of multiple mounts		1		1
Mean duration of multiple mounts		0,42		2,6
Mean interval first mount to first service		+6,2±0,0	+11,0±3,8	+6,0±0,3
Standing for other cows				
Incidence	0	2	2	1
Mean number of stands per oestrus		2,5±0,0	1,0±0,0	4,0
Incidence of multiple mounts		2		1
Mean duration of multiple stands		0,3±0,1		1,1
Mean interval from first stand to first mount		+9,3±3,2	+10,6±1,7	+5,4

Heterosexual activity

Mounting by cows

Partial weaning prior to insertion of a PRID significantly ($P<0,001$) reduced the incidence of cows mounting the bull in Mashona cows from 87% of oestrous periods in normally suckled cows to 14% (Table 54). A similar change in behaviour may have occurred amongst Afrikaner cows ($P<0,10$) but too few partially weaned Afrikaner cows were seen to be served for a reliable comparison to be made. Contrary to all previous results, most mounting of the bull by cows commenced after the first observed service was recorded and was observed from $8,2\pm1,2$ to $9,3\pm1,2$ hours after first service. This interval was similar in both groups of Mashona cows and partially weaned Afrikaner cows (Table 54).

Chin-resting

Chin-resting by the bull was observed in all oestrous periods in Afrikaner cows and in all except two oestrous periods in Mashona cows. The number of events per oestrus did not differ significantly between any of the four treatment groups and ranged from $6,7\pm1,2$ to $10,3\pm3,4$ hours (Table 54). Multiple (more than three) events were recorded in a similar proportion (70%) of oestrous periods in each treatment group. These results are consistent with earlier work and confirm that bulls were active with the cows that they found in oestrus but does not indicate how effective they were in detecting heat.

Duration of chin-resting from the first to the last event and the mean interval from first chin-rest to first service were similar across breeds and treatments (Table 54).

Table 54. Heterosexual activity during oestrus with observed service in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to the insertion of a PRID for nine days beginning 35 days post partum.

Breed	Afrikaner		Mashona	
Treatment	Normally suckled	Partially weaned	Normally suckled	Partially weaned
Full ovuls.	10	18	15	27
Observed services	10 P<0,001	3	15 P<0,01	14
Mounting the bull				
Incidence	7 P<0,10	0	13 P<0,0001	2
Mean number of mounts per oestrus	2,2±0,6		1,9±0,5	1,5±0,5
Incidence of multiple mounts (>1)	3	0	5	1
Mean duration of multiple mounts	2,1±1,2		2,8±1,0	0,9
Mean interval first mount to first service	+8,2±1,2		+9,3±1,2	+9,3±1,0
Chin-resting by the bull				
Incidence	10	3	15	12
Mean number of rests per oestrus	7,6±2,4	10,3±3,4	6,7±1,2	9,8±1,5
Incidence of multiple chin-rests	6	3	9	10
Mean duration of multiple chin-rests	5,4±1,5	4,0±0,4	5,3±0,9	5,5±0,9
Mean interval from first rest to first service	2,9±1,0	0,5±0,2	2,6±0,9	2,0±0,7

Nosing of the perineum

Nosing of the perineum of cows in oestrus was observed in 88% of oestrous periods. Neither breed nor suckling status modified any aspect of this behaviour (Table 55). The mean number of events per oestrus ranged from 3,7±0,6 to 5,8±1,1 over a period which lasted from 2,7±1,9 to 6,3±0,7 hours. Nosing of the perineum was observed for the first time from 2,1±0,7 to 2,9±,9 hours before the first observed service. The oestrus specific odours released prior to and during oestrus were clearly present and bulls were responding to them.

Flehmen

Flehmen was observed in 83% of oestrus periods during which oestrus was observed and occurred from 2,3±0,5 to 3,9±0,9 times over a period which lasted 4,0±1,9 to 5,8±1,3 hours (Table 55). The incidence of flehmen did not differ between groups. The first occurrence of flehmen occurred from 1,9±1,3 hours before to 0,8±1,2 hours after the first service. On average this was slightly later than the first incidence of nosing of the perineum.

