

**Farm Environmental Factors and Cow Physiological Traits Affecting the Gender
Ratio of Newborn Dairy Calves**

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ABSTRACT

Farm environmental factors in the KwaZulu-Natal Midlands were investigated to determine whether any of them had an influence on the calf gender ratio in dairy herds in this area. Heat detection score ($P < 0.001$), number of inseminators ($P < 0.001$), geographical location ($P < 0.001$), bull presence on the farm ($P < 0.001$), and timing of insemination ($P < 0.001$), had a highly significant effect on the calf gender ratio. The probability of female calves increased when heat detection included visual observation and two aids, three inseminators, geographical location around Boston, Kamberg, Umzimkulu or Underberg, when a bull was present on the farm and when timing of insemination was according to an assessment of oestrous behaviour of each cow (where insemination was immediate if a cow was thought to have been on standing heat for a period before being observed, or delayed until the next milking if this was not the case). These factors could be manipulated, where possible, in an attempt to skew the gender ratio of newborn dairy calves in favour of females, as this would lead to economic gain in a dairy enterprise due to the comparative worth of heifer calves compared to bull calves.

An additional experiment was conducted to examine the relationship between rectal and vaginal temperature with oestrus and ovulation in the cow. Rectal temperature was found to be the best predictor of both oestrus ($P < 0.001$) and ovulation ($P < 0.001$), when measured within 24 hours of the start of oestrus. Rectal temperature should, therefore, only be used to predict the onset of oestrus if the approximate time of oestrus was known from heat expectancy records. Rectal temperature could also be used to determine when to inseminate relative to estimated time of ovulation to increase the probability of male or female calves, if used in conjunction with the observation of oestrous behaviour.

I hereby certify that this research is the result of my own investigation. Where use was made of the work of others it has been duly acknowledged in the text. The results in this dissertation have not been submitted, in whole or in part, for a degree at any other University.

A handwritten signature in blue ink, appearing to read 'N. Mills'.

Nicola Claire Mills
December 2002

I hereby release this thesis for examination in my capacity as supervisor.

A handwritten signature in blue ink, appearing to read 'N. S. Ferguson'.

Prof. N. S. Ferguson
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"My soul glorifies the Lord and my spirit rejoices in God my Saviour"

Luke 1:46, 47

GENERAL INTRODUCTION

Reproduction technology has developed substantially in the past 15 years, and it is now possible to alter gender ratios artificially in cattle by sexing semen (Johnson *et al.*, 1994) or sexing embryos (Appa Rao *et al.*, 1994). Pregnant cows can also be selected based on the sex of the foetus they are carrying, which can be determined either by ultrasound technology (Reeves *et al.*, 1984) or amniocentesis (Leibo and Rall, 1987).

The sex of each calf is an important outcome in the dairy industry, as only heifer calves can be used for milking and are therefore more valuable than their male counterparts. Thus, economic gain will be found if the mechanisms underlying gender selection in dairy cows could be found and manipulated in order to ensure a bias towards female calves.

Discussions with numerous dairy farmers and veterinarians in the KwaZulu-Natal area suggested that, while some farms reared equal numbers of male and female calves, some farms experience gender ratios biased towards males. However, this may be a perceived problem due to the comparative worth of heifer calves. Semen sexing and embryo sexing procedures, as well as sexing ultrasound technology and amniocentesis, are costly, time consuming or require technical expertise and are not commonly used for predetermination of gender. This led to a study in which the objective was to identify whether any farm environmental variables cause a bias in the gender ratio towards females.

Farmers intent on adjusting gender ratios have employed ever more scientific approaches. One applied reproduction tool that has been researched in cattle is a vaginal probe, which is used to predict the time of ovulation. Timing of insemination in relation to ovulation is thought to affect the offspring gender ratio, with more females resulting from earlier insemination and more males from inseminations closer to ovulation (Wehner *et al.*, 1997). This hypothesis led to a second investigation in the present study, with the objective of

identifying whether the time of ovulation could be predicted from rectal and/or vaginal temperature. This was an attempt to find a more practical and cost-effective means of accurately timing insemination than the probe used in the experiment of Wehner *et al.* (1997).

Chapter 1 of this thesis is a literature review of the explanation of a natural 50:50 gender ratio, possible causes of deviation from this ratio, and of the methods used to manipulate this ratio.

Chapter 2 reports the results of the study, which looked at dairy farm production and management variables in the KwaZulu-Natal Midlands to identify whether they have an effect on calf gender ratio. It also discusses how these variables may be practically utilised to maximise the probability of producing female calves.

The relationship of vaginal and rectal temperature with oestrus and ovulation in dairy cows is the focus of Chapter 3. The possible use of cow temperature as a predictor of ovulation is discussed.

A general discussion linking the results of Chapters 2 and 3 and revisiting the objectives outlined in each chapter concludes this thesis.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Predetermining the gender of offspring has fascinated mankind throughout history. Many methods have been used, which are often based on superstition, in an effort to promote the chances of having a child of specific gender (Jafar and Flint, 1996). When left to nature, it seems the chances of giving birth to either sex are fifty percent. However, there has been much research into the controlling mechanisms of this phenomenon in order to try to manipulate the ratio, and this research will be discussed in this review.

The ability to predetermine the gender of offspring has important implications, not only in the livestock industry, but also in humans. There are over five hundred X-chromosome linked diseases in humans, including haemophilia and Duchenne muscular dystrophy, which can be prevented with the ability to predetermine the gender of offspring (Fugger, 1999). The preservation of endangered species may also rely on reproduction technology to predetermine gender as, if only a few individuals of an endangered species are available, chance production of a few males instead of a few females might diminish the chances of survival of the species. Gender pre-selection for particular matings also becomes essential in developing breeding systems that minimize inbreeding when only a few breeding animals are available (Seidel and Johnson, 1999).

With the advances in reproductive technology, there are now several ways in which the expected 50:50 ratio of males:females can be manipulated. These include fertilisation with sexed semen (Cran *et al.*, 1993; Johnson *et al.*, 1994; Seidel *et al.*, 1999), implantation into the uterus of sexed embryos (Williams,

1986; Booman *et al.*, 1989; Herr and Reed, 1991; Appa Rao *et al.*, 1994) and by timing fertilisation according to the time of ovulation (Wehner *et al.*, 1997).

There are many applications of gender predetermination in the livestock industry. Increasing the number of heifers from which to choose replacements would increase the selection intensity and therefore improve the rate of genetic gain (Rendel and Robertson, 1950). Only the best milking dairy cows could be used to produce replacement dairy heifers, with the remainder inseminated with semen from beef bulls to obtain male calves for beef production. This would allow greater profit as crossbred dairy/beef calves have a better growth rate compared to dairy calves (McElhenney *et al.*, 1986). There could also be facilitation of husbandry of single sex groups (Morrell *et al.*, 1988).

1.2 The 50:50 gender ratio

1.2.1 Explanation of the 50:50 gender ratio

An understanding of the reasons why male and female offspring are produced in equal numbers is important to researchers wishing to manipulate this ratio. When looking at the evolution of the gender ratio, Darwin (1894) stated that “In no case would an inherited tendency to produce both sexes in equal numbers, or to produce one sex in excess, be a direct advantage or disadvantage to certain individuals more than to others; for instance, an individual with a tendency to produce more males than females would not succeed better in the battle for life than an individual with an opposite tendency; and therefore a tendency of this kind could not be gained through natural selection.” He did, however, suggest that the problem was more intricate than this. Fisher (1930) first explained why, under natural selection, the two sexes are usually produced in approximately equal numbers, irrespective of the method of gender determination and Bull (1983) explained this with the following example:

Suppose in a population there are ten females for every male at conception. It follows that each male zygote will transmit more genes to the offspring (as it will

have on average ten times as many offspring) than the average female zygote. Thus, parents which overproduce sons will have more grandchildren, since a male will contribute more genes than a female, and a heritable tendency to overproduce sons will increase in the population, the number of male offspring will increase and thereby reduce the sex ratio bias, eventually leading to equilibrium, where there is an equal number of males and females at conception.

At the cellular level, it can be shown that it is the male gametes in cattle that determine the gender of the offspring. The chromosomal makeup of the female consists of two X chromosomes, while the male has an X and a Y chromosome. Thus, the gametes produced by the female both contain X chromosomes, while the gametes produced by the male contain either an X chromosome or a Y chromosome. If the oocyte is fertilized by a male gamete containing an X chromosome, the result is an XX individual (female), and if fertilized by a male gamete containing a Y chromosome, a XY individual (male) results (Figure 1.1).

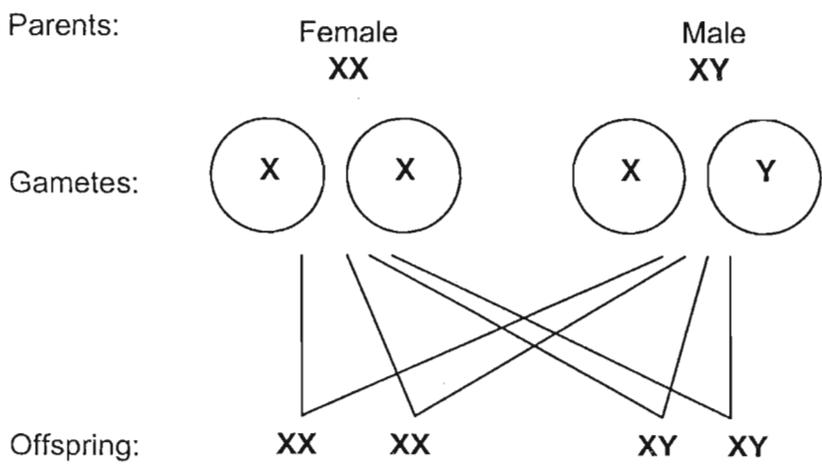


Figure 1.1 Diagrammatical representation of the mode of gender-determination of offspring in mammals

1.2.2 Causes of differential gender ratios

There do appear to be circumstances in which the gender ratio of offspring may not be 50 percent. As reviewed by Clutton-Brock and Iason (1986), variations in the gender ratio may occur as a result of variation in the relative fitness of sons and daughters, sibling competition for mates or resources, competition between parents and offspring, co-operation between parents and offspring, co-operation between siblings, gender differences in juvenile mortality during the period of parental investment, fluctuations in the adult gender ratio and inbreeding. Other scenarios also appear to cause deviations in the gender ratio, and these will be discussed in more detail.

1.2.2.1 Skewed ratios of X- or Y-bearing sperm

In the case of domestic livestock where artificial selection is practised and the breeder decides upon the selection criteria, the gender ratio has remained approximately equal. For example, from 513 624 Dairy Herd Improvement records in the USA, 52.94 percent of calves born were males (Powell *et al.*, 1975), 52.74 percent of 4245 Holstein calves were male (Gray and Hurt, 1979) and of 1109 lambs born, 48.2 percent were males (Karam, 1957). A search by Powell *et al.* (1975) for individual bulls with a skewed gender ratio of progeny failed, but Chandler *et al.* (1998) found that the percentage of sperm bearing the Y chromosome per individual ejaculate ranged from 24 (± 9.8) percent to 84 (± 9.8) percent, although the overall mean was 50.0 (± 4.6) percent. One fifth of the ejaculates differed significantly ($P < 0.05$) from the overall mean. These results suggest that X- and Y-bearing spermatozoa were unequally represented in ejaculates. Thus, ejaculates screened for the percentage of Y-bearing spermatozoa could enhance calf production with the desired gender ratio, or randomisation of straws from different ejaculates could negate the effect of biased gender ratios. Chandler *et al.* (1998) suggested that the variation in gender ratios among ejaculates could be a result of epididymal storage function or perhaps some as yet undefined testicular function.

1.2.2.2 *Age of oocytes*

Mittwoch (1973) stated that, as early as 1860, a theory existed that the gender of offspring is determined by the age of the egg at the time of fertilisation. Early fertilisation was thought to result in female calves, while delayed fertilisation of the egg was supposed to result in bull calves.

The gender ratio of 2-cell bovine embryos after *in vitro* fertilisation depends on the timing of insemination. Zygotes derived from oocytes inseminated immediately after selection for the polar body are predominantly female, while those derived from oocytes inseminated after an 8 hour delay are predominantly male (Dominko and First, 1997). The same tendency is observed when the cleaving zygotes are allowed to develop to the 8-cell stage, but since the skewing of the gender ratio already existed at the 2-cell stage, the time kinetics of spermatozoa and oocyte interaction was suggested as a possible cause for the deviation from the expected 50:50 ratio, i.e. the maturity of an oocyte at the time of insemination may be responsible for preferential processing of either X- or Y-bearing spermatozoa.

Unfortunately, attempts to use this method to skew the gender ratio in favour of females have failed. When oocytes are inseminated immediately after polar body extrusion, the majority of embryos degenerate. Only 19 percent progressed to the 8-cell stage or beyond, in comparison to 55 percent of those oocytes inseminated after 8 hours (Dominko and First, 1997).

1.2.2.3 *Multiple births*

Several studies in sheep have looked at whether the gender ratio of offspring changes with twin or triplet births. Karam (1957) and Napier and Mullaney (1974) found no significant difference in the gender ratio of single or twin births in sheep. Chapman and Lush (1932) found no significant difference in the percentage of males in singles, twins or triplets in 1019 lambs born from 1915 to 1930. However, Kent (1995) found significantly more males were born as

singles than as triplets over nine lambing seasons (53.04 percent vs. 45.54 percent; $P < 0.05$).

1.2.2.4 *pH of the female reproductive tract*

Sperm cells carrying either an X or Y chromosome differ in physical and chemical characteristics, which may explain the differential fertilisation rates of X- and Y-bearing sperm at different times in the cycle.

Gordon (1958) separated X- and Y-bearing rabbit sperm by electrophoresis. With no voltage, sperm move about at random, but as soon as voltage is applied to the semen, they move toward either the anode or the cathode. Inseminating with semen from the anode resulted in a higher proportion of female offspring, and more male offspring resulted from inseminating with sperm from the cathode. The suggested explanation for this phenomenon is that there are differences in the protein content of cells. All proteins carry both positive and negative charges, their net charge depending on the pH of their environment. When the pH is low, they act as positive ions and, when it is high, as negative ions. In between is a value of pH at which they are neutral (the isoelectric point, which differs between proteins). Thus, it is suggested that the proteins in X- and Y-bearing sperm cells are different, and therefore their isoelectric points are different. If placed in a solution where the pH lies between the two isoelectric points, one type will be negatively charged and move towards the anode, the other positively charged and move towards the cathode.

Because the pH of the female reproductive tract changes over the oestrous period, with a decrease from 7.41 to 7.32 on the day of oestrus (Lewis and Newman, 1984), the X- and Y-bearing sperm may exhibit different fertilising capabilities at different times, depending on the pH.

1.2.2.5 *Maternal-biased gender selection*

Trivers and Willard (1973) postulated that, as females deviate from average condition, they show an increased tendency to bias the production of their young toward one gender or another. The assumptions for this hypothesis are:

- a female in good condition is better able to bear and nurse a calf, so the healthiest, strongest and heaviest calves at weaning will tend to be offspring of females who were in the best condition.
- there is a tendency for differences in the condition of calves at weaning.
- such differences in condition affect male reproductive success more strongly than female reproductive success, for example by excluding other males from breeding and inseminating females.

Under these circumstances, an adult female in good condition that produces a son will leave more surviving grandchildren than a similar female who produces a daughter. However, an adult female in poor condition who produces a daughter will leave more surviving grandchildren than a similar female that produces a son. Therefore, it would be more beneficial to produce sons when body condition is good, and daughters when body condition is poor, and thus, the gender ratio of offspring may be influenced by the female's body condition, which is a direct result of nutritional status.

Several studies have confirmed the Trivers-Willard hypothesis. Kohlmann (1999) showed that female elk in above-average condition (as measured by a kidney fat index) are more likely to conceive a son than a daughter. Monard *et al.* (1997) found the birth gender ratio in horses to vary according to nutritional stress. Mares have less chance of producing males after a poor year than a good one. Cameron *et al.* (1999) also showed that mares that had a male foal in one year, and a female in another were in significantly poorer condition when they conceived their female foal ($P < 0.05$). Cameron *et al.* (1999) assumed that biases were caused by differential conception of males and females and not by differential foetal loss during gestation, since live foal rates were similar

regardless of condition at conception and there was no evidence of selective abortion of male fetuses. Furthermore, by mid-gestation, maternal condition was no longer a predictor of offspring gender ratio, supporting differential conception of male and female fetuses.