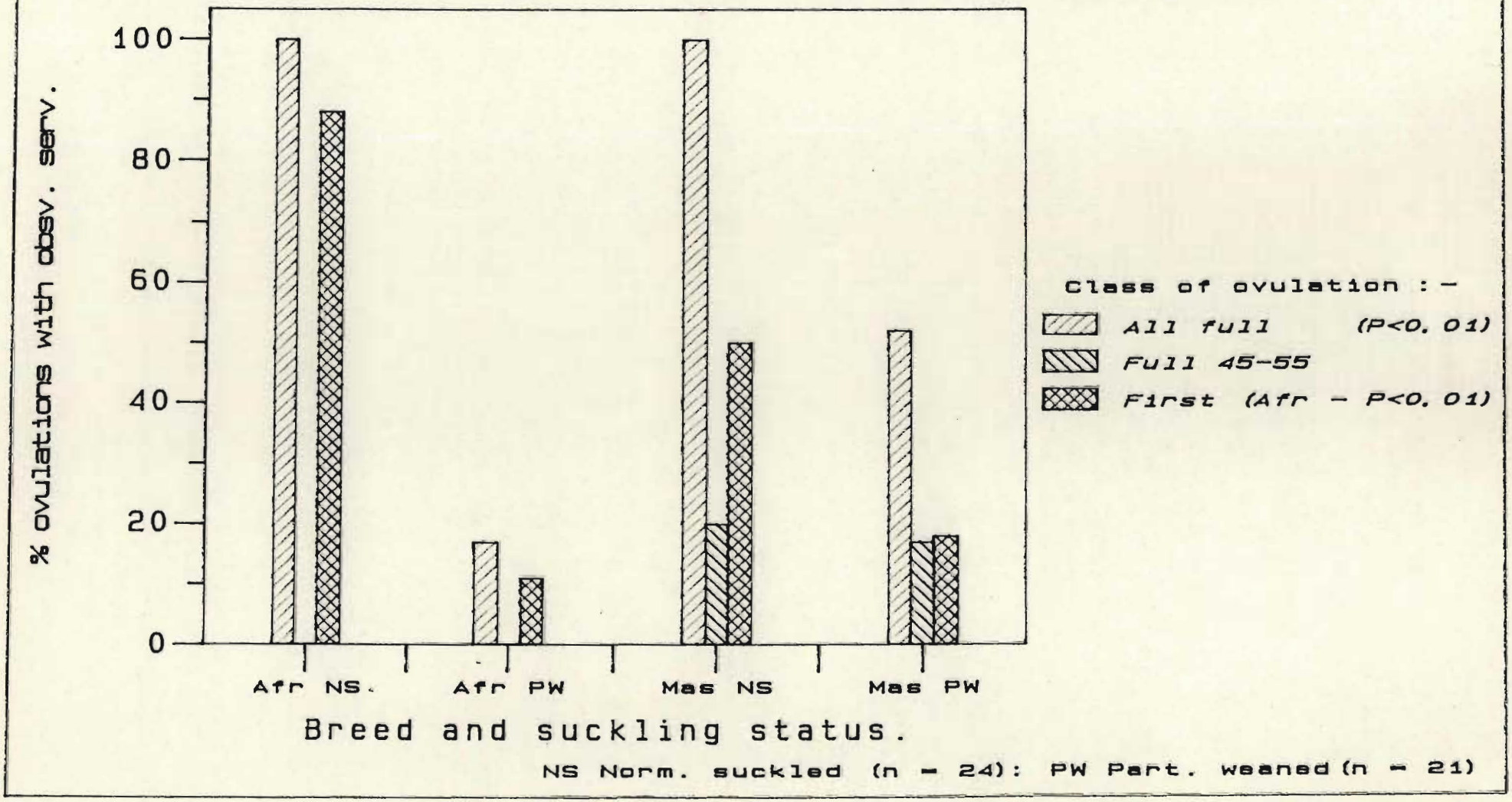
Observed service

In Afrikaner cows, partial weaning prior to the insertion of a PRID induced nine full ovulations between 45 and 55 days post partum compared to one over this period in normally suckled cows (Table 56). The provision of progesterone prior to these ovulations had no effect on the expression of oestrus in the Afrikaner since no observed services were recorded against any of the ovulations recorded during this period (Table 56, Figure 31).

Table 55. The incidence and characteristics of nosing of the cow's perineum by the bull and flehmen during oestrous periods with observed service in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to exposure to exogenous progesterone for nine days.

Breed	Afrikaner		Mashona	
Treatment	Normally suckled	Partially weaned	Normally suckled	Partially weaned
Full ovuls.	10	18	15	27
Observed services	10 P<0,001	3	15 P<0,001	14
Nosing of perineum				
Incidence	10	2	14	11
Mean number of sniffs per oestrus	3,7±0,6	4,0±2,0	4,2±2,0	5,8±1,1
Incidence of multiple (3) events	3	1	8	9
Mean duration of multiple events	4,7±1,4	2,7±1,9	6,3±0,7	6,1±1,2
Mean interval first sniff to first service	2,3±1,5	0,6±0,1	2,9±0,9	2,1±0,7
Flehmen by the bull				
Incidence	10	1	13	11
Mean number of flehmen per oestrus	3,9±0,9	3,0	3,1±0,8	2,3±0,5
Incidence of multiple (1) flehmen	8		9	4
Mean duration of multiple flehmen	5,2±1,3		5,8±1,3	4,0±1,9
Mean interval from first flehmen to first service	1,9±1,3	0,4	1,6±1,2	0,8±1,2

Figure 31. The mean incidence of observed service in NS and PW Afr and Mas cows up to 100 days PP.



In Mashona cows a similar number of ovulations were recorded between 45 and 55 days post partum in normally suckled (five ovulations) and partially weaned (six ovulations) cows. Observed service was recorded against only two of these ovulations (one in each group, Table 56, Figure 31). The provision of progesterone prior to these early ovulations had clearly not improved the expression of oestrus in either Afrikaner or Mashona cows.

The mean interval to first full ovulation was shortened ($P<0,001$) by 23 days with partial weaning (Table 21, Chapter 6). In contrast to the three previous trials, these first full ovulations in the present study were clearly behaviourally deficient in both Afrikaner and Mashona cows. Less than 20% of these first full ovulations in partially weaned cows (with a mean post-partum interval to conception of 45 ± 2 days) were accompanied by observed service, a level of incidence which was significantly ($P < 0,01$) lower than in normally suckled cows (Figure 31). The effect was more pronounced in Afrikaner cows (more effectively programmed for the target period) than in Mashona cows.

These results were surprising considering the high incidence of bull attention during oestrus, particularly in the Mashona cow (chin-resting, nosing and flehmen).

The incidence of observed service prior to conception in Mashona cows and normally suckled Afrikaner cows was 100%, in contrast to the 29% recorded in partially weaned Afrikaner cows ($P<0,05$, Table 56). This also contrasts sharply with earlier results in that partially weaned cows appear to have been served more frequently at night in this study.

Table 56. The incidence of observed oestrus in Afrikaner and Mashona cows which were either normally suckled or partially weaned (from 25 days post partum) and exposed to exogenous progesterone from a PRID inserted 35 days post partum.

Breed	Afrikaner		Mashona	
Treatment	Normally suckled	Partially weaned	Normally suckled	Partially weaned
Group no.	12	10	12	11
Full ovulations occurring 45 to 55 days PP.	1 P<0,001	9	5	6
Acompanied by observed service 45 to 55 days PP.	0	0	1	1
First full ovulations	8	9	12	11
Accompanied by observed service	7 P<0,01	1	6 P<0,10	2
Total number of full ovulations	10	18	15	27
Accompanied by observed service	10 P<0,001	3	15 P<0,001	14
Confirmed conceptions	7	7	9	8
Observed conceptions	7 P<0,05	2	9	8
Mean PPI to observed conception	92,7±2,3a	91,5±3,5a	74,8±4,5b	75,9±5,8b

a--b Breed means differ significantly P<0,01.

A summary of all behaviour observed with service

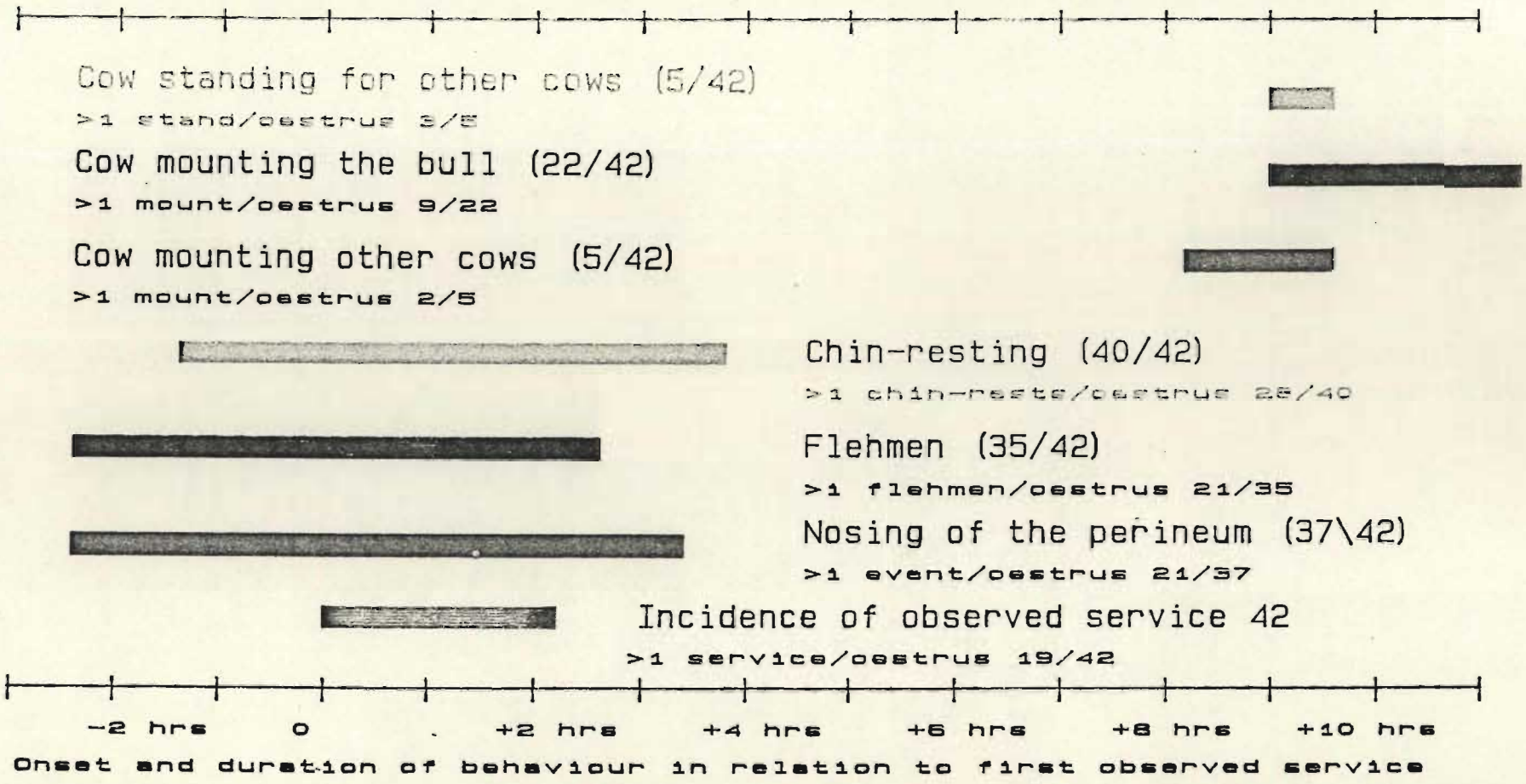
The consolidated picture of oestrus presented in Figure 32 and Table 57 illustrates two major features. Firstly all oestrous activity initiated by cows commenced after cows had been mounted by the bull, beginning five to six hours after the last service and lasting from one to two hours (Figure 32). This was most unusual and does not agree with earlier work in both breeds.