Paul and Kuester (1987) found differences in the gender ratio of offspring of Barbary macaques (*Macaca sylvanus*) (primates) to be associated with maternal rank. High-ranking females (in better condition) produced significantly more male offspring ($P < 0.02$) and low-ranking females (in poorer condition) produced significantly more female offspring ($P < 0.05$).

In mountain goats, young females produced significantly more daughters than older females ($P < 0.001$), which also supports the Trivers-Willard hypothesis, if older mothers are considered to be better mothers because of higher social rank and experience (Côté and Festa-Bianchet, 2001). Interestingly, the overall gender ratio did not differ from unity over the nine-year study period.

In sheep, Kent (1995) observed that the gender ratio changes from female-biased to male-biased with an increase in flock age, and remains male-biased up to a certain point, after which it becomes female-biased again. This supports the Trivers-Willard hypothesis if ewes of intermediate age are those in the best condition and start to lose rank/condition after a peak period. These parents of intermediate age are able to expend more energy and other resources on the production of sons, which will then have added advantages in establishing themselves in the dominance hierarchy during the mating season.

Meikle *et al.* (1993) found maternal rank to have a significant effect on the proportion of male births in pigs. The mean proportion of males born was greater for high-ranking females than for low-ranking females ($P < 0.001$). However, Mendle *et al.* (1995) observed no effect of social status on birth gender ratio. Maternal weight gain during pregnancy (measured as an indicator of nutritional status) also has no effect on birth gender ratio of pigs.

Hoefs and Nowlan (1994) also reported gender ratios favouring females in captive elks ($P < 0.005$), reindeer ($P < 0.025$), mountain goats ($P < 0.005$), Dall's sheep ($P < 0.005$), Stone's sheep ($P < 0.01$) and caribou ($P < 0.05$). The bias in ratios was attributed to improved nutrition, which was quantified in a number of measured characteristics such as early age at first breeding, high productivity, large body sizes and improved antler or horn growth (and increased twinning rates in goats).

1.3 Predetermining gender in cattle

From the preceding discussion, it is clear that there are a number of different factors that cause differential gender ratios, providing at least a partial explanation of why one gender may be favoured in certain conditions. Practical ways in which to exploit an understanding of these factors may allow manipulation of the gender ratio in favour of the desired gender. There have been attempts to manipulate the gender ratio in cattle, with the methods used based on differences in X- and Y-bearing sperm, the changing conditions of the female reproductive tract at the time of oestrus and the age of gametes. Unfortunately success rates have been variable.

1.3.1 Timing of insemination

Since artificial insemination is typically delayed until late oestrus (am-pm rule), whereas natural mating must occur at the time of oestrus, Rorie (1999) suggested that if the time of insemination influences gender ratio, a higher percentage of male offspring would occur in offspring produced by artificial insemination than in offspring produced from natural mating. However, results from an earlier study, looking at gender ratios after artificial insemination showed a gender ratio close to that obtained by natural mating (Gardner, 1950).

Results from 1000 calvings showed that no significant deviations from the normal gender ratio emerged when inseminations were timed either 0 to 12 hours or 12 to 24 hours after standing oestrus (Ballinger, 1970). Rorie *et al.*

(1999) found no difference in the gender ratios of two groups inseminated early (>20 hours before expected ovulation) or late (≤ 10 hours before expected ovulation). Time of ovulation was assumed to be 28 to 32 hours after the onset of oestrus.

Pursley *et al.* (1998) observed significantly more female than male calves born when insemination was timed early (0 hours after synchronisation) ($P < 0.05$). However, more female calves ($P < 0.05$) also resulted from late insemination (32 hours after synchronisation). The calf gender ratios resulting from inseminations performed at 8, 16 and 24 hours after synchronization were not different from the expected 50:50 ratio. An explanation of the predominance of females in the 32-hour group was offered by Rorie (1999), who suggested that it could be due to the increase in embryonic death of males, as pregnancy and calving rates were lower for cows inseminated at this time.

Guerrero (1974) reported the proportion of human male births to increase from 0.386 in mothers inseminated three or more days before supposed ovulation to 0.621 in mothers inseminated on the day of the supposed ovulation ($P < 0.001$). However, the opposite trend was observed in cases where natural insemination took place, although this may be due to differences in the frequency of sexual intercourse with natural mating.

The gender ratio in a population of Barbary macaques (primates) was thought to be modified by timing of mating in relation to ovulation (Paul and Kuester, 1987). The interval between mating and birth was measured since, if the chances of fertilisation by Y-bearing sperm are increased when matings occur near ovulation, the interval between mating and birth should be shorter for male offspring. This was found to be the case, with the interval between mating and birth significantly shorter for male offspring ($P < 0.02$). Paul and Kuester (1987) also measured the interval between mating and onset of deturgescence of anogenital swelling. If the shorter mating to birth interval of males is due to a shorter interval between mating and ovulation, and not just a shorter gestation length, deturgescence should occur earlier after mating in females who give

birth to male offspring, since the deturgescence is associated with the rise in progesterone level after ovulation. This was found to be the case, where deturgescence occurred significantly earlier ($P < 0.05$) when males were conceived.

When cows were bred either $20 (\pm 3)$ hours pre-ovulation or $10 (\pm 2)$ hours pre-ovulation, there were significant differences in the gender ratio between the two groups of offspring ($P < 0.005$), with a predominance of females (1 male:13 females) in the group inseminated earlier and a predominance of males (11 males:1 female) in the group inseminated later at a time closer to ovulation (Wehner *et al.*, 1997). Ovulation in this study was confirmed by rectal palpation.

Verme and Ozoga (1981) studied white-tailed deer and suggested that the ability to produce more females from early mating and more males from late mating is an adaptive mechanism to ensure survival of the species. If males are plentiful, early mating in most of the females will result, ensuring that more female offspring are born, whereas late mating suggests a scarcity of males, and more male offspring will be the result.

The varied results in manipulating gender ratio in the above experiments may be due to the inaccuracy of the expected time of ovulation after onset of oestrous behaviour. Oestrous behaviour can be difficult to detect. Some of the aids available for oestrus detection are discussed below.

1.3.1.1 *Oestrus detection*

During natural mating, the bull will mount when the cow is expressing signs of oestrous behaviour. These signs are external indicators of the physiological changes that occur as ovulation approaches, and reflect the co-ordination of follicular development, ovulation and luteal function (Bearden and Fuquay, 2000). Oestrus detection aids improve visual observation and include mount detectors (Williamson *et al.*, 1972), radiotelemetric pressure-sensitive, rump-mounted devices (Stevenson *et al.*, 1996), oestrogen-treated steers with chin

ball harnesses (Sawyer *et al.*, 1986), androgenised heifers (Mortimer *et al.*, 1990) and monitoring by pedometry (a measurement of physical activity) (Kiddy, 1977). Pedometry has been advocated as a means of oestrus detection, as activity has been found to be at least two standard deviations above the mean ($P < 0.001$) on day 0 (oestrus) than days -3 to -1 and days +1 to +5 of the oestrous cycle (Lewis and Newman, 1984; Schofield *et al.*, 1991). Changes in heart rate might be expected to parallel the increase in activity around the time of oestrus but, in fact, the slowest heart rates are recorded at oestrus. However the drop in heart rate appears to be too small a change to be a valuable indicator of oestrus (Lewis and Newman, 1984).

Horrell *et al.* (1984) suggested that milk yield at oestrus shows greater fluctuations than at other times of the cycle. Many cows show a drop in milk production at the onset of oestrus, with a rebound enhancement at the next milking. If a cow produces less than 75 percent of its usual yield, there is a 50 percent chance that it is in oestrus. A sure, but rare, sign of oestrus is when a cow produces 25 percent more than its usual yield (Horrell *et al.*, 1984). To use milk yield as the only sign of oestrus is not advocated, but it provides a useful indicator if used in conjunction with other signs of oestrus.

Rajamahendran *et al.* (1989) and Schofield *et al.* (1991) found no significant difference in milk yield at oestrus compared to other days of the cycle. Schofield *et al.* (1991) found milk fat content to be significantly reduced at the morning milking on the day of oestrus compared to the subsequent morning milking, although no significant differences in milk protein levels were found.

The degree of cellular hydration of vaginal and vulvar tissue has been measured during oestrus and di-oestrus by morphometric analysis, which determines the cytoplasmic-to-nuclei ratio per unit area of tissue. The ratios are significantly different ($P < 0.05$) during oestrus and di-oestrus, with higher values obtained at oestrus (Smith *et al.*, 1989).

It has been determined that changes in progesterone and oestrogen seem to regulate the changes in the degree of hydration of vulvar tissue, and hydration of vulvar tissue appears, in turn, to govern changes in the resistive properties of the tissue (Lewis *et al.*, 1989; Smith *et al.*, 1989). Thus the outcome of the differences in the degree of cellular hydration mean that the resistive properties of the vaginal tissue change during oestrus (see section 1.3.1.2.4).

Alliston *et al.* (1958) examined the crystallisation patterns of cervical mucus, thought to be attributable to sodium chloride (NaCl). Aboul-Ela *et al.* (1983) also found mucus ferning patterns to be positively and significantly correlated with sodium and chloride concentrations in the mucus (while showing a reciprocal relationship to electrical resistance values). In the case of Alliston *et al.* (1958), the mucus was spread on a slide, and dried at a low temperature over an alcohol burner. Fernlike patterns of crystallisation were found to occur in the mucus around the time of ovulation. The crystallisation patterns were at a minimum during the luteal phase of the oestrous cycle and the average first indication of the fernlike pattern occurred 84 hours prior to oestrus. The incidence of fernlike patterns gradually increased to a maximum at the time of oestrus and began a decline prior to the average time of ovulation (determined by rectal examination). Aboul-Ela *et al.* (1983) observed a similar pattern with ferning values rising significantly on day -1, rising further on the day of oestrus, followed by a decrease to low di-oestrus values after day +1.

Analysis of crystallisation patterns requires labour and restraint of animals for collection of mucus and the techniques and apparatus are not familiar or available to commercial dairy farmers. Thus, this method is not used for routine detection of oestrus (Williamson *et al.*, 1972). It does, however, demonstrate the changes occurring in the mucus at the time of oestrus.

It has been reported that the viscosity of vaginal mucus changes with the onset of oestrus. One day before oestrus, there is an increasing liquefaction of vaginal mucus, as measured by a decrease in viscosity (Schilling and Zust, 1968). A minimum viscosity is measured immediately before ovulation, followed by a

rapid increase during the next two days, back to dioestrus levels. Nuclear magnetic resonance spectroscopy has been used to evaluate bovine cervical mucus. The spectra obtained of cervical mucus during oestrus closely resemble that of distilled water, while a transition occurs to a lower moisture content during the luteal phase (Merilan, 1983).

1.3.1.2 *Determining the time of ovulation*

Despite aids in detecting oestrous behaviour, there are varying reports in the literature on the time interval from oestrus to ovulation. Ninety five percent of heifers ovulate within 36 hours after the onset of oestrus (Christenson *et al.*, 1975) and it has been reported that 78 percent of cows ovulate 29 (± 6) hours after the onset of oestrus (Swanson and Hafs, 1971), and within 40 hours after the onset of oestrus (Walker *et al.*, 1996). Rajamahendran *et al.* (1989) found the time interval from oestrus to ovulation to be dependent on parity. Pluriparous and biparous cows ovulated within 24 and 30 (± 3.2) hours, respectively, from the onset of standing oestrus.

The mean time of ovulation was not found to differ between animals in which oestrus was induced with prostaglandin $\text{PGF}_{2\alpha}$ and those in which oestrus occurred spontaneously. However, Nkuuhe and Manns (1985) found that the intervals to the LH peak and ovulation after $\text{PGF}_{2\alpha}$ treatment are significantly shorter in heifers than in cows, suggesting that timed inseminations should be made earlier in heifers than in cows. The mean time from $\text{PGF}_{2\alpha}$ injection to the start of oestrus was 61 (± 2) hours (Gonzalez *et al.*, 1985) and 90 (± 10) hours after the second $\text{PGF}_{2\alpha}$ injection, given 12 days after the first injection, in a two-injection synchronisation regime (Rajamahendran *et al.*, 1989). Synchronisation with a nine-day progesterone implant and an injection of $\text{PGF}_{2\alpha}$ had no effect on the relationship among the onset of standing oestrus, the LH surge and ovulation following implant removal. Nor was there any decrease in fertility of heifers in this trial (Rajamahendran and Taylor, 1991).

Thus, in addition to the above methods used for oestrus detection, ovulation could be measured more accurately in the following ways.

1.3.1.2.1 *Rectal palpation/ultrasound of ovaries*

Rectal palpation or trans-rectal ultrasonography of the ovaries can be performed to give an indication of ovulation, which is associated with the disappearance of the dominant follicle (Pierson and Ginther, 1984). The presence of a corpus luteum is only detected four days after ovulation, and thus cannot be used as a reliable indicator of ovulation time (Edmondson *et al.*, 1986).

1.3.1.2.2 *Hormone assays*

The luteinising hormone (LH) surge has been closely correlated to the time of ovulation ($R=0.81$) (Rajamadendran *et al.*, 1989). The time from LH surge to ovulation has been shown to be consistent, with reported values of 27.3 (± 1.6) hours (Bernard *et al.*, 1983); 22 (± 2) hours (pluriparous cows) and 31 (± 2) hours (biparous cows) (Rajamahendran *et al.*, 1989) and 21.43 (± 4.31) hours (Mosher *et al.*, 1990). However, this method requires blood sampling on a regular basis, as well as immediate analysis, to be of any benefit in timely determination of ovulation.

1.3.1.2.3 *Temperature changes*

Wrenn *et al.* (1958) found the vaginal temperatures in cows to be lowest just before oestrus, high on the day of oestrus, low again at the time of ovulation, and high during the luteal phase. Significant differences in temperature were recorded between day -2 and the luteal phase, day 0 and days -1 and -2, as well as day +1.

When temperature of the vagina was measured at four minute intervals with the use of a radiotelemetric system (identity-coded temperature sensitive

radiotransmitters, radio receiving antenna, radio receiver and personal computer), a depression prior to oestrus and a dramatic elevation in vaginal temperature at oestrus were also observed, with another decrease a day later at presumed ovulation (Kyle *et al.*, 1998). Ovarian status was determined by progesterone assays and transrectal ultrasonography of the ovaries. Temperature peaks were defined as an increase of 0.4°C for three or more consecutive hours using the corresponding hourly means of a two or three day baseline.

Rectal and vaginal temperatures have been found to be correlated ($R=0.95$), but only the vaginal temperature rose to greater than twice the standard deviation above the basal value (Rajamahendran *et al.*, 1989). Peak vaginal temperature has also been found to be highly correlated ($P<0.05$; $R=0.74$) with the time of ovulation (Rajamahendran *et al.*, 1989). Clapper *et al.* (1990) observed that the preovulatory LH peak precedes the onset of a temperature spike by eight to twenty hours, and the relationship between the LH surge and temperature is more consistent than the relationships between temperature and both oestradiol and progesterone. Thus it was concluded that the temperature spike is a better indicator of the LH surge than other physiological parameters such as the decline in serum progesterone or rise in serum oestradiol. Mosher *et al.* (1990), in a companion paper, further showed the temperature spike to be a reliable predictor of ovulation, which was measured by laparoscopy or a combination of laparoscopy and rectal palpation. The temperature spike in this case was observed 16 to 33 hours before ovulation, while the LH peak occurred before, during or after the temperature spike (13 to 27 hours before ovulation). The discrepancy between the time of the temperature peak in relation to the time of the LH surge between Clapper *et al.* (1990) and Mosher *et al.* (1990) was attributed to the different experimental methods.

Lewis and Newman (1984) cautioned that if changes in body temperature are to be used to predict oestrus, they must be adjusted for ambient temperature, as ambient temperature in their own trial accounted for more of the variance than did the day of the oestrous cycle.