The second point of interest is that bull activity was by far the most frequently observed component of oestrus. Instances of nosing, chin-resting and flehmen occurred 5±1, 8±1 and 3±1 times respectively during oestrus (Table 57). This feature is consistent with earlier findings and along with the high incidence rates (better than 83% of oestrous periods) confirms that bulls were active in interacting with cows in oestrus, but does not confirm the effectiveness of their heat detection.

Table 57. A summary of behaviour observed during oestrus with service in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with exogenous progesterone for nine days from a PRID inserted 35 days post partum.

	Incidence	Events per oestrus	Incidence mult.	Duration of mult. events	Interval to first service
Homosexual activity					
Mounting	5	2,0±0,8	2	1,5±1,1	+8,0±1,7
Standing	5	2,2±0,6	2	0,7±0,4	+9,0±1,5
Heterosexual activity					
Nosing	37	4,5±0,8	29	5,4±0,7	2,4±0,6
Chin-rest	40	8,1±0,9	28	5,3±0,5	2,3±0,5
Flehmen	35	3,1±0,4	21	5,2±0,8	0,9±0,7
Mounting	22	2,0±0,4	9	2,4±0,7	+9,0±0,8

Figure 32. A summary of the incidence, time of onset and duration of each of 5 components of oestrous behaviour recorded with observed service.



A summary of all oestrous behaviour (with and without service)

Of 70 ovulations recorded in this study 42 were accompanied by observed service and the remaining 18 followed oestrous periods during which service was not observed. These additional 18 cases broadened the data base and they have been included in Tables 58 and 59 and Figures 33 and 34, which summarise all oestrous behaviour observed.

Homosexual activity

This larger data base confirms earlier anomalous results reported in Table 53, specifically that the incidence of homosexual behaviour was not improved by partial weaning (Figure 33). The consistency of this finding in both breeds would confirm that this unusual development is not a chance effect.

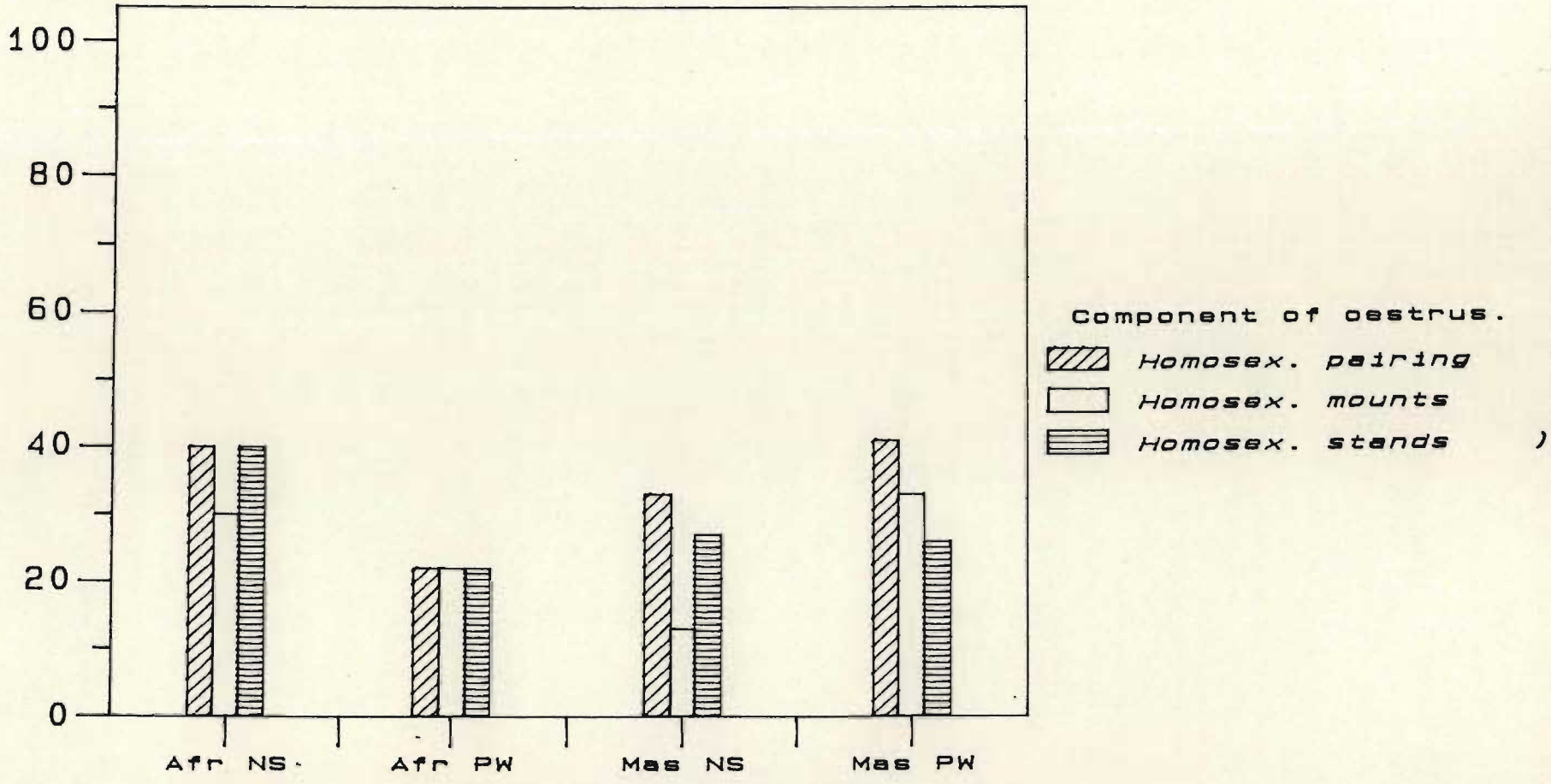
Although duration of pairing was highly variable, partially weaned cows may have spent more time in sexually active groups and done more mounting and standing for other cows than normally suckled cows. (Table 58). Again the mean differences between suckling treatments were small and the high variability of this data masked any effects partial weaning may have had on the time cows spent in sexually active groups.

The number of homosexual stands and mounts observed in one oestrous period was highly variable. The standard errors of some of the treatment means confirm this finding and inspection of the original data shows that five cows (all partially weaned, one Afrikaner and four Mashona) stood to be mounted from 23 to 36 times during a single oestrus (data not shown). Further

Table 58. A summary of all homosexual activity in normally suckled and partially weaned Afrikaner and Mashona cows exposed to exogenous progesterone for nine days from a PRID inserted 35 days post partum.

Breed	Afrikaner		Mashona	
Suckling status	Normally suckled	Partially weaned	Normally suckled	Partially weaned
Group number	12	10	12	11
Full ovuls.	10 P<0,001	18	15 P<0,001	27
Two or more ovulations	1 P<0,01	6	5 P<0,05	10
Oestrus prior to full ovulation	10 P<0,01	9	15	24
Oestrus prior to primary ovulation	2	0	1	0
Pairing				
Incidence	4	4	5	11
Mean Duration	1,0±0,2	2,4±1,1	0,3±0,1	2,2±0,7
Mounting				
Incidence	3	4	2	9
Mean number of mounts per oestrus	4,7±3,2	6,8±2,5	4,0±2,8	13,0±4,5
Standing				
Incidence	4	4	4	7
Mean number of stands per oestrus	6,0±1,5	12,8±7,8	1,3±0,3	16,7±4,8

Figure 33. The incidence of homosexual activity in NS and PW Afr and Mas cows up to 100 days PP.



NS Norm. suckled (n = 24): PW Part. weaned (n = 21)

inspection of this sub-sample shows that with one exception (27 days post partum) this behaviour was recorded on day 46 or 47 after calving. All PRIDs which had been inserted 35 days post partum were removed 44 or 45 days post partum. Mounting activity in these cows was not as consistent with two of the cows mounting 40 and 25 times and the remainder mounting less than 8 times during oestrus. Partial weaning tended to cause a higher incidence of homosexual mounting than recorded in normally suckled cows although a pooled breed comparison was not significant.

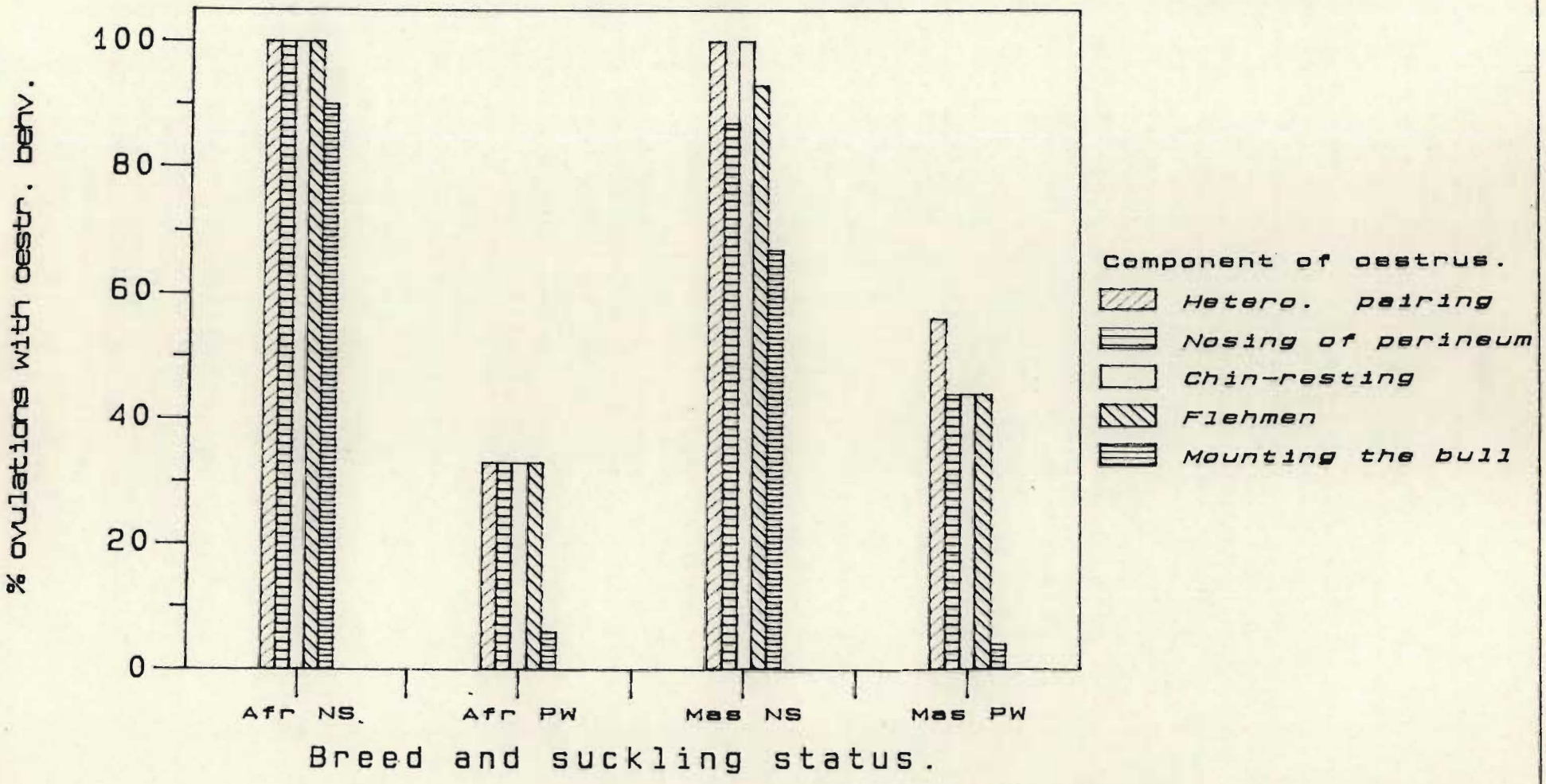
Heterosexual activity

The same pattern of behaviour seen in the expression of homosexual behaviour was observed with all components of heterosexual activity, with bulls being less interested in Afrikaner and Mashona cows which had been partially weaned. This adverse trend was not as strong in Mashona cows as it was in Afrikaner cows (Figure 34).

The one component of heterosexual activity which was most affected by partial weaning was heterosexual mounting by cows. This component of oestrus was almost non-existent in partially weaned Afrikaner and Mashona cows (Figure 34). The significance of this dramatic change is not immediately apparent.