1.3.1.2.4 *Electrical resistance*

There have been conflicting reports as to whether the conductivity of vaginal and/or cervical mucus changes during oestrus. Williamson *et al.* (1972) used an ohmmeter attached to a human rectal probe, which was inserted into the vagina of cows. They found no differences in vaginal conductivity between cows in oestrus and di-oestrus. Peters (1989) found that vaginal resistance values decreased in 10 out of 13 cows after synchronisation with prostaglandin. It was determined (by plasma progesterone concentration) that luteolysis was successful in these three cows, and vaginal resistance values thus showed false negative results. In three other cases, false positive results were obtained where progesterone concentrations did not decrease, indicating failed luteolysis, but vaginal resistance values did decrease. Feldmann *et al.* (1978) observed an increase in conductivity when electrodes were implanted into vaginal tissue, which was assumed to be due to congestion of the blood vessels. When used as a method for optimal timing of insemination, there is no significant difference in conception rates of cows bred according to vaginal resistance patterns and those bred by visual observation of oestrus (Canfield and Butler, 1989).

Despite the problems associated with this method, more promising results have been obtained. Vaginal and vulvar resistance values were found to be lower at oestrus than during the other phases of the cycle (Scipioni *et al.*, 1982; Aboul-Ela *et al.*, 1983; Lewis *et al.*, 1989). The resistance values have been found to be significantly lower ($P < 0.001$) at the afternoon milking on the day of oestrus observation than any other day of the cycle at both morning and afternoon milking times (Schofield *et al.*, 1991). Lewis *et al.* (1989) found resistance to increase from about 160Ω , five days prior to oestrus, to about 166Ω , three days prior to oestrus. It then decreased to less than 155Ω at the onset of oestrus, after which an increase back to 160Ω was observed. However, animal-to-animal variation in baseline resistance and the associated decline following luteolysis has been observed (Canfield and Butler, 1989). The lowest vaginal resistance value and the LH surge were found to be synchronous (Aboul-Ela *et al.*, 1983;

Canfield and Butler, 1989) with the lowest resistance value being within six hours of the LH surge in 79 percent of the animals, and within twelve hours of the LH surge in 89 percent of animals. This was found to be a valuable method for predicting the LH surge in animals that exhibited limited or undetected behavioural signs of oestrus.

Although the time of ovulation was not determined, a closer relationship between conductivity values and ovulation has been suggested than between conductivity values and the onset of oestrus (Smith *et al.*, 1989). This is because it takes several hours after a definite conductivity threshold is exceeded (indicating the time of oestrus) before peak conductivity is reached. Conductivity values indicating the time of oestrus also continued for substantially longer than the time-span of oestrous behaviour.

From a practical standpoint, Rust *et al.* (1996) concluded that a heat-detecting probe used to measure electrical impedance can only be put to practical use when oestrus has been synchronised. Canfield and Butler (1989) suggested that measurements should be taken at twelve-hour intervals or more, which would fit in with dairy routine, but they expressed concern over the additional record-keeping load. A given numerical value for vaginal resistance is not necessarily indicative of oestrus or the accompanying LH surge without monitoring the relative changes within an animal. Rather each animal has its own baseline resistance level and associated decline during oestrus, and individual records need to be kept for each animal (Canfield and Butler, 1989).

Electrical conductivity values also appear to indicate abnormalities in the reproductive tract. When electrodes were implanted into vaginal tissue, daily inspections of the wounds revealed that a decrease in conductivity coincided with the healing process (Feldmann *et al.*, 1978).

Wehner *et al.* (1997), who successfully manipulated the gender ratio by timing insemination according to ovulation, used the Ovatec instrument (Profitable

Breeding Corporation)¹. This measures a combination of electrical resistance and capacitance parameters in the calibration and sensing of ion fluxes to determine the time of ovulation.

1.3.2 *Differential development rates of male and female embryos*

The skewing of the gender ratio of early embryos has been attributed to different developmental rates of males and females, with a predominance of males in the earliest developing embryos (Longergan *et al.*, 1999). Xu *et al.* (1992) observed the same tendency, with a ratio of 9.67 males to 1 female ($P < 0.001$) from 32 embryos at the most advanced stages, as did Gutiérrez-Adán *et al.* (1996), who obtained a ratio of 1.37 males to 1 female in bovine embryos at 10 days of *in vitro* culture ($P < 0.01$). This suggests that the differences between male and female embryos in developmental ability are due to events that occur after cleavage, such as a faster growth rate in male embryos. Because males have to undergo their development in the female hormonal environment of the uterus, in which oestrogen levels rise throughout pregnancy, and because oestrogen is known to interfere with male development, the accelerated growth of male embryos increases the probability of the testes being established and imposing a male phenotype before this male phenotype would be damaged by high oestrogen levels (Mittwoch, 1996).

The genetic disposition for increased growth of males can be seen in birth weights of calves, in which males are 8.5 percent heavier than females (Kertz *et al.*, 1997). Significantly higher birth weights in male calves have been found by Prior and Laster (1979) ($P < 0.01$); Echterkamp (1993) ($P < 0.01$) and Kars *et al.* (1994) ($P < 0.001$), and thus a greater incidence of dystocia has been reported for heifers giving birth to bull calves than heifer calves (Davis *et al.*, 1998). Echterkamp (1993) and Prior and Laster (1979) reported that the increased birth weight of male calves was not associated with a difference in placental weight, cotyledonary number, area or weight, and Bellows *et al.* (1990)

¹ 9877 Simmonds Rd, Corfu, NY 14036, USA.

suggested that the larger male foetus size could be attributed to genetic growth factors arising from gender of calf early in gestation.

One of the outcomes of the increased growth rate of males is that there is a positive linear relationship between calf birth weight and subsequent milk yield of the dam (Erb *et al.*, 1980; Thatcher *et al.*, 1980; Collier *et al.*, 1982), and therefore there is a possibility that milk yield of cows would be greater after producing a bull calf. Therefore, if sexing is used to produce heifer calves from top-producing cows for replacements, and bull calves from the lower-producing cows for beef production, an additional benefit of an increase in the milk yield of the lower-producing cows might be seen. If there is a loss in milk production from the high yielding cows due to the birth of female calves this may be counterbalanced. Contradictory results were found where there were no significant differences from gender of calf on subsequent 200- and 300-day yields of milk, fat and total solids were found by Chew *et al.* (1981).

If males have a faster growth rate early in gestation, they might be more susceptible than females to stressors, such as a lowered nutrition level. However, this was not found to be the case in sheep where there was a non-significant difference in the gender ratio of stillborn lambs (Chapman and Lush, 1932).

Even in those species that have temperature-dependent gender determination, such as lizards, turtles and crocodiles, the temperature-sensitive period occurs during embryogenesis at about the time when gonadal development occurs. This suggests that temperature-dependent gender determination could affect the sexual phenotype by controlling the rate of growth (Mittwoch, 1996).

Therefore, the gender ratio of offspring could be manipulated in embryo transfer programmes, according to their rates of development. By removing the fastest developing embryos, and transferring those that take longer to develop, the chances of obtaining a female calf will be improved.

1.3.3 Early foetal gender determination in cattle

Early diagnosis of foetal gender would be of use in any cattle-breeding programme since cows are costly animals and have a long gestation. Additional benefit may also be obtained if early diagnosis is used in conjunction with one of the methods to manipulate the gender ratio. Being able to determine foetal gender during gestation would allow selection of cows and heifers according to the gender of their offspring, i.e. a heifer in calf with a heifer may be more valuable than a heifer in calf with a bull. There may be circumstances in which induced abortion of embryos of an unwanted gender may have economic merit. Foetal gender determination is also a valuable research tool, and will be discussed as such here. Early foetal gender determination can be achieved by ultrasonography or DNA testing of cells obtained by amniocentesis.

1.3.3.1 Ultrasonography

The fluid in the uterus associated with pregnancy can be seen by transrectal ultrasonography at 22 days after conception in cows, but it is difficult to distinguish the fluid of early pregnancy from the fluid seen at oestrus (Fissore *et al.*, 1986). Accurate detection of pregnancy by this method has been reported 28 days after conception, where all pregnancies were later confirmed by rectal palpation (Reeves *et al.*, 1984). Fissore *et al.* (1986) first detected the presence of an embryo at 27 days after breeding. The embryo is seen as an echogenic structure in the nonechogenic fluids of pregnancy.

Besides its use in pregnancy diagnosis, ultrasonography can be of use in determining the gender of the foetus. Differentiation of males and females is made on the basis of the genital tubercle, which is the embryonic structure that differentiates into the penis in males and the clitoris in females. This structure moves from the initial position between the hind limbs towards the umbilical cord in males and towards the tail in females (Figure 1.2). The ultrasonic appearance of the genital tubercle is similar in both sexes, appearing as a hyperechogenic, bilobar structure, with each lobe elongated and oval shaped,

recognisable despite the presence of other echogenic structures (Curran *et al.*, 1989) (Figure 1.3).

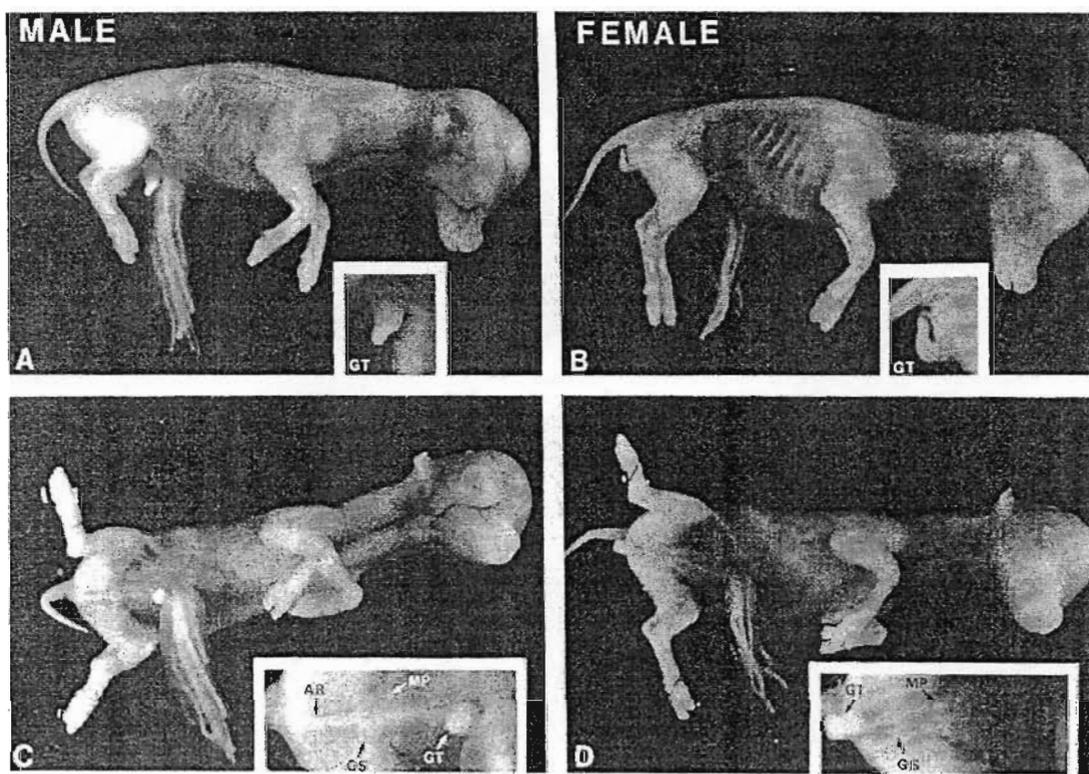


Figure 1.2 Gross appearance and relative locations of the external genitalia in male (A, C; day 62) and female (B, D; day 67) bovine foetuses. AR, anogenital raphe; GS, genital swelling (forerunner of scrotum); GT, genital tubercle (forerunner of penis in male and clitoris in female); MP, mammary papillae (from Curran *et al.*, 1989).

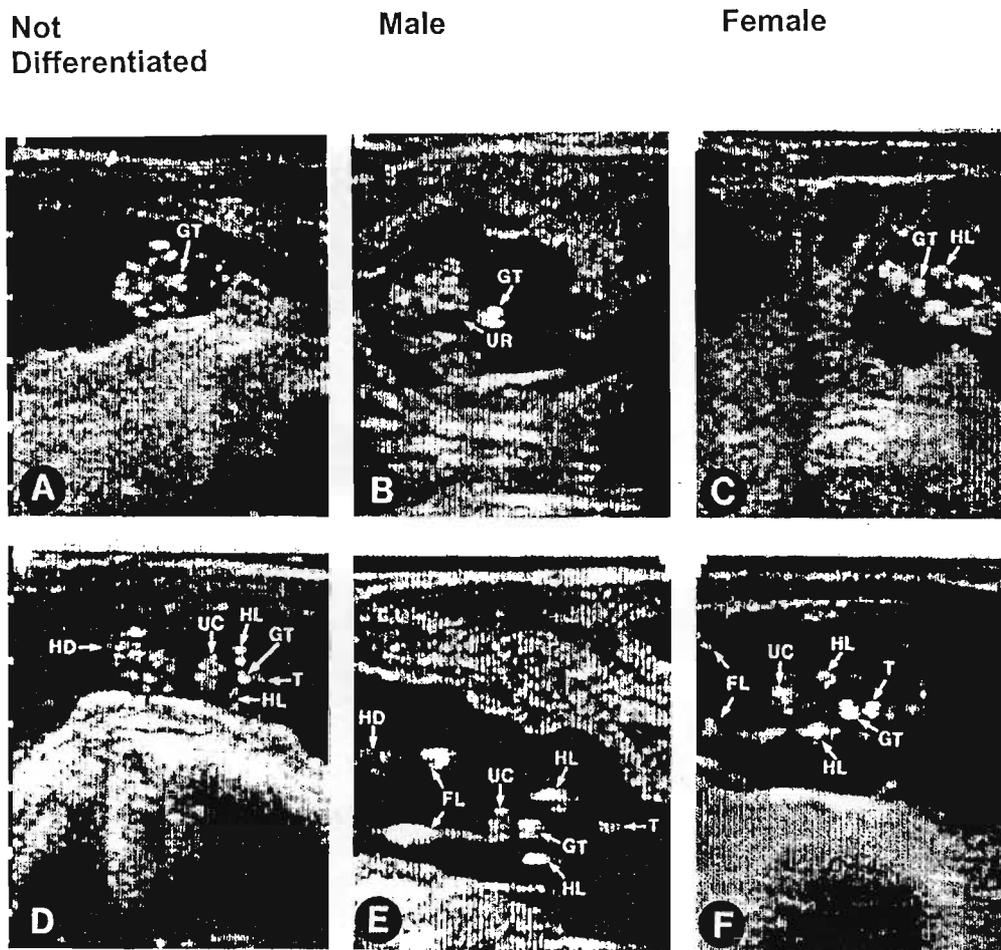


Figure 1.3 Ultrasonograms of the genital tubercle in undifferentiated (gender not determinable; A, day 51; D, day 47), male (B, day 69; E, day 60), and female (C, day 55; F, day 56) bovine foetuses. The images were taken in cross-sectional (A, B, C) and frontal (D, E, F) planes to show ultrasonic morphology and location of the genital tubercle relative to other structures. *HD*, head; *HL*, hind limbs; *GT*, genital tubercle; *T*, tail; *UC*, umbilical cord; *UR*, urachus (from Curran *et al.*, 1989).

Other internal structures of the foetus seen in cross section are helpful in locating the genital tubercle (Figure 1.4). For example, the urachus (nonechogenic structure) is located in the area of the genital tubercle in male foetuses (Curran *et al.*, 1989), and foetal landmarks such as the beating heart, the pulsating umbilical cord, the rear legs and tail may be used for orientation (Curran and Ginther, 1991).