The number of events during oestrus of each component of behaviour did not differ significantly with breed or suckling treatment (Table 59). The pattern of chin-resting differed from that of nosing of the perineum and flehmen. Neither of the latter two components showed any indication of changes between breed and between treatments. Chin-resting however seemed to

Figure 34. The incidence of heterosexual activity in NS and PW Afr. and Mas. cows up to 100 days PP.



NS Norm. suckled (n = 24): PW Part. weaned (n = 21)

occur more frequently during oestrus in Afrikaner than in Mashona cows. Within each breed, partially weaned cows may have solicited this response from the bull more often during oestrus than normally suckled cows.

Table 59. A summary of heterosexual activity in Afrikaner and Mashona cows which were either normally suckled or partially weaned prior to treatment with exogenous progesterone for nine days from a PRID inserted 35 days post partum.

Breed	Afrikaner		Mashona	
Suckling status	Normally suckled	Partially weaned	Normally suckled	Partially weaned
Full ovuls.	10 P<0,001	18	15 P<0,001	27
Oestrus prior to full ovulation	10	9	15	24
Pairing				
Incidence	12	6	16	15
Mean duration	6,5±4,6	4,6±0,6	4,8±0,9	3,3±0,7
Nosing of the perineum				
Incidence	12 P<0,001	6	13 P<0,01	12
Mean number of events per oestrus	3,8±0,4	4,5±1,0	4,1±0,6	4,7±0,9
Chin-resting				
Incidence	11 P<0,001	5	16 P<0,01	12
Mean number of events per oestrus	11,2±2,3	13,4±2,5	6,3±1,3	9,1±1,7
Flehmen				
Incidence	12 P<0,001	6	14 P<0,01	12
Mean number of events per oestrus	3,9±1,0	2,5±0,6	2,9±0,8	2,2±0,4
Cow mounting the bull				
Incidence	9 P<0,0001	1	10 P<0,0001	1
Mean number of mounts per oestrus	3,2±0,6	5	2,5±0,8	14

DISCUSSION

From an endocrine aspect the reduced suckling treatment imposed in this study was effective. The mean post-partum interval to first full ovulation was reduced by 23 days to 45±3 days in Afrikaner and Mashona cows suckling their calves once each day beginning 25 days post partum (Table 21, Chapter 6). This earlier resumption of ovarian activity was induced by an earlier increase in gonadotropin activity consistent with a reduction in suckling intensity (Carruthers and Hafs, 1980; Peters, Lamming and Fisher, 1981; Walters et al., 1982b; Edwards, 1985).

Earlier work had confirmed that the first induced ovulations in partially weaned and non-suckled Afrikaner cows were largely infertile despite a normal expression of oestrus (Chapters 3 to 5). The greatest concern was that conception did not follow these early ovulations because of the absence of service during oestrus. The results of Chapters 8 to 10 indicated that this was not the case and that the low conception rates to these early ovulations was due to a failure of events after insemination.

The work of Ramirez-Godinez et al. (1981) indicated that progesterone priming prior to first ovulation post partum improved first service conception rates and eliminated the incidence of shortened luteal phases at the resumption of ovarian activity. In this latter study however, cows were more than 48 days post partum when they were weaned and this could explain the improvement in conception in response to weaning and simultaneous progesterone treatment. All studies in this series have confirmed that ovulations earlier than 50 days post partum are less fertile than later ovulations (Chapters 3 and 4).

In the present study, cows were 25 days post partum when they were weaned and 35 days post partum when treated with progesterone. None of the ovulations following the removal of the PRIDs in Afrikaner cows were accompanied by observed service. Summaries of all oestrous behaviour show conclusively that cows which had been partially weaned prior to progesterone treatment had oestrous periods of a very low intensity (Figure 34). Ultimately, partial weaning induced more ovulations than in normally suckled cows but in nearly 50% of these ovulations the intensity of expression of oestrus was extremely low.

Although homosexual behaviour was recorded in only 35% to 40% of ovulations, the incidence of this behaviour was similar in both normally suckled and partially weaned cows. However all components of heterosexual behaviour were markedly depressed in cows which had been partially weaned. In particular the incidence of heterosexual mounting by cows was reduced from 90% of ovulations in normally suckled Afrikaner cows to less than 10% of ovulations in partially weaned Afrikaner cows. This dramatic decline in activity indicates one of two possibilities. Either the opportunity for heterosexual interaction was severely reduced in partially weaned cows due to the logistics of suckling a penned calf daily, grazing and watering or the opportunities for heterosexual association were adequate but the stimuli driving the expression of this component of oestrus in the cow were depressed in partially weaned cows treated with progesterone.

One factor which has not received attention is the incidence of vaginitis with the use of intra-vaginal devices in beef cattle. In the present study there was a more than 80% incidence of

vaginitis in the trial. Since odours specific to oestrus originate from the cow on the approach of oestrus (Kiddy and Mitchell, 1981; Ladewig and Hart, 1981) and are involved in the attraction of males to oestrous females (Fletcher and Lindsay, 1968) a high incidence of vaginitis could detract from the effectiveness of these pheromones in attracting males or even discourage cow/bull interaction, since the initial components of oestrus include nosing of the perineum followed by chin-resting.

The incidences of bull-cow interactions show clearly that the 55% to 65% of ovulations in partially weaned Afrikaner and Mashona cows did not stimulate nosing of the perineum, chin-resting and flehmen (Figure 34). A critical question is whether or not the incidence and intensity of response in each of these components of bull behaviour are a function of the intensity or nature of pheromones derived from the cow. If they are, then this evidence in conjunction with the very low incidence of bull mounting in partially weaned Afrikaner and Mashona cows points to events (endocrine ?) at and around oestrus which cause these early ovulations to be accompanied by "covert oestrous periods". These deficiences in bull mounting by the cow might be caused by subtle receptor changes which modify behaviour centre thresholds of response to endocrine signals with the approach of ovulation.

The lack of conception to the first ovulation in Afrikaner cows following progesterone and partial weaning, appears to have been due to the absence of natural insemination. This statement is made with some reservation since the number of confirmed conceptions accompanied by observed oestrus (33%) would indicate that in this group there was a higher incidence of oestrous

activity outside the surveillance period than in the other three groups. Haresign (1985) has suggested that following synchronised oestrus the decline in progesterone concentrations is atypically rapid. This induces unnaturally rapid increases in LH concentrations, a factor which might be involved in an abnormal temporal control of oestrus, impaired sperm transport, abnormal oestradiol secretion, and mucous secretion and subfertility prior to the induced ovulation (Smith and Robinson, 1970; Smith and Allison, 1971; Echternkamp, Bolt and Hawk, 1976). Thus the failure of oestrus following progesterone withdrawal is likely to have been compounded by these abnormal endocrine changes.

A high incidence of homosexual activity in this study was associated with a lower incidence of heterosexual interaction and in particular observed service. In 12% of oestrous periods when cows were served they stood to be mounted and mounted other cows. In contrast to this situation, of a total of 28 oestrous periods during which cows were not served, 13 and 14 of these saw cows mounted by other cows standing for other cows respectively (these proportions differ significantly $P < 0,001$). This might be evidence of unsatisfactory cow-bull contact in keeping with the observations of the last chapter where cows partially weaned prior to the breeding season showed a higher incidence of homosexual activity in the absence of the bull. Alternatively homosexual activity declines in the presence of the male by virtue of its futility.