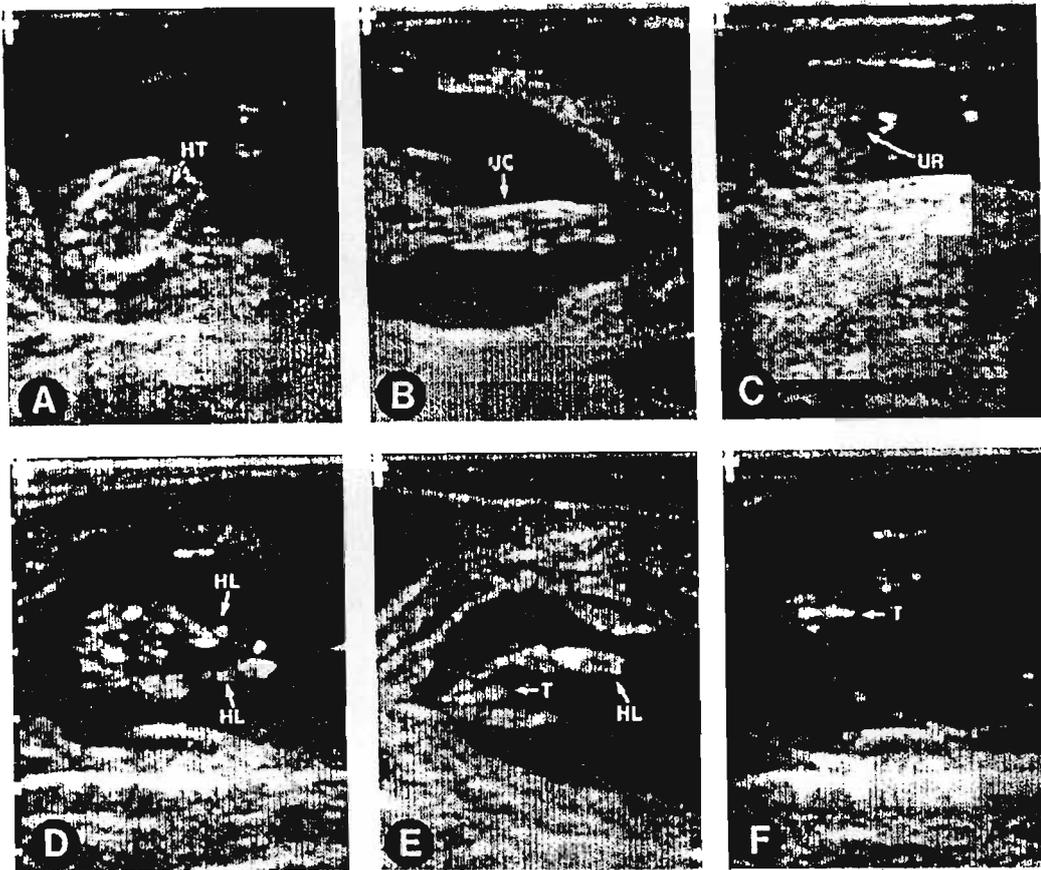


Figure 1.4 Ultrasonograms of Day 58 to 68 bovine fetuses showing cross-sectional ultrasonic morphology at various levels (A, chest; B, longitudinal view of umbilical cord; C, urachus; D, pelvis and hind limbs; E, caudal portion of hind limbs and proximal portion of tail; F, distal portion of tail). The identified structures are helpful in determining the location of the sequential planes of view for systematically searching for the genital tubercle. *HL*, hind limb; *HT*, heart; *T*, tail; *UC*, umbilical cord; *UR*, urachus (from Curran *et al.*, 1989).

The diagnosis of foetal gender becomes accurate for all fetuses on day 53 and is consistently correct thereafter (Curran *et al.*, 1989). It was concluded that ultrasonography can be used accurately from days 50 to 60 post ovulation (Curran *et al.*, 1989). In a later study, Curran and Ginther (1991) confirmed that the optimum time for gender diagnosis on the basis of the genital tubercle is day 55 to 64 postovulation in cattle. Two situations occur once the foetus reaches about 70 days and onwards. Firstly, as the foetus becomes larger, it becomes

more difficult to move the transducer relative to the foetus to attain the desired ultrasound image and, secondly, the gravid horn is more likely to have descended ventrally into the abdominal cavity, making the foetus inaccessible (Beal *et al.*, 1992). It was suggested that the optimum time for sexing cows of larger-framed breeds and/or older cows is between days 55 to 70 and between days 60 and 80 for cows of smaller-framed breeds and/or younger cows (Beal *et al.*, 1992). It may be difficult to see some foetuses any older than this. However, if an adequate view is obtained with older foetuses, gender diagnosis is accurate (Curran and Ginther, 1991).

Müller and Wittkowski (1986) identified gender of the foetus by ultrasonic determination of the scrotal swellings (2 to 14 mm diameter) or mammary teats from day 70 to 120 post-insemination. It should, theoretically, be possible to see the vulvar swelling in female embryos. However, in this experiment, the tail vertebrae provided a confused image of the perineal region (Müller and Wittkowski, 1986).

The average time required for an experienced operator to determine gender of the foetus by identification of location of the genital tubercle by ultrasound, under farm conditions, is less than two minutes, with a range of 16 seconds to 8 minutes 30 seconds (Curran and Ginther, 1991). In a trial looking for scrotal swelling as an indication of gender, examination time did not exceed five minutes per individual (Müller and Wittkowski, 1986).

Baxter and Ward (1997) found that the use of ultrasound to detect early pregnancy (days 30 to 40) did not increase the rate of foetal loss. Curran and Ginther (1991), after performing ultrasonic gender determination, observed an abortion rate of three percent, and Müller and Wittkowski (1986) did not cause a single abortion with the use of ultrasound scanning when looking for the scrotum and mammary glands at day 70 to 120 of gestation.

1.3.3.2 Amniocentesis

Amniocentesis is the collection of foetal amniotic fluid and has been performed successfully in different ways:

- by accessing the pregnant uterus through an incision comparable in size and location to that used in surgical embryo transfer (Leibo and Rall, 1987)
- by inserting a needle encased in a blood collection tube into the vagina, through the cervix and into the uterus with the aid of rectal manipulation of the pregnant uterus (Bongso and Basrur, 1975)
- by inserting a needle through the cervix with the aid of a speculum (Sprecher and Kaneene, 1992)
- by inserting a needle via transvaginal ultrasound-guided aspiration between days 70 to 100 of gestation (Garcia and Salaheddine, 1997; Kamimura *et al.*, 1997).

Fluid from the amniotic sac is aspirated and the amniotic cells analysed to determine the foetus gender. Gender determination of the amniotic cells can be determined in various ways:

- DNA analysis, following culture of cells, by observation of metaphase plates to distinguish between X- and Y-chromosomes. In the bovine, the X-chromosomes are readily distinguishable from the autosomal chromosomes as they are metacentric and among the largest, whereas the autosomes are acrocentric (Leibo and Rall, 1990).
- Electrophoresis of polymerase chain reaction (PCR)-amplified products of foetal fluid, by synthesis of oligonucleotides on the basis of both male-specific and gender-neutral DNA sequences as primers for PCR (Kamimura *et al.*, 1997).

Leibo and Rall (1990) successfully diagnosed foetal gender in 93 percent of 302 fetuses in which amniotic fluid was collected surgically from cows between

days 49 to 154 of gestation. However, they concluded that it is reasonable to perform amniocentesis as soon as possible after pregnancy has been diagnosed if the aim is to determine foetal sex as early as possible, because delaying amniocentesis only improves the medium growth rate by five days. However, aspiration of the required amount of 15 to 20 ml of fluid through the vaginal route was most easily obtained between days 70 to 100 of gestation (Bongso and Basrur, 1975).

Leibo and Rall (1990) failed to sex 23 out of 325 (seven percent) pregnancies by the method of amniocentesis, because the amniotic cells did not grow in culture. Twenty-one of these pregnancies ended in spontaneous abortion, and it was suggested that the foetuses were dead or dying at the time of amniocentesis, which would explain why the amniotic cells did not grow.

One of the problems associated with this method of sexing is that of maternal cell contamination of the amniotic cells (Leibo and Rall, 1990). Thus, some male calves were predicted to be females, due to the presence of maternal cells. All the cows predicted to be carrying male calves gave birth to (or aborted) male calves (although only 26 out of 79 foetuses from induced abortions were recovered), while seven out of 149 cows (five percent) predicted to be in calf with heifers, gave birth to bulls (Leibo and Rall, 1990). Gender identification by PCR correctly identified 16 out of 16 females, but 13 out of 15 males (Kamimura *et al.*, 1997). Garcia and Salaheddine (1997) minimized maternal cell contamination by advancing the needle through maternal tissue with a stylette in place.

There is an increased risk of abortion with amniocentesis when performed in the non-sterile environment of standard cattle handling facilities (Leibo and Rall, 1990), as well as in those cows that required multiple needle penetration (Kamimura *et al.*, 1997). Cows that were subjected to the same procedures as those undergoing amniocentesis, without needle penetration, had significantly fewer abortions ($P=0.04$) (Sprecher and Kaneene, 1992). There seems to be no adverse effects of amniocentesis being manifested after birth as none of the

187 calves whose gender was verified at birth died prior to weaning (Leibo and Rall, 1990).

Ultrasonography and amniocentesis are appropriate tools in the early determination of foetal gender, which can be useful to both dairy and beef farmers. The method of ultrasonography is more practical and applicable without the degree of veterinary expertise that amniocentesis requires, and is less likely to cause subsequent abortion, although experience in using ultrasound to determine gender is necessary.

1.4 Conclusions

Although it appears that the gender ratio of offspring remains at 50 percent under most conditions, there are reports in the literature of deviations from unity in this ratio, and a number of theories have been proposed to explain what causes these deviations. The cattle industry would benefit greatly from a practical method to predetermine gender. However, the most practical method, which is to time insemination according to the time of ovulation, has had variable success rates. The success of this method is limited by the ability to predict the time of ovulation, which in turn is dependent on the following. Firstly, oestrous behaviour, which is an indication of ovulation, is not always easy to detect; secondly, the timing of ovulation after onset of oestrous behaviour is variable; and, thirdly, some methods to determine the time of ovulation cannot be used to accurately *predict* the time of ovulation. This prediction is important if insemination is to occur at a specific time period before ovulation. A more extreme method of gender selection is that of selective abortion after early gender determination by ultrasound or amniocentesis. These two procedures can also be useful in providing information for culling/selling decisions of pregnant animals, based on the gender of the calf they are carrying.

CHAPTER 2

THE POTENTIAL USE OF PRODUCTION AND MANAGEMENT VARIABLES TO MANIPULATE THE CALF GENDER RATIO IN DAIRY CALVES IN THE KWAZULU-NATAL MIDLANDS

2.1 Introduction

The calf gender ratio (CGR), expressed as a percentage of males, has been reported to be close to 50 percent in cattle (Powell *et al.*, 1975; Gray and Hurt, 1979). However, some dairy farmers in the KwaZulu-Natal Midlands have reported skewed gender ratios, in favour of males, in the calves born in their herds (Mullins, 2001 - personal communication). There is no apparent explanation for these skewed ratios.

Due to the comparative worth of heifer calves in a dairy enterprise, it would be economically valuable if the cause of such a bias towards male calves could be identified and manipulated in order to prevent the bias and, further, to cause a bias in the direction of female calves.

The purpose of this study was to attempt to identify management or production variables that may influence CGR, and to investigate how the variable(s) could be manipulated to improve the probability of female offspring.

2.2 Materials and methods

Farm records were collected from 20 farmers in the south-western KwaZulu-Natal Midlands area. A computer programme was written to extract the necessary information on farms with computerised records. Manual retrieval of the required information from cow cards was necessary where computer records were unavailable. As many variables as possible were included in the

data collection to improve the chances of identifying which variable(s) may influence CGR. The following variables were recorded:

- calf gender (male or female or, if twins, the combination of genders) (CG).
- calving date (CD).
- dam's month of birth (DOB).
- dam's parity (P).
- dam's age at each calving (AC).
- dam's inter-calving period (ICP).
- number of services required to conceive (NS).
- month of dam's last service date (LSD).
- 300 day milk production (300dP).
- lactation length (L).
- geographical location of farm (GA) recorded as one of the following regions: Balgowan, Boston, Creighton, Donnybrook, Highflats, Ixopo, Kamberg, Umzimkulu or Underberg.
- timing of insemination after standing heat (TAI), recorded as immediate insemination after observation of standing heat (1), insemination ± 12 hours after observation of standing heat (3), i.e. if a cow is seen on standing heat in the morning milking, insemination will take place at the afternoon milking and vice versa (am-pm guideline) or a combination of these (2), i.e. immediate insemination of heifers and assessment of cows showing standing heat, leading to immediate insemination of cows perceived to have been on standing heat for a period before being observed, or insemination at the next milking of those cows perceived to have just come on standing heat.
- heat detection score (HDS) (visual only (1) visual and one aid (2) or visual and two aids(3)).
- number of inseminators (NI) (1, 2 or 3).
- whether or not a bull(s) is/are present on the farm (BP)(1=yes and 0=no).
- milking frequency (MF)(2 or 3 times daily).

Minimum and maximum temperatures on the day of insemination (T_{\min} and T_{\max}) were obtained for all geographical areas.

Analyses were conducted on 18 958 complete records using GenStat® 6th edition (2002). If any variable for a given calf was missing, the entire calf record was excluded from the analysis, so that each calf analysed was represented by a complete set of variables. Discriminant function analysis was undertaken as an exploratory tool to identify any separation between males, females, and twins (male twins, female twins and mixed twins). This is the appropriate statistical procedure for testing the hypothesis that the group means of a set of independent variables for two or more groups are equal. This results in a discriminant Z score for each individual in the analysis which, when averaged result in the group mean (Hair *et al.*, 1998). Two groups (males and females) showed discrimination and therefore logistic regression is preferred to discriminant analysis (Hair *et al.*, 1998). Hence, a stepwise multiple logistic regression was performed (with GenStat®). Terms with a variance ratio greater than 3 were included in the model, to identify which variables significantly affect CGR. The variable with the greatest contribution is added first and variables are then selected for inclusion based on their incremental contribution over the variable(s) already in the equation (Hair *et al.*, 1998). In this study, the percentage of females rather than males was considered to be important, thus the CGR was a measure of female offspring (female = 1; male = 0). Predictions of a female calf were then determined from the variables that were significant in the model, using the predict directive in GenStat® which forms predictions from a generalised linear model. Because the outcome of logistic regression is binary, it directly predicts the probability of an event occurring (Hair *et al.*, 1998). However, not all combinations of each variable were represented by the available data set, therefore some of the predictions were based on estimations.

2.3 Results and discussion

Reallocation of variables in the discriminant analysis yielded no separation between groups of bulls, heifers, bull twins, bull and heifer twins and heifer twins. Therefore, bull twin data were merged with single bull data, and heifer twin data merged with single heifer data. This left 3 groups, namely bulls, heifers and bull and heifer twins. Again, reallocation of variables did not show separation of groups. However, after excluding bull and heifer twin data (187 out of 18958 records) the two remaining groups of bulls and heifers did show separation. The means of the latent vectors were 0.08562 and -0.09244 for bulls and heifers respectively, implying that the variables used in determining the averages cause separation between bulls and heifers and should be considered further.

The latent roots provide an estimate of the amount of shared variance between the respective variates and it was apparent that some variables had a negligible influence on CGR. However, some variables appeared to be more influential (Table 2.1).

Table 2.1 Management variables that appear to influence CGR in dairy calves

Variable	Latent Roots
Heat detection score (HDS)	-1.2657
Bulls presence (BP)	-1.1711
Number of inseminators (NI)	0.3244
Timing of AI (TAI)	0.1861
Geographical area (GA)	-0.1713
Number of services (NS)	0.1489

Thus, it appears that some of the variables tested may influence the gender ratio in dairy calves. Stepwise multiple logistic regression analysis confirmed these results, with HDS, BP, NI, TAI and GA showing significance ($P < 0.001$). Because there were nine geographical areas, the regression analysis was run separately nine times, with each area being the area of reference to which each other area was compared. This process allowed areas that were not

significantly different from each other to be grouped (Table 2.2). Three groups resulted, within which areas were not different to each other, but significantly different to all other areas not included in the group. The three groups are as follows: Area group 1 – Balgowan and Creighton, Area group 2 – Boston, Kamberg, Umzimkulu and Underberg and Area group 3 – Donnybrook, Highflats and Ixopo (Figure 2.1)

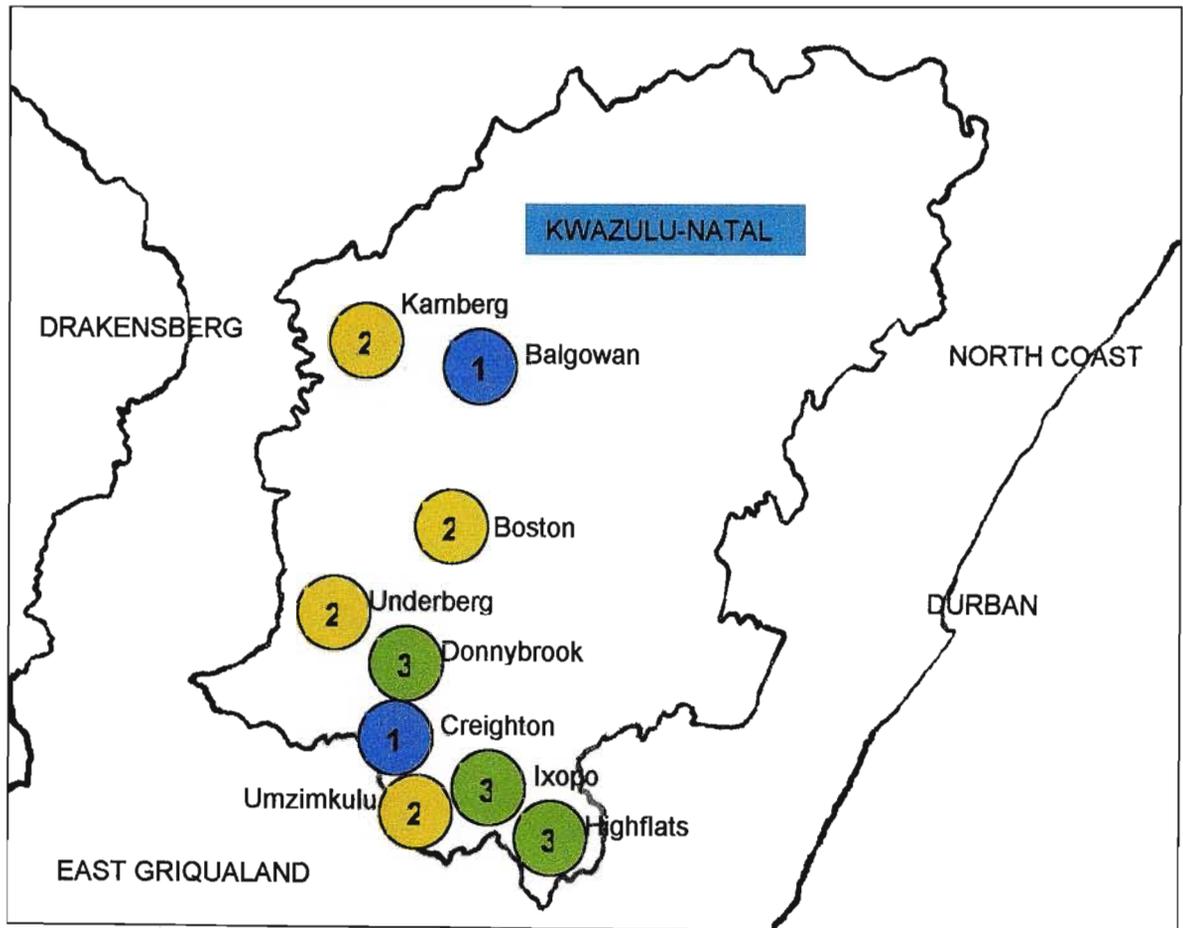


Figure 2.1 Map of the KwaZulu-Natal Midlands, showing Area group 1 (Balgowan and Creighton), Area group 2 (Boston, Kamberg, Umzimkulu and Underberg) and Area group 3 (Donnybrook, Highflats and Ixopo)

Table 2.2 Differences between the nine areas, with each area used as the reference point in the regression analysis

	Balgowan	Boston	Creighton	Donnybrook	Highflats	Ixopo	Kamberg	Umzimkulu	Underberg
Balgowan		**	ns	**	**	**	**	**	**
Boston	**		**	**	**	**	ns	ns	ns
Creighton	ns	**		**	**	**	**	**	**
Donnybrook	**	**	**		ns	ns	**	**	**
Highflats	**	**	**	ns		ns	**	ns	**
Ixopo	**	**	**	ns	ns		**	ns	**
Kamberg	**	ns	**	**	**	**		ns	ns
Umzimkulu	**	ns	**	**	ns	ns	ns		ns
Underberg	**	ns	**	**	**	**	ns	ns	

Stepwise logistic regression analysis yielded the following model, with $P < 0.001$ for each term.

$$\text{CGR} = -2.224 (\pm 0.203) + 0.328 (\pm 0.0289) \text{HDS} + 0.3214 (\pm 0.0532) \text{BP} + 1.077 (\pm 0.159) \text{Area group 2} + 0.809 (\pm 0.156) \text{Area group 3} - 0.402 (\pm 0.112) \text{NI 2} + 0.3386 (\pm 0.0645) \text{NI 3} + 1.179 (\pm 0.148) \text{TAI both} + 0.2068 (\pm 0.0625) \text{TAI am-pm}$$

(Residual mean deviance = 1.374; 18759 df)

Where:

HDS is the heat detection score

BP is bull presence on the farm

Area 2 is Boston, Kamberg, Umzimkulu and Underberg

Area 3 is Donnybrook, Highflats and Ixopo

NI 2 is two inseminators

NI 3 is three inseminators

TAI both is immediate or am-pm insemination depending on the cow

TAI am-pm is insemination based on the am-pm guideline

The reference levels used in this model were:

- BP = 0
- Area group 1 (Balgowan and Creighton)
- NI = 1 (one inseminator)
- TAI = 1 (immediate insemination)

Using this model, predictions for a female calf were made (Table 2.3). The levels for each term in the model were set (HDS at 1, 2 and 3; BP at 0 and 1; Area group at 1, 2 and 3; NI at 1, 2 and 3; and TAI at 1, 2 and 3). The scenario giving the highest probability of a female calf was that of Area group=2, BP=1, NI=3, TAI=2 and HDS=3. This combination of variables gave a probability of 0.8424 ± 0.0222 of a female calf. Therefore, a farm with the

Table 2.3 Probabilities of a female calf under different combinations of Area group, bull presence (BP), number of inseminators (NI), timing of AI (TAI) and heat detection score (HDS)

Area group	BP	NI	TAI	HDS						
				1*	s.e.	2*	s.e.	3*	s.e.	
1	0	1	1	0.1306	0.0209	0.1726	0.0239	0.2245	0.0269	
			2	0.3281	0.0184	0.4040	0.0184	0.4848	0.0199	
			3	0.1559	0.0213	0.2041	0.0241	0.2626	0.0269	
		2	1	0.0913	0.0211	0.1224	0.0256	0.1622	0.0305	
			2	0.2462	0.0237	0.3120	0.0246	0.3863	0.0256	
			3	0.1100	0.0237	0.1464	0.0286	0.1924	0.0339	
		3	1	0.1741	0.0253	0.2264	0.0279	0.2889	0.0301	
			2	0.4066	0.0187	0.4874	0.0178	0.5690	0.0187	
			3	0.2059	0.0268	0.2646	0.0293	0.3331	0.0317	
	1	1	1	0.1716	0.0249	0.2234	0.0274	0.2854	0.0297	
			2	0.4024	0.0149	0.4832	0.0137	0.5648	0.0152	
			3	0.2031	0.0265	0.2613	0.0292	0.3293	0.0317	
		2	1	0.1217	0.0276	0.1614	0.0328	0.2108	0.0383	
			2	0.3106	0.0291	0.3847	0.0295	0.4647	0.0298	
			3	0.1456	0.0316	0.1913	0.0374	0.2472	0.0433	
		3	1	0.2252	0.0315	0.2875	0.0338	0.3591	0.0354	
			2	0.4858	0.0220	0.5674	0.0208	0.6455	0.0207	
			3	0.2633	0.0349	0.3317	0.0375	0.4079	0.0393	
	2	0	1	1	0.3061	0.0243	0.3798	0.0248	0.4595	0.0257
				2	0.5891	0.0391	0.6656	0.0378	0.7343	0.0355
				3	0.3517	0.0150	0.4296	0.0153	0.5111	0.0178
			2	1	0.2279	0.0235	0.2906	0.0242	0.3625	0.0246
				2	0.4896	0.0243	0.5711	0.0247	0.6489	0.0254
				3	0.2663	0.0203	0.3350	0.0203	0.4116	0.0209
3			1	0.3823	0.0193	0.4622	0.0173	0.5440	0.0171	
			2	0.6680	0.0326	0.7363	0.0304	0.7950	0.0278	
			3	0.4322	0.0089	0.5138	0.0082	0.5946	0.0121	
1		1	1	0.3783	0.0185	0.4579	0.0166	0.5397	0.0165	
			2	0.6642	0.0310	0.7330	0.0293	0.7921	0.0271	
			3	0.4280	0.0091	0.5095	0.0087	0.5905	0.0126	
		2	1	0.2893	0.0261	0.3610	0.0256	0.4396	0.0250	
			2	0.5695	0.0219	0.6474	0.0215	0.7182	0.0217	
			3	0.3336	0.0254	0.4100	0.0252	0.4910	0.0253	
		3	1	0.4605	0.0171	0.5424	0.0141	0.6219	0.0137	
			2	0.7351	0.0274	0.7939	0.0248	0.8424	0.0222	
			3	0.5122	0.0143	0.5931	0.0139	0.6692	0.0158	
3		0	1	1	0.2523	0.0235	0.3191	0.0251	0.3941	0.0272
				2	0.5231	0.0406	0.6036	0.0408	0.6789	0.0398
				3	0.2933	0.0183	0.3656	0.0197	0.4444	0.0225
			2	1	0.1842	0.0208	0.2386	0.0222	0.3032	0.0235
				2	0.4232	0.0228	0.5046	0.0241	0.5857	0.0260
				3	0.2173	0.0194	0.2782	0.0205	0.3485	0.0221
	3		1	0.3214	0.0170	0.3966	0.0155	0.4771	0.0157	
			2	0.6061	0.0329	0.6812	0.0320	0.7478	0.0306	
			3	0.3680	0.0097	0.4470	0.0092	0.5288	0.0129	
	1	1	1	0.3176	0.0185	0.3925	0.0177	0.4728	0.0182	
			2	0.6020	0.0326	0.6774	0.0319	0.7446	0.0306	
			3	0.3640	0.0137	0.4428	0.0140	0.5245	0.0169	
		2	1	0.2374	0.0230	0.3018	0.0233	0.3750	0.0235	
			2	0.5030	0.0192	0.5842	0.0200	0.6610	0.0216	
			3	0.2769	0.0237	0.3471	0.0245	0.4246	0.0256	
		3	1	0.3951	0.0127	0.4755	0.0087	0.5572	0.0088	
			2	0.6797	0.0274	0.7466	0.0261	0.8035	0.0243	
			3	0.4454	0.0121	0.5272	0.0120	0.6075	0.0150	

* Numbers in bold are those probabilities greater than 0.5 and thus likely to result in a female calf

above conditions would have a high probability of producing predominantly female calves.

2.3.1 Heat detection score (HDS)

The positive relationship between CGR and HDS suggests that as heat detection improves on a farm (assumed to be the case when more heat detection aids are used), the CGR becomes closer to one (i.e. a female). Six scenarios, taken randomly from the prediction table, were plotted (Figure 2.2). Regardless of the specific probability scores, the general trend is for the probability of a female calf to increase as heat detection improves.

If heat detection is poor, a cow is more likely to be served later, after oestrous behaviour is first detected and thus, closer to ovulation. Wehner *et al.* (1997) found that earlier insemination (20 hours pre-ovulation) favours female calves ($P < 0.025$) whereas late insemination (8 hours pre-ovulation) favours male calves. Verme and Ozoga (1981) also reported significant deviations ($P < 0.001$) from a 50% gender ratio, according to the time of mating in white-tailed deer (72.9% females from matings occurring within 36 hours of oestrus, as opposed to 69.7% males resulting from delayed breeding). The results of the study by Wehner *et al.* (1997) were attributed to the probable physiologic shifts in ion concentration in the cervical mucus at the time of oestrus. They postulate, from research conducted on the importance of ion concentration in sperm capacitation, that spermatozoa carrying a Y chromosome capacitate earlier post-insemination than spermatozoa carrying an X chromosome, due to a greater sensitivity to uterine ion concentration. Therefore, gender selection could result from the uterine-oviduct environment at the time of insemination. If early insemination encourages capacitation of Y-bearing spermatozoa, and then their death (or decapacitation), only X-bearing spermatozoa would survive at ovulation. This could serve as a mechanism to maintain equilibrium within herd gender ratios in natural-mating populations. If there is a shortage of males they will take longer to serve all the females, and therefore some females will be served later in their oestrous cycles, resulting in the delayed breeding and the birth of more males, and *vice versa*.

If the assumption that insemination is performed earlier due to improved heat detection with more aids, it can also be said that if visual heat detection takes place at increased intervals, without necessarily using aids, the same trend for an increase in female calves could be expected.

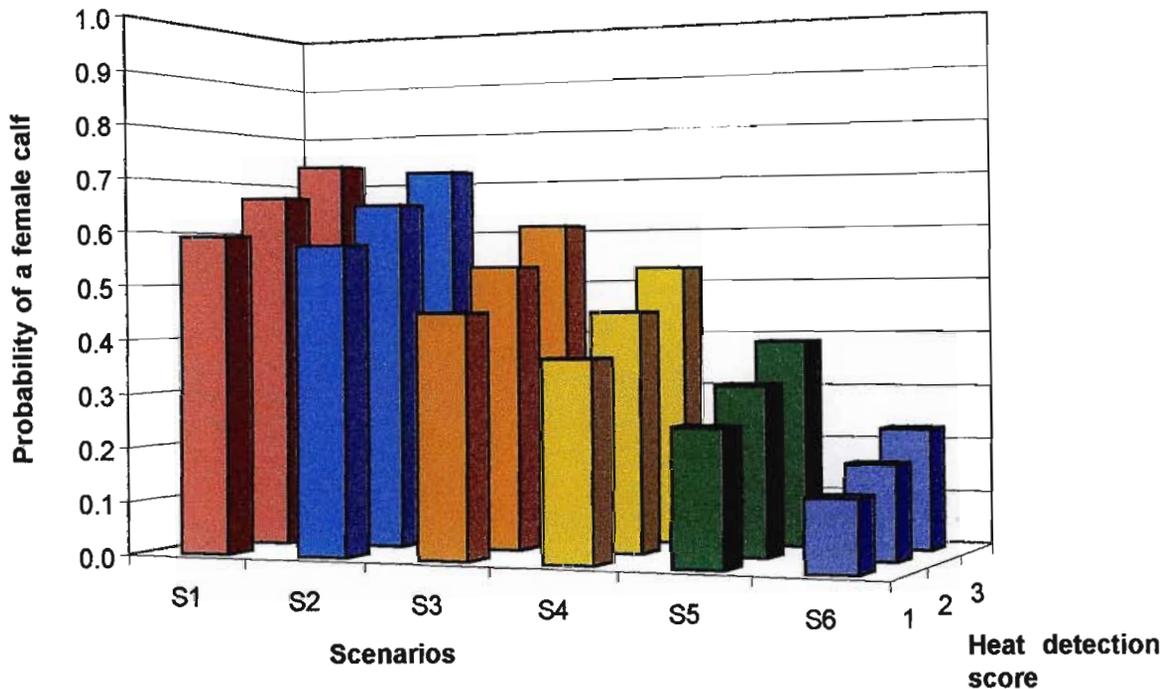


Figure 2.2 The probability of a female calf over different heat detection scores (visual only (1), visual and one aid (2) and visual and two aids (3)), under six different scenarios:
 S1 one inseminator, Area group 2, both immediate/am-pm AI and no bulls on farm;
 S2 two inseminators, Area group 2, either immediate/am-pm AI and bulls on farm;
 S3 three inseminators, Area group 3, am-pm AI and bulls on farm;
 S4 one inseminator, Area group 3, am-pm AI and bulls on farm;
 S5 two inseminators, Area group 1, either immediate/am-pm AI and no bulls on farm;
 S6 one inseminator, Area group 1, immediate AI and no bulls on farm.