The adverse results in this trial are not conclusive proof of the inadvisability of the use of progestagens in Afrikaner cows. Under less stressful conditions, there may well be a case for

mimicing the short luteal phase with an exogenous progesterone source. The long mean intervals to conception in the Afrikaner and Mashona (75±2) bear witness to the fact that these animals were under considerable stress during this study. The results also confirm that even partial weaning will not improve conception rates and shortened the mean post-partum interval unless minimum levels of gonadotropin activity, nutrition, comfort and opportunity for heterosexual interaction are achieved.

CONCLUSIONS

Partial weaning from 25 days post partum significantly increased the number of Afrikaner cows ovulating before 55 days post partum. The provision of progesterone from 35 to 44 days post partum failed to increase the proportion of cows being served within 10 days of PRID removal. Thus the absence of conception to these first full ovulations was due almost entirely to a deficiency in oestrous behaviour rather than fertilisation failure, lack of luteal support or early embryonic death.

Patterns of bull-initiated oestrous behaviour indicated that early ovulations induced by partial weaning, failed to induce pre-coital bull-cow interaction, (nosing of the perineum, chin-resting and flehmen). This might have been due to insufficient or altered pheromones being released which had no effect on the bull or oestrus detection by bulls was poor due to environmental stresses.

The high incidence of vaginitis with the insertion of a PRID into

the cows in this trial may have detracted from the potency of their pheromones, essential for partner seeking by bulls in large paddocks. Either the principle of progesterone priming improving conception rates to an induced ovulation is not consistent in all situations or the timing of treatment application in this study was not physiologically correct.

Chapter 12

GENERAL DISCUSSION

The object of this series of studies was to investigate and assess the influence that suckling had on the resumption of ovarian activity in the Afrikaner cow.

The suckling stimulus is comprised of two components. The first is the stimulus experienced by the cow during a single suckling event and the second is the total stimulation from all suckling bouts during 24 hours. The amount of milk removed from the udder at one time is thought to be primarily a function of calf appetite (Gifford, 1949 and 1953, Drewry *et al.*, 1959).

Single suckling bouts consisted of an extraction phase and a stripping phase. Each phase was characterised by distinct patterns of behaviour which appeared to be related to the amount of milk removed from the udder. Although the sample number was small, it appeared unlikely that variation in patterns of behaviour within each suckling event were closely associated with endocrine activity *post partum* and in particular the interval to first ovulation. Where differences in the resumption of ovarian activity *post partum* were due to the suckling stimulus *per se*, the parameter of interest was most likely to have been the number of suckling events per 24 hours. No references have been found concerning the specific relationship between daily suckling frequency and post-partum reproductive function. Thus, it is impossible to confirm whether the differences between cows in post-partum interval to first ovulation are attributable to either total suckling stimulation per day or differences in sensitivity of the hypothalamo-pituitary axis to the suppressive

effects of similar levels of suckling frequency per 24 hours.

From Figures 13 and 14 it can be seen that calf live mass at 75 days post partum was not correlated to post-partum interval to first ovulation. Variation in calf mass at 75 days is a function of milk production and genotype of sire. Since calf mass at 90 days is negatively correlated to suckling frequency (Drewry et al., 1959) suckling frequency per se is unlikely to be correlated to post-partum interval to first ovulation. The suckling studies of Chapter 7 involved too few calves to investigate the relationships between daily suckling frequency, calf growth rate and post-partum interval to first ovulation. Nevertheless Figure 13 clearly illustrated that normal suckling delays first ovulation by 15 days compared to cows suckled once a day. The essence of the problem appears to be the sensitivity of individual cows to the suppressive effects of suckling on the hypothalamo-pituitary axis rather than the frequency of suckling.

Sensitivity to suckling is undoubtedly a major cause of the lower reproductive performance of the Afrikaner cow compared to the Mashona cow. Suckled once a day or not at all, these breeds have similar conception rates and similar mean post-partum intervals to conception. However, when compared to the Mashona under normal suckling intensities, the Afrikaner has a 25% lower conception rate and a 15 day delay to mean date of conception post partum. The absence of breed differences in the resumption of ovarian activity when cows are not suckled implies that either the level of suckling stimulus in the normally suckled Mashona is below some critical level or the breed differences noted with unrestricted suckling levels in the Afrikaner are a consequence

of a higher number of suckling bouts per day. Although the results (Chapter 7) did not indicate any breed differences in suckling frequency per 14 hours the sample number was too small to provide conclusive evidence of differences in patterns of suckling behaviour in the Afrikaner and Mashona. Nevertheless there was some evidence to indicate that daily suckling frequency in the Afrikaner showed a higher variation than in the Mashona.

By 30 days post partum in both Afrikaner and Mashona cows, the hypothalamo-pituitary-ovarian axis is capable of inducing and supporting fertile ovulations and sustaining persistent cyclic ovarian activity (Table 3, Chapter 3). Any deviation from this pattern is due to active intervention from the cerebral cortex involving down regulation of receptors governing GnRH release. In the normally suckled cow, compared to the non-suckled cow, this first ovulation is delayed by 30 days in the Afrikaner and by 15 days in the Mashona cow. In cows suckled once a day, breed differences are eliminated and the first ovulation is delayed by 10 to 15 days.

In all studies in this series, these early ovulations (less than 50 days after calving) in both Afrikaner and Mashona cows on reduced suckling treatments, were sub-fertile with conception rates of less than 20% to first ovulation. This reduced probability of conception was undoubtedly endocrine in origin since the failure of conception to these early ovulations was clearly not the result of any behavioural deficiencies (Figure 17, Chapter 8). In three out of the four studies, reducing the suckling stimulus increased the probability that the first ovulation post partum would be accompanied by at least one observed service. In the last of the four studies however,

partial weaning prior to progesterone treatment actually reduced the incidence of observed service during oestrus but this must be attributed in part to the treatment of cows with PRIDS. No satisfactory explanation can be offered for this finding based on the data available, but it seems likely that the provision of exogenous progesterone adversely affected patterns of gonadotropin and steroid release prior to ovulation.

Evidence for two areas within the hypothalamus, one controlling gonadotropin release and the other mediating sexual behaviour have been recorded in sheep and cats (Michael, 1973). In cattle an endocrine control centre appears to drive the hypothalamus based on information from both the external (suckling intensity, oestrous activity, climatic conditions, photoperiod) and internal environments (post-partum interval, metabolic status, uterine condition) of the cow. A behaviour centre controlling the expression of oestrus is stimulated by endocrine inputs and appears to be modified by higher brain function.

The resumption of ovarian activity post partum seems to be determined primarily by an inherent rhythmic control which is not substantially different in the Afrikaner and the Mashona. The activity of this centre is readily modified by factors such as metabolic stress, climate and, depending on breed, suckling status. This pre-hypothalamic control centre cannot be activated permanently in anovulatory cows with the use of exogenous peptide or steroid hormones (Holness and Hale, 1980) unless it is close to spontaneous activity, as indicated by an unprompted increase in gonadotropin levels following the low levels observed soon after calving.

The activity of these two centres in the bovine is linked via endocrine messengers which interact with neural inputs from the cortex prior to the expression of oestrus. For this reason oestrus in relation to ovulation may be absent or delayed or modified by chance interventions of other animals or situations. Given the restricted period of cow receptivity in the bovine (less than three hours, Figures 18, 23 and 32) such situations can seriously reduce the probability of conception. Since the Afrikaner would appear to have a low intensity of expression of oestrus, this might be reinforced favourably with the use of teaser bulls, the proximity of other cows in oestrus and breeding paddocks of a size which would facilitate cow-bull interaction. Good visibility across all areas within the paddock, and watering points in proximity to adequate grazing could improve the probability of efficient oestrus detection by bulls.

The components of oestrus appear to manifest themselves based on a priority system. Proceptive behaviour (mounting other cows, mounting the bull) would appear to have a lower priority rating than receptive mounting behaviour including standing for other cows and for the bull. The most common form of oestrus related behaviour was initiated by the bull (chin-resting, flehmen and nosing of the perineum) presumably in response to pheromones released by the cow. Homosexual behaviour, even at the height of oestrus appears to be readily displaced by heterosexual behaviour in the presence of bulls. Homosexual mounting behaviour by cows was lowest on the priority list of mounting activities and was observed frequently in cows in di-oestrus. The priority listing for components of oestrous behaviour may depend on different steroid thresholds for the onset of expression of each component. Social factors and particularly social dominance play a major

part in the expression of these components and change dramatically as cows come into oestrus (Mylrea and Beilharz, 1964).

The expression of oestrus is readily influenced by inputs from the cerebral cortex. Stresses such as heat, exhaustion and social subordination depress the expression of heat (particularly heterosexual mounting activity in both sexes) whilst reduced suckling intensity will enhance the expression of oestrus-related behaviour. Improved expression of oestrus (higher proportion of ovulations preceded by overt oestrous behaviour and more activity during observed oestrus) could be a result of either higher concentrations of oestradiol impinging on behaviour centres or an increase in the responsiveness of these centres to endocrine inputs.

For conception to be achieved before 50 days post partum a coordinated improvement in post-partum endocrine function must occur. Weaning is known to induce early ovulations, normal oestrous behaviour and adequate luteal function following ovulation. Yet conception rates are still limited by unknown factors following these first full ovulations in weaned cows. To date no explanation is apparent for the failure of conception to first ovulations after calving. Eduvie et al. (1985) reported that uterine involution in Bunaji (Bos indicus) cattle was complete by 20 to 25 days post partum and that the absence of suckling had no effect on the rate of uterine regression. If uterine involution is not limiting reconception 30 to 40 days post partum in the weaned cow, then factors such as ovum fertility, timing of insemination (natural or artificial), gamete

transport and embryo mortality might be adversely affected and be responsible for the low conception rates.

These problems which limited very early conceptions in the weaned cow, did not arise in the normally suckled cow ovulating 20 days later. Although ovulation rates per treatment group were lower, conception rates per ovulation were higher than in cows on a limited suckling program. The higher conception rates with later ovulations are undoubtedly linked to post-partum interval. Perhaps the key to the failure of early conceptions lies with the interaction of the spontaneous increase in LH activity after calving and the supplementary increase which follows the removal of or a reduction in the intensity of suckling stimulus.

Although no direct measurements of gonadotropin activity have been made in the Afrikaner and Mashona cows in this study, the response of these two breeds to a reduction in suckling stimulus is most likely to have been due to the earlier increase in gonadotropin activity in weaned cows post partum than in their normally suckled contemporaries . In 90% of cases, suckled Mashona cows appear to escape from the suckling suppression of initial hypothalamic stimulation by 25 to 35 days post partum. In the Afrikaner cow, up to 30% of cows may not experience any increase in gonadotropin activity by 100 days after calving. In both breeds, the pituitary probably contains adequate stores of gonadotropins, which can be released with exogenous release factors and will induce ovulations in a high proportion of anovulatory cows (Holness and Hale, 1980). Although ovulation and luteal activity may be induced in the Afrikaner cow in this manner, it is unlikely to result in persistent cyclic ovarian activity if applied too early post partum. In contrast, partial

weaning 25 days post partum (and possibly earlier) will induce persistent cyclic ovarian activity within 10 to 20 days in 90% of Afrikaner cows, supporting the hypothesis that the mechanism of resumption of ovarian activity is driven by some pre-hypothalamic centre which cannot be permanently activated by the premature use of exogenous steroid or peptide hormones.

Higher brain centre involvement in the control of reproduction in the bovine is evidenced by the ram effect in sheep, the enhancement of homosexual activity in partially weaned cows, the decline in homosexual activity with the introduction of bulls, seasonal effects on reproductive behaviour and cow-mounting by pre-pubertally castrated bulls (Folman and Volcani, 1966). Thus breed differences probably exist in the responsiveness of the centre controlling hypothalmo-pituitary axis to similar extero-stimuli. Holness et al. (1979) demonstrated that the Afrikaner will become anovulatory following an 18% loss in live body-mass, whilst the Mashona will lose up to 33% of live body mass before ceasing cyclic ovarian activity.

The sensitivity of the Afrikaner to a depletion of body reserves would be of considerable benefit in harsh environments. This sensitivity would also appear to occur in centres regulating the breed's endocrine system. During the luteal phase, total estimated plasma progesterone is significantly lower in the Afrikaner than in the Mashona cows. This observation was recorded following both fertile and non-fertile services. Hale and Robey (unpublished data) have also demonstrated that in response to the same GnRH challenge Afrikaner cows release more LH than Mashona cows and Holness et al. (1980) have shown that

the ovaries of Afrikaner cows show a markedly greater response to a standard dose of PMSG than Mashona cows. The Afrikaner might therefore be more responsive to both endocrine and extero-sensory stimuli than the Mashona. Perhaps this is why the normally suckled Afrikaner conceives later (+8 days), has a lower conception rate (-25%) than the Mashona cow, and yet has similar levels of reproductive performance to the Mashona when placed on a restricted suckling regime.

Both the Afrikaner and the Mashona have developed in environments where winter nutrition provides the greatest single limit to reproductive performance. The Afrikaner would appear to combat this potential threat by directing all available resources to the calf at foot rather than to reconceiving within 100 days. The more vigorous the calf, the more persistently it will attack the udder and the longer might be the post-partum interval to reconception. The Mashona on the other hand places a higher priority on conception, possibly at her own expense, in extreme environments. Both of these mechanisms will ultimately ensure the continued survival of the breed. Unfortunately, this survival mechanism is not readiy suppressed in the modern Afrikaner, which places the breed in a poor position under improved management systems.

Based on the findings from this study, the control of ovarian function post partum in the Afrikaner cow is profoundly influenced by the suckling stimulus. The term "hardiness" is difficult to quantify but this term must include characteristics such as heat tolerance, mobility, tick tolerance, resistance to tick born diseases and draught capability. "How much better is the Afrikaner than its contemporaries in these qualities?" and

"Of what importance are these traits under good managment which can eliminate the major threat of stress in these areas?" are questions which for the present remain unanswered. The breed is hardy and is more sensitive to the suppressive effects of suckling than the Mashona, a more fertile breed. Although the Afrikaner has the same potential for post-partum reproduction as the Mashona it appears that the breed's lower fertility is an active state induced by the cerebral cortex delaying the resumption of hypothalamic activity after calving based on either some inherent rhythm or adverse environmental factors.