2.3.2 Bull presence

The positive relationship of CGR with BP suggests that more female offspring result if there is a bull (or bulls) on the farm. The presence of a bull on the farm consistently increased the probability of female offspring (Figure 2.3).

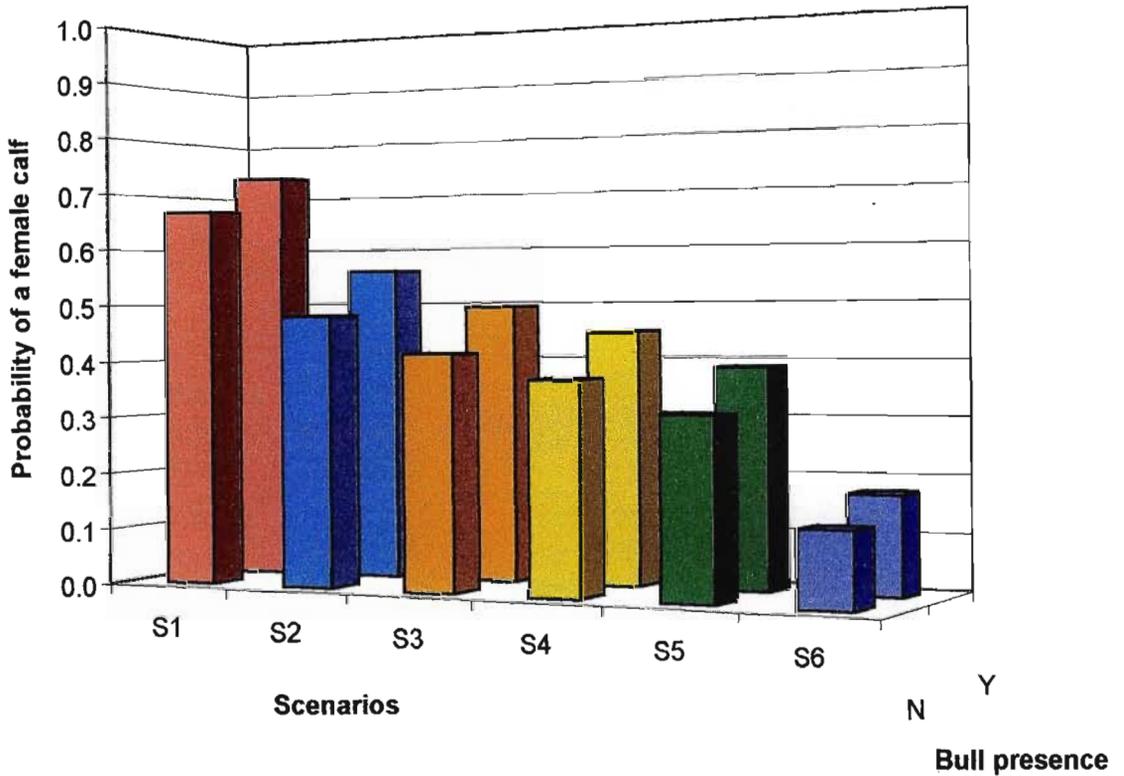


Figure 2.3 The probability of a female calf with a bull(s) present on farm or not, under six different scenarios:
 S1 ■ Area group 2, one inseminator, either immediate/am-pm AI and heat detection score 2;
 S2 ■ Area group 3, three inseminators, immediate AI and heat detection score 3;
 S3 ■ Area group 2, two inseminators, am-pm AI and heat detection score 3;
 S4 ■ Area group 3, three inseminators, am-pm AI and heat detection score 1;
 S5 ■ Area group 1, two inseminators, either immediate/am-pm insemination and HDS 2;
 S6 ■ Area group 1, one inseminator, immediate AI and heat detection score 1.

On dairy farms that use natural mating instead of artificial insemination, the CGR appears to be close to 50 percent and even ratios in favour of females (Corbishley, 2002; van Huysteen, 2002 - personal communication).

The inclusion of bull presence in this analysis was not intended as a comparison of natural mating versus artificial insemination, but to investigate whether the presence of the male of the species has a more subtle effect on CGR. Hence, BP was positive if a farmer simply keeps one or more bulls on the farm. This would be the case for example, if a bull is used to “clean up”, i.e. cover those cows not conceiving with artificial insemination. A beef bull might also be kept to cover lower producing cows to produce calves for the meat market, as these calves would have better growth rates (McElhenney *et al.*, 1986).

Therefore, the conclusion here is not that the bull serving a cow (instead of AI) leads to more females (this has not been found to be true (Wehner *et al.*, 1997)), but rather that the actual bull presence, or lack thereof, affects the CGR. Perhaps the low probability of females when bulls are absent is an attempt to establish gender equilibrium or a return to equilibrium, whereby a shortage of males somehow triggers a mechanism ensuring that the next generation overcompensates for males. Evidence for this theory has been shown in humans, where the gender ratio after the first and second World Wars was biased towards male offspring. Lummaa *et al.* (1998) suggested that humans are capable of adjusting the gender ratio of their offspring in response to the operational gender ratio, so as to maximise the reproductive success of their progeny (i.e. producing more sons when males are rare). Although the newborn male surplus would only reach a reproductive age once the present females would have passed their optimum reproductive age, it is thought that the present situation provides the only cue about future mating opportunities (Lummaa *et al.*, 1998). This means that if the operational gender ratio is currently biased, it may be the best indicator of the gender ratio, and hence mating opportunity, in the next generation.

2.3.3 Area

Area groups 2 and 3 consistently showed increased probabilities of female offspring, while the probabilities of female offspring in Area group 1 are consistently lower than the other areas (Figure 2.4).

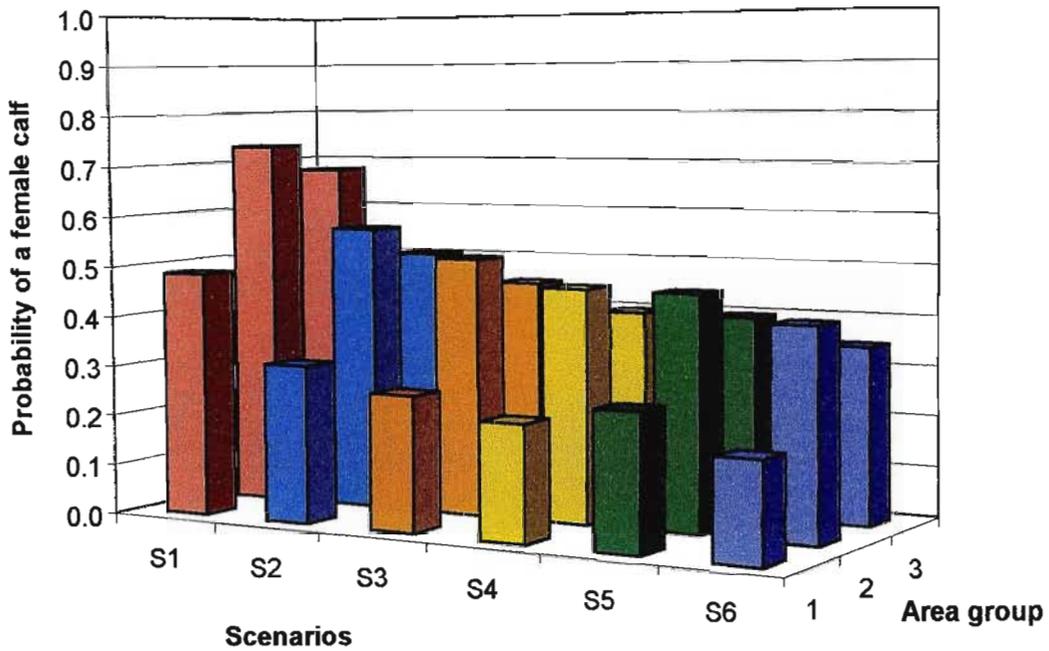


Figure 2.4 The probability of a female calf in different area groups (Balgowan and Creighton (1), Boston, Kamberg, Umzimkulu and Underberg (2) and Donnybrook, Highflats and Ixopo (3)), under six different scenarios:
 S1 no bulls on farm, one inseminator, either immediate/am-pm AI and heat detection score 3;
 S2 no bulls on farm, three inseminators, am-pm AI and heat detection score 2;
 S3 no bulls on farm, one inseminator, either immediate/am-pm AI and heat detection score 3;
 S4 bulls on farm, three inseminators, immediate AI and heat detection score 1;
 S5 no bulls on farm, one inseminator, immediate AI and heat detection score 3;
 S6 bulls on farm, two inseminators, am-pm AI and heat detection score 2.

There appears to be no logical geographical separation of groups and the minimum and maximum temperatures on the day of conception for dams in each of these groups had no effect on CGR. The explanation for the

difference is thought to be due to other environmental factors not taken into consideration in this study.

2.3.4 *Timing of artificial insemination*

Due to the response in CGR to changes in heat detection score, it might be expected that if artificial insemination was performed as soon as the cow was seen in standing heat, more female offspring should result than from those cows inseminated 12 hours later. However, the results show a greater chance of obtaining female calves when there is am-pm timing (TAI=1) and either immediate or am-pm timing of AI (TAI=2). Farmers falling into the latter category do not have a rigid rule of inseminating immediately or waiting 12 hours, but usually inseminate heifers immediately and will judge timing of AI on cows by assessing the stage of heat. If cows are assessed to have been on standing heat for a period of time before being observed, then AI will be immediate. However, if it appears that the occurrence of standing heat has just begun, insemination will be delayed until 12 hours later. This practice of timing insemination after assessing the cow for the stage of standing heat resulted in the greatest probability of a female calf (Figure 2.5).

Immediate timing of insemination should have resulted in the greatest probabilities of a female calf, due to earlier insemination preovulation (Wehner *et al.*, 1997). However, these predictions consistently resulted in the lowest predictions of a female calf. A possible explanation for this is that the predictions were estimated from the available data set and accurate predictions for a female calf when timing of insemination is immediately after standing heat could not be made.

For all other terms in the model, the opposite trends to that of female probabilities were observed when predicting the probability of a male calf (Table 2.4), which was expected, except for the timing of AI (Figure 2.6). It can be seen that the predictions for a male calf increase as timing of AI is delayed. Therefore, immediate timing of insemination resulted in the lowest

predictions for both female and male calves, and could therefore be a characteristic of this data set.

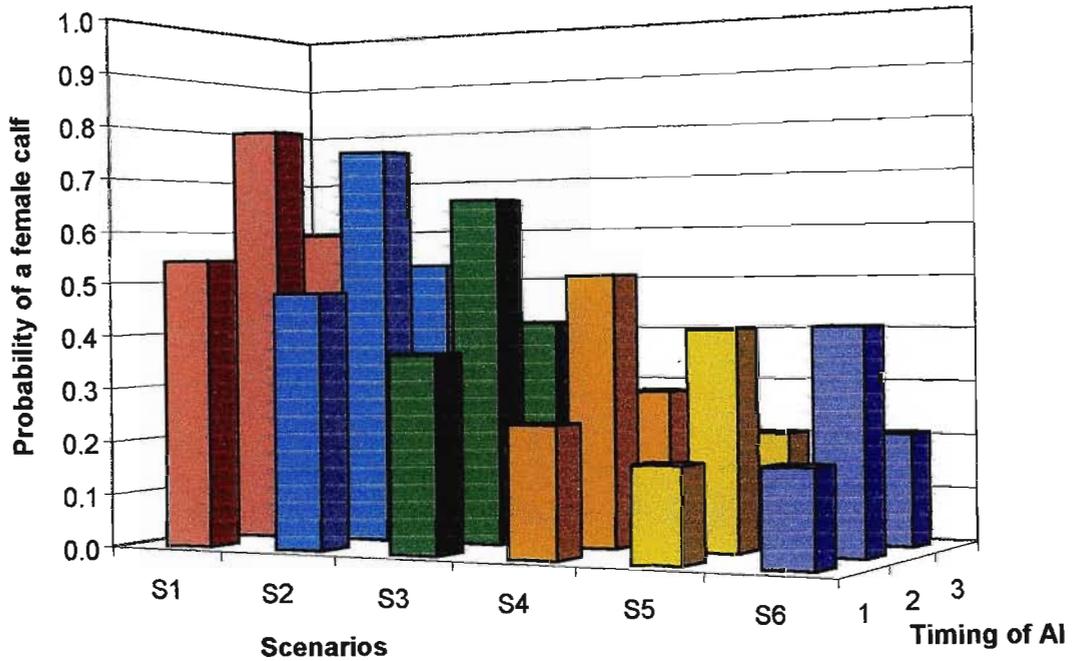


Figure 2.5 The probability of a female calf with the timing of AI, under six different scenarios:
 S1 ■ Area group 2, bulls on farm, one inseminator and heat detection score 3;
 S2 ■ Area group 3, bulls on farm, three inseminators and heat detection score 2;
 S3 ■ Area group 2, no bulls on farm, two inseminators and heat detection score 3;
 S4 ■ Area group 3, no bulls on farm, two inseminators and heat detection score 2;
 S5 ■ Area group 1, bulls on farm, one inseminator and heat detection score 1;
 S6 ■ Area group 1, no bulls on farm, three inseminators and heat detection score 1.

Another explanation for the low probabilities of a female calf from immediate insemination could also be due to heat detection methods, and how the farmer assessed how long the cow had been in heat. If heat detection is not done at regular intervals, a cow may be inseminated immediately after standing heat is observed, but, insemination could actually be closer to ovulation than anticipated if the cow has been showing undetected signs of heat for a period of time.

Table 2.4 Probabilities of a male calf under different combinations of Area group, bull presence (BP), number of inseminators (NI), timing of AI (TAI) and heat detection score (HDS).

Area group	BP	NI	TAI	HDS					
				1*	s.e.	2*	s.e.	3*	s.e.
1	0	1	1	0.6719	0.0184	0.5960	0.0184	0.5152	0.0199
			2	0.8441	0.0213	0.7959	0.0241	0.7374	0.0269
			3	0.8694	0.0209	0.8274	0.0239	0.7755	0.0269
		2	1	0.7538	0.0237	0.6880	0.0246	0.6137	0.0256
			2	0.8900	0.0237	0.8536	0.0286	0.8076	0.0339
			3	0.9087	0.0211	0.8776	0.0256	0.8378	0.0305
		3	1	0.5935	0.0187	0.5126	0.0178	0.4310	0.0187
			2	0.7941	0.0267	0.7354	0.0293	0.6669	0.0316
			3	0.8259	0.0253	0.7736	0.0279	0.7111	0.0300
	1	1	1	0.5976	0.0149	0.5169	0.0137	0.4352	0.0152
			2	0.7969	0.0265	0.7387	0.0292	0.6707	0.0317
			3	0.8284	0.0249	0.7766	0.0274	0.7147	0.0296
		2	1	0.6894	0.0290	0.6153	0.0295	0.5353	0.0298
			2	0.8544	0.0316	0.8087	0.0374	0.7527	0.0433
			3	0.8783	0.0275	0.8386	0.0328	0.7892	0.0382
		3	1	0.5142	0.0220	0.4326	0.0208	0.3545	0.0208
			2	0.7366	0.0349	0.6683	0.0374	0.5921	0.0393
			3	0.7748	0.0315	0.7125	0.0338	0.6409	0.0354
2	0	1	1	0.4109	0.0391	0.3344	0.0377	0.2657	0.0355
			2	0.6483	0.0150	0.5704	0.0153	0.4889	0.0178
			3	0.6939	0.0243	0.6202	0.0248	0.5405	0.0257
		2	1	0.5104	0.0243	0.4289	0.0246	0.3511	0.0254
			2	0.7337	0.0203	0.6650	0.0203	0.5884	0.0209
			3	0.7721	0.0235	0.7094	0.0242	0.6375	0.0246
		3	1	0.3320	0.0325	0.2637	0.0303	0.2051	0.0278
			2	0.5678	0.0089	0.4862	0.0082	0.4054	0.0121
			3	0.6177	0.0193	0.5378	0.0173	0.4560	0.0171
	1	1	1	0.3359	0.0310	0.2670	0.0293	0.2079	0.0271
			2	0.5720	0.0091	0.4905	0.0087	0.4095	0.0126
			3	0.6217	0.0185	0.5421	0.0166	0.4603	0.0165
		2	1	0.4305	0.0219	0.3526	0.0215	0.2818	0.0217
			2	0.6664	0.0254	0.5900	0.0251	0.5090	0.0253
			3	0.7107	0.0261	0.6390	0.0256	0.5604	0.0249
		3	1	0.2649	0.0274	0.2061	0.0248	0.1576	0.0222
			2	0.4878	0.0143	0.4069	0.0140	0.3308	0.0158
			3	0.5395	0.0170	0.4577	0.0141	0.3781	0.0137
3	0	1	1	0.4769	0.0406	0.3964	0.0408	0.3212	0.0398
			2	0.7067	0.0183	0.6345	0.0196	0.5556	0.0225
			3	0.7477	0.0235	0.6810	0.0251	0.6059	0.0271
		2	1	0.5768	0.0228	0.4954	0.0241	0.4143	0.0260
			2	0.7827	0.0194	0.7218	0.0205	0.6515	0.0221
			3	0.8158	0.0208	0.7614	0.0222	0.6968	0.0235
		3	1	0.3939	0.0328	0.3189	0.0320	0.2522	0.0305
			2	0.6320	0.0097	0.5556	0.0225	0.4712	0.0129
			3	0.6786	0.0170	0.6034	0.0155	0.5229	0.0157
	1	1	1	0.3980	0.0325	0.3226	0.0319	0.2554	0.0306
			2	0.6360	0.0137	0.5572	0.0140	0.4755	0.0169
			3	0.6824	0.0185	0.6075	0.0177	0.5272	0.0182
		2	1	0.4970	0.0192	0.4159	0.0200	0.3390	0.0216
			2	0.7231	0.0237	0.6529	0.0244	0.5754	0.0256
			3	0.7626	0.0230	0.6982	0.0233	0.6250	0.0234
		3	1	0.3203	0.0274	0.2534	0.0260	0.1965	0.0243
			2	0.5546	0.0140	0.4728	0.0120	0.3925	0.0150
			3	0.6049	0.0127	0.5245	0.0087	0.4428	0.0088

* Numbers in bold are those probabilities greater than 0.5 and thus likely to result in a male calf

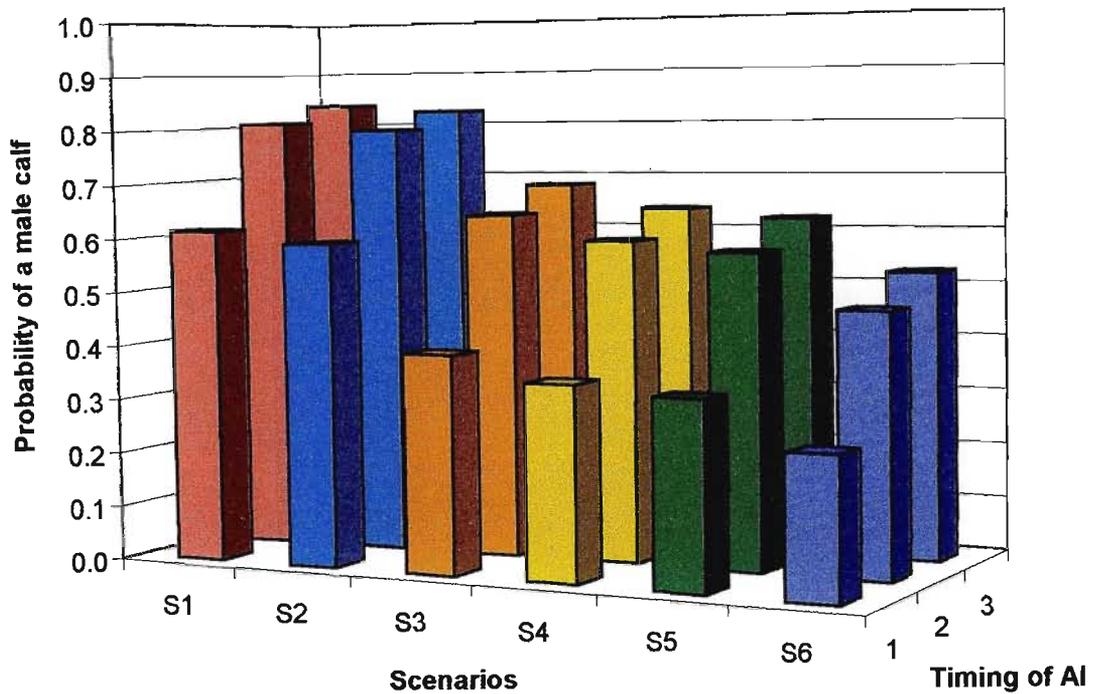


Figure 2.6 The probability of a male calf with timing of AI, under six different scenarios:

- S1 ■ Area group 1, no bulls on farm, heat detection score 3 and two inseminators;
- S2 ■ Area group 1, no bulls on farm, heat detection score 1, and three inseminators;
- S3 ■ Area group 3, bulls on farm, heat detection score 2 and one inseminator;
- S4 ■ Area group 2, no bulls on farm, heat detection score 3 and two inseminators;
- S5 ■ Area group 2, bulls on farm, heat detection score 1 and one inseminator;
- S6 ■ Area group 3, bulls on farm, heat detection score 1 and one inseminator.

2.3.5 Number of inseminators

Most farmers offer an incentive scheme, such that the inseminator receives a bonus according to the number of cows successfully inseminated. Therefore, it would benefit inseminators to improve heat detection in order to achieve this, and it could be expected that insemination would be performed earlier when there are more inseminators on a farm, increasing the probability of a female. However, an increase in the probability of females as the number of

inseminators increases was not found in this study. The highest probabilities of female calves resulted from farms having either one or three inseminators, with lower probabilities of female calves from farms having two inseminators (Figure 2.7). In this study, those farms with two inseminators usually included the farmer himself and a labourer. In this case, the farmer might observe a cow in oestrus and leave insemination to be done by the labourer at the next milking, which would result in insemination closer to ovulation, and therefore a lower probability of a female calf.

When the model was run with the male as the response variate (male=1; female=0), the predictions generated from the significant terms in the model were generally greater (closer to 1) than the predictions obtained for female calves (Table 2.4), i.e. the model was better able to predict male calves than female calves. The greatest probability of a male was obtained in Area group 1, no bulls on farm, two inseminators and am-pm timing of AI, with a probability of 0.9087 (± 0.02). This also resulted in the lowest probability of a female calf (0.1100 ± 0.02). Therefore it is easier to use this model to increase the chances of a bull calf, which is not beneficial in a dairy situation, but could possibly be applied to a beef situation. However, most of the terms used in the model do not apply to beef enterprises. Natural service is usually used in a beef enterprise, hence the presence of a bull(s), which also negates the need for heat detection. There is also no need for inseminators, and timing of insemination is not under managerial control. Therefore these variables could not practically be manipulated to increase the probability of obtaining male calves in a beef situation, unless AI was practised.

The benefits of obtaining heifer calves in a dairy enterprise are clear, but, dairy farmers in the KwaZulu-Natal Midlands have expressed concerns that a possible surplus of heifers might result from being able to skew the gender ratio in favour of female calves. However, even if the replacement rate were to remain constant, the greater number of heifers available to choose replacements from would increase the selection intensity and allow for faster genetic progress within a herd (Rendel and Robertson, 1950). The degree of skewing towards females cannot be expected to be greater than 84 percent

(greatest prediction of a female), while most predictions above 50 percent were below 70 percent, which is unlikely to result in a surplus of heifers.

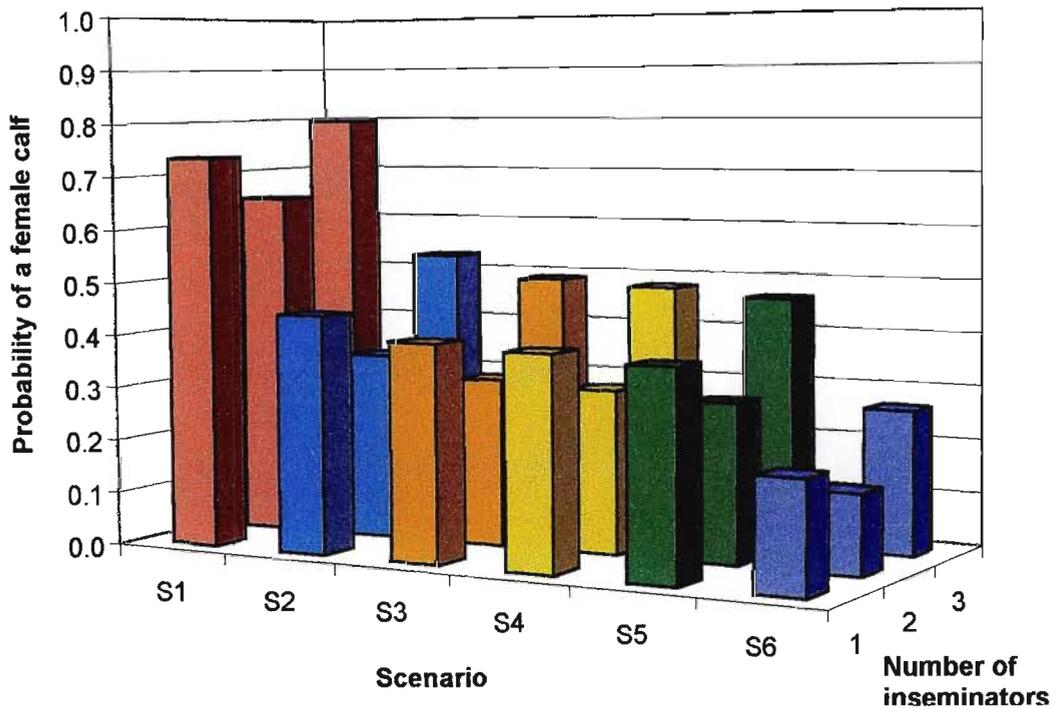


Figure 2.7 The probability of a female calf with the number of inseminators on a farm under six different scenarios:
 S1 ■ Area group 2, no bulls on farm, both immediate/am-pm AI and heat detection score 3;
 S2 ■ Area group 3, no bulls on farm, am-pm AI and heat detection score 3;
 S3 ■ Area group 1, bulls on farm, both immediate/am-pm AI and heat detection score 1;
 S4 ■ Area group 3, bulls on farm, immediate AI and heat detection score 2;
 S5 ■ Area group 2, no bulls on farm, immediate AI and heat detection score 2;
 S6 ■ Area group 1, bulls on farm, am-pm AI and heat detection score 1.

2.4 Conclusions

The results of this study suggest that there are factors that can be manipulated in order to improve the chances of obtaining more female calves in a dairy, which would be favourable economically. An improvement in heat detection, and an insemination guideline of assessment of the stage of standing heat, with immediate insemination if a cow appears to have been showing signs of heat for some time, would allow earlier insemination in relation to ovulation and improve the probability of female offspring. However, the low probabilities of females resulting from immediate timing of AI does not support this hypothesis, but the fact that probabilities of a male calf were also low when timing of insemination was immediate suggest that this is a finding of the available data.

Keeping a bull on a farm where AI is routine requires increased levels of management and involves a high initial expense, but the chance of obtaining female calves will be increased due to bull presence. An added advantage of keeping a bull is that the bull can naturally inseminate cows having difficulty conceiving with artificial insemination. A beef bull could also be used to produce calves from low yielding cows, which are useful only for their milk production and not their genetic contribution to the herd.

It appears that some areas of the KwaZulu-Natal Midlands are intrinsically disposed to the production of more female calves than others, and should possibly be favoured when deciding on the location of a dairy farm. These areas include Boston, Donnybrook, Highflats, Ixopo, Kamberg, Umzimkulu and Underberg. Balgowan and Creighton appear to be areas to avoid. The results of this study also suggest that the use of one or three inseminators results in more female calves than the use of two inseminators.

CHAPTER 3

THE RELATIONSHIP OF RECTAL AND VAGINAL TEMPERATURE TO OESTRUS AND OVULATION IN DAIRY COWS: IMPLICATIONS FOR CALF GENDER RATIOS

3.1 Introduction

One of the methods available for manipulating the gender ratio of dairy calves is that of inseminating at a specific time before ovulation. Significantly more female offspring are reported to result from inseminations twenty hours pre-ovulation rather than eight hours pre-ovulation (Wehner *et al.*, 1997). However, this method requires knowledge of the exact time of ovulation. Oestrous behaviour in the dairy cow, and in particular standing heat, is the signal that ovulation will follow and experiments have been conducted to estimate the time of ovulation after standing heat. The average oestrus to ovulation interval has been reported to be 23.3 (\pm 1.4) hours (Mikeska and Williams, 1988) and 26.6 (\pm 0.44) hours in beef cows (Pinheiro *et al.*, 1998) and 24 (\pm 3.2) hours (Rajamahendran *et al.*, 1989), 26.4 (\pm 4.2) hours (Lopez *et al.*, 2002) and 27.6 (\pm 5.4) hours in dairy cows (Walker *et al.*, 1996). However, there are some reports of a large variation in this figure, e.g. Walker *et al.* (1996) reported that 22 percent of cows had not ovulated within 40 hours of the onset of oestrus. Thus, the expression of oestrous behaviour cannot be used to predict the time to ovulation with the accuracy required for insemination to bias the gender ratio.

Wehner *et al.* (1997) utilised the Ovatec² unit, which measures a combination of electrical resistance and capacitance parameters in the calibration and sensing of ion fluxes in the vaginal mucus, in order to predict the time of ovulation. Trans-rectal ultrasonography of the ovaries can also be performed to give an indication of ovulation, which is associated with the disappearance

² Profitable Breeding Corporation, 9877 Simmonds Rd, Corfu, NY 14036, USA.

of the dominant follicle (Pierson and Ginther, 1984). However, these methods are both costly and time-consuming, and cannot be easily performed on commercial dairy farms.

The purpose of this experiment was to investigate whether the time of ovulation can be predicted using an alternative, cheaper method, in order for gender ratios to be manipulated through appropriate timing of insemination. Vaginal temperature has been found to increase at the time of oestrus (Wrenn *et al.*, 1958, Lewis and Newman, 1984). An increase in physical activity is observed at oestrus (Lewis and Newman, 1984; Schofield *et al.*, 1991; Walton and King, 1986), which could cause the increase in temperature at this time. The time interval from body temperature rise to ovulation seems to show consistency, with ovulation occurring between 22 (\pm 3.5) hours (pluriparous cows) and 27 (biparous cows) (\pm 3.5) hours after the vaginal temperature peak (Rajamahendran *et al.*, 1989). Mosher *et al.* (1990) observed ovulation 21.14 (\pm 6.07) hours after vaginal temperature peak. However, measuring vaginal temperature leads to the risk of introducing infections into the reproductive tract. Body (rectal) temperature is a common, easy and less risky measurement than vaginal temperature. Therefore, this study aimed to investigate the relationship between body (rectal) temperature (RecT) and vaginal temperature (VagT) to oestrus and ovulation.

3.2 Materials and methods

Six cyclic, non-lactating, multiparous Holstein cows weighing an average of 564.4 (\pm 29.9) kg were used in this experiment. Oestrus was synchronised initially using a double injection of 2 ml of a synthetic prostaglandin (Estrumate[®])³ ten days apart. Vaginal and rectal temperatures were measured at approximately two-hourly intervals beginning at the second injection, until after ovulation had occurred. Visual observations of oestrous behaviour were also made at these times. Microlife[®] digital pen-type electronic thermometers (MT 1681)⁴ with an accuracy of 0.1°C were used to measure VagT and RecT,

³ Schering-Plough (PTY) LTD, 54 Electron Avenue, Islando, 1600, RSA

⁴ Advanced Health Care, 20 Boland Street, P.O. Box 3852, Honeydew, 2040, RSA

one used solely for vaginal temperatures and the other for rectal temperatures. The thermometer used for vaginal temperature measurement was rinsed in a 2 percent chlorhexidine (disinfectant) solution and dried between readings on successive cows to lessen the chances of spreading infection. Environmental temperature was recorded using an Oregon Scientific digital thermo-hygrometer (model ETHG913R)⁵. A Pie Medical ultrasound machine (Scanner 200)⁶ with a 5 MHz rectal probe was used to observe the follicle status of the ovaries at four-hourly intervals, starting 10 hours after the observation of standing heat. The follicle diameters were measured, and ovulation was determined by the disappearance of the largest (dominant) follicle (Rajamahendran *et al.*, 1989). This procedure was performed after the temperature measurements were recorded, and dishwashing liquid was used as a lubricant when entering the rectum. These measurements were repeated over four consecutive oestrous cycles in each cow. It was hoped that the cows would remain synchronised after the initial treatment with Estrumate[®], but the second oestrous cycles were too spread out to maintain the intensive recordings, so oestrus was again synchronised with a single injection of Estrumate[®] for the third and fourth cycles.