It seems probable that the Afrikaner with its protracted post-partum anovulatory period will not readily conform to a 365 day intercalving interval and better than 85% conception. However the breed's sensitivity to a reduction in the suckling stimulus offers the Afrikaner breeder an extremely effective tool for dealing with the anovulatory post-partum cow which is within a cycle or two of spontaneous ovulation.

Chapter 13

GENERAL SUMMARY AND CONCLUSIONS

A single suckling event for the Afrikaner cow comprises two phases which in this thesis have been termed an extraction phase and a stripping phase. The extraction phase is associated with a rapid removal of milk from the udder and lasts 6±0,5 minutes during which time the calf will change teats 25±2 times. The stripping phase lasts 9±0,5 minutes and the calf will change teats 145±7 times in an effort to remove the remaining milk from the udder. During the whole suckling event the calf will butt the udder 34±3 times ($P<0,01$), which is more often than calves of either Mashona or Hereford dams. Although patterns of behaviour changed with the amount of milk available, the differences in total udder stimulation at each suckling bout appeared negligible by comparison to differences in total udder stimulation recorded between cows suckled from one to six times per 14 hour observation period (0500 to 1900).

The limited amount of data compiled on specific characteristics of suckling behaviour indicated that frequency of suckling per 24 hour period was the only component likely to be correlated with post-partum reproduction.

In the Afrikaner cow, the nursing calf with continuous access to its dam, is responsible for delaying the first ovulation after calving by 27-30 days when compared to the non-suckled cow. The removal of the suckling stimulus facilitates the spontaneous increase in gonadotropin activity which normally occurs 5 to 30 days after calving to levels which will induce folliculogenesis and ovulation. This apparent "escape" from the suppressive

efforts of suckling is due to an increased stimulation of the hypothalamus and an increased responsiveness (possible up-regulation of GnRH receptors) of the pituitary to GnRH stimulation.

The reproductive performance of the unsuckled Mashona (±90% conception rate at 46±7 days post partum) is almost identical to that of the unsuckled Afrikaner (80% conception rate at 46±3 days post partum), indicating that the hypothalamo-pituitary axis is very similar in Afrikaner and Mashona cows in terms of the temporal control of the initial stimulation of the hypothalamus, pituitary and gonadotropin activity and responsiveness of the ovary to gonadotropin stimulus.

The above evidence points to the Afrikaner being particularly sensitive to the suckling stimulus, a factor which delays first ovulation after calving and lowers conception rates within 100 days of calving to a greater degree than in fertile breeds such as the Mashona, maintained under similar conditions. This study revealed conceptions (confirmed by progesterone profiles and calving date) by 10 days after calving in Mashona cows and by 22 days after calving in the Afrikaner cow. Such early conceptions are evidence of the high variation in post-partum potential for reproduction between cows and the incredible flexibility of the reproductive system after calving.

The suppressive effects of suckling on the hypothalamo-pituitary axis appear to be proportional to the intensity of suckling stimulation. Reducing the suckling stimulus to once a day shortened the post-partum interval by 11 days. However in the

partial weaning study there was a higher incidence of anovulatory cows for 100 days post partum than in the previous study when calves were completely weaned. Thus factors were present in the environment in that season which delayed the unprompted (as distinct from prompted by reduced suckling) increase in GnRH activity and confirm that a spontaneous increase in gonadotropin activity must precede any successful response to a reduction in the suckling stimulus. Since suckling has a suppressive effect on hypothalamo-pituitary activity its removal in the post-partum cow is not a stimulus but the removal of a suppression. This underlines the necessity of a potential increase in GnRH activity before any response to a decrease in suckling stimulus will occur.

An interesting and consistent observation recorded with the use of reduced suckling has been the significant ($P<0,01$) increase in the incidence of observed service to first full ovulation after calving in the absence of suckling and a small but non-significant increase with once a day suckling. The higher incidence of observed services prior to first full ovulations may have been caused by either an increase in the responsiveness (to oestradiol) in the hypothalamic centres controlling oestrus expression or a decrease in nocturnal oestrous activity with a restricted suckling programme. Increased oestradiol-17-beta concentrations would not seem to be responsible for the higher incidence of observed service (Pope, 1980).

Despite a higher incidence of observed service and shorter post-partum interval to ovulation in cows on limited suckling programmes, the probability of conception to these early ovulations was usually less than 20%, if these ovulations

occurred before 50 to 60 days post partum. A definitive explanation of this observation is not forthcoming from either the literature or data compiled from these studies. Progesterone priming prior to ovulation would appear to be involved. Only after primary ovulations was luteal function noticeably increased by up to 110% in response to a reduction in suckling stimulus. Since abnormal patterns of plasma concentrations of progesterone around the time of oestrus have been implicated in poor fertility (Maresign, 1985), this increased luteal function in weaned cows prior to first full ovulation might have depressed fertility following adverse changes in gonadotropin secretion prior to ovulation. After primary ovulation with reduced suckling, total plasma progesterone concentrations were increased by 120% in weaned cows compared to control cows. Since the duration of these short cycles was similar (7±0,6 days) in both control and weaned cows, luteal activity was significantly higher in non-suckled and in partially weaned cows than in controls. However, the higher total progesterone concentrations during short cycles in weaned cows are more likely to be a symptom of an abnormal endocrine environment at this time than the primary cause of poor fertility.

The lower conception rates in cows ovulating before 50 days post partum was not a major concern, since the net result of a reduction in suckling intensity in Afrikaner cows was an improvement in conception rate (+25%) and a reduction in the mean post-partum interval to conception (up to 20 days shorter) compared to normally suckled cows.

The provision of exogenous progesterone to mimic the short cycle

and improve conception to ovulations earlier than 55 days post partum was unsuccessful. The lack of success was largely a result of the apparent absence of service to ovulations occurring within 10 days of progesterone removal. In addition, the progesterone release was about 100% more than during the first short cycle in normally suckled cows which may have presented the same problems associated with higher luteal function after primary ovulation reported above. In addition the level of general oestrous behaviour following PRID removal was lower (in cows ovulating within the target period of 45 to 55 days post partum).

The two most striking features of oestrus in the Afrikaner cow have been firstly the low incidence of homosexual behaviour (less than 30% of ovulations) and heterosexual behaviour (Figure 15, 20) and secondly the short (three to four hours) duration of heterosexual activity prior to ovulation (Figure 18, 23). Whether these factors play any part in the reduced probability of conception in the Afrikaner under free range conditions is difficult to assess, but with any artificial breeding program, the detection of oestrus in the absence of a bull is unlikely to give satisfactory results. Ovulation rates and probabilities of conception might be improved with the use of low cow/bull ratios during breeding and compact calving and breeding periods to ensure that oestrous activity is maximal during breeding.

The Afrikaner cow, in the absence of suckling, might be as fertile as any breed. However the suckling stimulus appears to delay first ovulation after calving by as much as one to two cycles compared to the non-suckled cow. This sensitivity to the

suckling stimulus might be linked to the breed's apparent sensitivity to potentially stressful stimuli such as live mass loss. Ultimately though, this sensitivity may be a fundamental component of the hardiness of the Afrikaner cow.

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