The cows had an adverse reaction to the dishwashing liquid that was used as a lubricant during trans-rectal ultrasound examination during data collection in the first measured cycle, which made it very uncomfortable for the cow each time an examination of the ovaries was made. Due to the cows' reaction it was not possible to determine the time of ovulation with accuracy. The reaction to the dishwashing liquid could have affected temperature; therefore, the data obtained from cycle one was not included in the analysis. As oestrus was not synchronised for the second cycle, there were two cows whose oestrus and subsequent ovulation were missed, and they were thought to have cycles shorter than 21 days. One of these cows did not show signs of heat, nor ovulate in the fourth cycle either, and therefore only contributed data for one cycle, hence, all data from this cow were excluded from the analysis. Another cow in the fourth cycle did not show signs of oestrous behaviour nor

⁵ Oregon Scientific, Inc. Portland, Oregon, USA

⁶ Philipsweg 1, 6227, AJ Maastricht, The Netherlands

ovulate, and another ovulated, but no standing heat was observed, although there was a bullstring present, which was recorded as oestrus. Either this cow showed no signs of standing heat, or standing heat was less than two hours long, and therefore not observed at the two-hourly interval observation times. The duration of standing heat in this cow was recorded as 0 hours. Therefore, data from five cows were included in the analysis, and each cow was treated as an experimental unit (replicate).

Statistical procedures were performed with Genstat® 6th edition (2002). Analyses of variance (ANOVA, no blocking) were performed for both RecT and VagT in relation to the time of oestrus as well as in relation to the time of ovulation, with time, cow and cycle as factors. The ANOVA's were performed to identify if there were significant differences in RecT and VagT both between and within cows, different cycles and at different time increments before either oestrus or ovulation.

When associating RecT and VagT with oestrus, the 2-hourly time increments starting 24 hours before oestrus until the time of oestrus were used. Because there was no significant effect of either cow or cycle, the data were pooled and the means used in the subsequent analysis. There were also no cycle x time interactions, but the main effect of time was significant. Therefore, linear regression analyses were used to determine the relationship between time and both RecT and VagT leading up to oestrus.

Similar analyses were conducted for both RecT and VagT in relation to the time of ovulation. Again, there were no significant effects of cow or cycle from the ANOVA, so data were pooled, and the means used in the subsequent analyses. Linear regression was performed using the 2-hourly time increments from 24 hours before ovulation until the time of ovulation. A limit of 24 hours before ovulation was set, as it was assumed that cows would have ovulated 24 hours after oestrus. A multiple regression was also performed with both RecT and VagT considered in the model.

Temperatures before and after these time periods were not considered, as the aim was to determine if the time of ovulation could be predicted from temperature and not to investigate the use of temperature as a heat detection aid.

3.3 Results and discussion

3.3.1 *Standing heat*

The mean duration of standing heat in this experiment, as determined by visual observation of a cow standing to be mounted, was 8 hours 19 minutes (± 58 minutes), with a minimum of <2 hours, and a maximum of 16 hours. The mean time from oestrus to ovulation was 19 hours 41 minutes (± 1 hour 44 minutes) with a minimum of 10 hours and a maximum of 32 hours.

3.3.2 *Environmental temperature*

There were low correlations between dry bulb ambient temperature and rectal and vaginal temperatures ($R=0.36$ and 0.32 respectively) and therefore, dry bulb temperature was assumed to have no effect on rectal and vaginal temperature in this study at the range of 7°C to 32°C . For the influence of effective ambient temperature, the climatic factors of wet-bulb temperature, air movement, radiation and temperature of the surroundings needs to be taken into consideration (Esmay, 1978), but unfortunately these were not measured in this study.

3.3.3 *Relationship of rectal and vaginal temperature with oestrus*

When using RecT to explain the time to oestrus, the best fitting model obtained accounted for 94.6 percent of the variance and is given in the following equation:

$$\text{Hours before oestrus} = -1172.7 (\pm 80.0) + 30.14 (\pm 2.08) \text{ RecT} \\ (\text{s.e. } \pm 1.81, R^2=94.6 \text{ percent, } n=13)$$

As this response was obtained with temperature data 24 hours before the onset of oestrus, it limits the application of the equation to this period of the cycle. This may therefore imply that a knowledge of the approximate time of the onset of oestrus, e.g. from heat expectancy records, is required. Thus, misleading predictions of the time of oestrus could be made using this equation without knowing the approximate time of the onset of oestrus. For example, a RecT of 38.4°C in the middle of the oestrous cycle would result in an erroneous prediction of 15 hours to oestrus using the above equation. However, its use in conjunction with heat expectancy records could be useful. Once a RecT of 38.91°C is recorded, it is likely that the cow will be showing behavioural signs of oestrus. The accuracy of prediction will improve with increased frequency of temperature measurements. If the farmer has accurate heat expectancy records, increased visual observation at this time is probably adequate in observing the cow in heat, with a resultant increase in conception rates. However, measuring RecT is a way to improve this.

The linear regression of VagT before the time of oestrus resulted in the following equation:

$$\text{Hours before oestrus} = -1459 (\pm 121) + 37.77 (\pm 3.17) \text{ VagT}$$

(s.e. ± 2.18 , $R^2=92.1$ percent, $n=13$)

The percentage variance accounted for by this fit is 92.1 percent. Thus, the vaginal temperature may also be used in predicting the time of oestrus, but only during the 24-hour period before the onset of oestrus.

Rectal and vaginal temperatures in this study were highly correlated ($R=0.83$), although a higher correlation has been reported ($R=0.95$) (Rajamahendran *et al.*, 1989). From a practical standpoint, it is easier and more hygienic for a farmer to measure rectal temperatures.

3.3.4 Relationship of rectal and vaginal temperature with ovulation

There is a linear decrease in RecT from the beginning of oestrus to ovulation, described by the following model:

$$\text{Hours before ovulation} = 740.5 (\pm 77.5) - 19.44 (\pm 2.0) \text{ RecT} \\ (\text{s.e.} \pm 2.63, R^2=88.6 \text{ percent}, n=13)$$

A similar response was observed for VagT:

$$\text{Hours before ovulation} = 925 (\pm 139) - 24.38 (\pm 3.61) \text{ VagT} \\ (\text{s.e.} \pm 3.59, R^2=78.8 \text{ percent}, n=13)$$

The above models can only be used to predict the time of ovulation when measurements are taken from the period of standing heat. Rectal temperature is a more practical measurement, than VagT, and was also found to be more accurate than combined measurements of RecT and VagT that were determined by the multiple regression ($R^2=87.6$ percent).

This model would be useful in predicting the time of ovulation to increase the chances of obtaining a female calf. If insemination needs to take place 20 hours before ovulation for a female calf (Wehner *et al.*, 1997), it should be done at a stage after the standing heat is first observed when RecT is 39.12°C (Figure 3.1), and VagT is 38.72°C. In most cases, this occurs while the cow is still in standing heat. By following the am-pm guideline for insemination, the chances of obtaining a male calf are greatly increased, considering the average time from the first observation of standing heat to ovulation was on average less than 20 hours. This was after observations had been taken every two hours. Therefore the chances of delaying insemination increase as the periods between heat observations increase, as the first time a cow is seen in standing heat could be hours after the actual first moment of standing heat. If the prediction of ovulation is less than 20 hours, the value of obtaining a female calf should be considered. It may be worth increasing the inter-calving interval by a cycle length (and insemination for a female attempted at

the next cycle), or it may be worth inseminating immediately, even though the chances of a male calf increase.

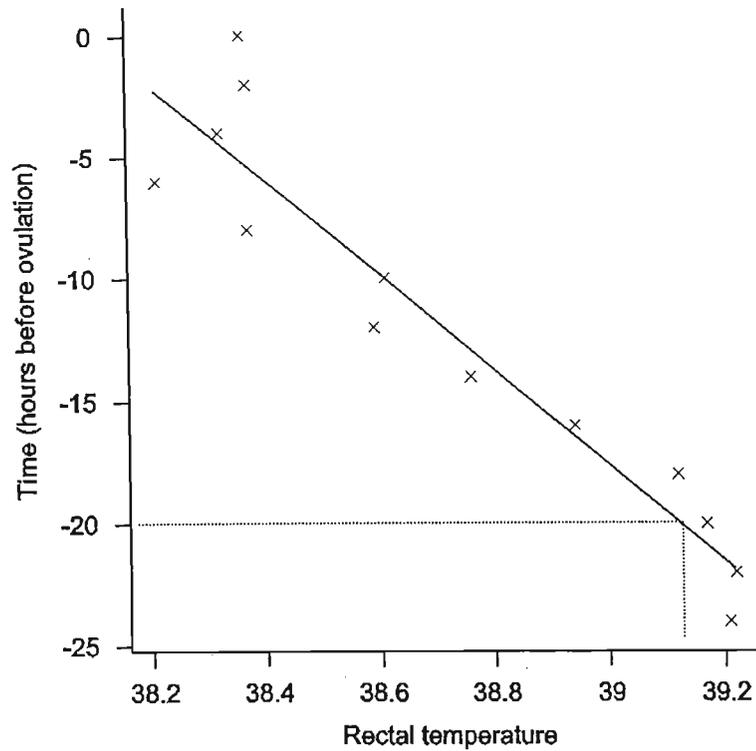


Figure 3.1 Relationship between rectal temperature and the time of ovulation from (useful approximately 24 hours before ovulation)
 x = actual
 — = predicted
 ---- = example of the time before ovulation when rectal temperature is 39.1°C

The models presented describe the pattern of rectal and vaginal temperature around the time of oestrus and ovulation as a linear increase to oestrus and a linear decline to ovulation. There are conflicting reports in the literature about this relationship. Wrenn *et al.* (1958) reported a decrease in vaginal

temperature the day before oestrus, an increase on the day of oestrus, and a decline a day later to presumed ovulation. However, temperature was only measured daily. In another report in which temperature was measured daily (Lewis and Newman, 1984), the lowest temperature was obtained on the day before oestrus, with an increase of 0.1°C on the day of oestrus. Temperature then increased for the next six days, and no drop before ovulation was reported.

Clapper *et al.* (1990) measured the vaginal temperature hourly, and considered prolonged (≥ 3 hours) elevated rises in temperature ($\geq 0.3^\circ\text{C}$) compared to the average at that time of the previous day as a "temperature spike". However, this was not related to behavioural oestrus or ovulation, but rather to the LH peak. Mosher *et al.* (1990), in a companion paper, measured the temperature at 15-minute intervals, and considered the same criteria for a temperature spike. Again this was not related to behavioural oestrus, however ovulation was determined with laparoscopy or rectal palpation and was found to occur 21.14 ± 2.3 hours after the temperature spike, however comparisons can not be made if the time of behavioural oestrus is not known.

Measurement of temperature spikes in this study was not considered, as the linear response was considered to be a more practical consideration for the farmer. If the interval in which temperature is measured is longer than 3 hours, it is possible that the temperature spike would be missed. However, with the observed linear relationship, the time to oestrus and ovulation can be predicted when the measurement interval is longer than three hours.

3.4 Conclusions

The results of this study permit the recommendation that rectal temperature rather than vaginal temperature can be measured at increased intervals close to the time of expected oestrus to determine the time of ovulation. This would allow for insemination to increase the chance of obtaining female calves.

GENERAL DISCUSSION

A natural gender ratio of 50 percent has long been accepted as the norm in most species, the explanation of which is attributed to the production of both X- and Y-bearing sperm by the male, which are thought to have an equal opportunity to fertilise the female oocytes, which all contain only X chromosomes. However, there appear to be circumstances that alter the equal opportunity of fertilisation by X- and Y-bearing sperm, and theories have been proposed to explain the mechanisms which might alter the 50 percent gender ratio. Probably the most cited hypothesis is that of Trivers and Willard (1973). They postulate that females in good condition are more likely to produce male offspring, while those females in poorer condition are more likely to produce female offspring. Another hypothesis, which has been the basis of a large proportion of this thesis, is that of Wehner *et al.* (1997), which suggests that earlier inseminations before ovulation result in more female offspring, and inseminations closer to ovulation result in more males. Wehner *et al.* (1997) base this assumption on the changing conditions of the female reproductive tract near the time of ovulation having a differential effect on the capacitation abilities of X- and Y-bearing sperm.

One of the aims of this study was to investigate which production and management variables on dairy farms might influence the calf gender ratio, in order to explore alternatives to technologies such as semen and embryo sexing in gender predetermination. The results showed promising alternatives to these expensive technologies, and the manipulation of heat detection, bull presence, number of inseminators, timing of insemination and geographical location of farms in the KwaZulu-Natal Midlands could allow farmers to improve the probability of obtaining female calves. The manipulation of heat detection, timing of insemination and the number of inseminators can be explained on the basis of the theory proposed by Wehner *et al.* (1997). As heat detection improved, the probability of a female calf being born improved. This suggests that cows are observed in heat sooner when more heat detection aids are used, and therefore inseminated earlier when conditions in

the female tract are more favourable to X-bearing sperm. The same argument applies to the timing of insemination, where more female calves resulted when the cow had been assessed to determine how long the signs of behavioural oestrus had been displayed. If a cow was thought to have shown behavioural oestrus for some time before being observed, immediate insemination was performed, rather than waiting until the next milking. Therefore, earlier insemination would again result in the favouring of X-bearing sperm. However, farms where there was a policy of immediate insemination had a low probability of a female calf. In these instances, it was thought that predictions could not be made accurately from the available data, because when looking at male predictions, the immediate insemination procedure also resulted in the lowest predictions for a male calf compared to the other ways of timing of insemination.

The proposed theory of Wehner *et al.* (1997) may also explain that having two inseminators on the farm (in comparison to one or three) resulted in a greater probability of males. On farms with two inseminators, it is possible that a cow seen in heat by the farmer in the morning may have been left to be inseminated by a labourer at the next milking, and this could have resulted in delayed insemination. However, the explanation is more likely to be attributed to another unknown factor such as the size of the farm. The significant effect of geographical area could not be explained.

An interesting result of this study was the greater probability of female calves on those farms that kept a bull. There appears to be an unknown mechanism that aims to keep the gender ratio of a population at equilibrium so that when there is a perceived shortage of one sex, that sex will be overproduced in the next generation. Further research into the mode and action of this perception may reveal how this can be manipulated in order to moderate its effect on farms that solely utilise AI for fertilisation.

By manipulating heat detection score and timing of insemination to increase the probability of a female calf, it is assumed that insemination will occur earlier, before ovulation. Therefore, the probability of female calves may also

be increased by predicting the time of ovulation, and inseminating accordingly. Since ovulation can only be associated with, and not accurately predicted from behavioural oestrus on most farms, rectal and vaginal temperatures were assessed as more accurate predictors of ovulation. This was to allow a method of timing insemination according to accurate predictions of ovulation, as more female calves were produced from insemination at specifically 20 hours preovulation (Wehner *et al.*, 1997).

Rectal and vaginal temperatures were highly correlated and both were found to be accurate predictors of ovulation if measured from the time of standing heat (which in turn can be accurately predicted from 24 hours previously). The measurement of vaginal temperature would necessitate good hygiene procedures to minimise the risk of infection to the reproductive tract. Therefore, rectal temperature would be a more practical measurement to perform. These measurements represent practical and inexpensive tools to dairy farmers wishing to improve the gender ratio bias in favour of female calves.

Being able to manipulate calf gender ratios will be a valuable tool to most dairy farmers. The increased value of heifer calves would improve financial status, and would also allow for faster expansion of a herd or increased selection intensity of replacement heifers. This would lead to more rapid genetic progress in a herd (Rendel and Robertson, 1950).

REFERENCES

- Aboul-Ela, M. B., J. H. Topps, and D. C. Macdonald. 1983. Relationships between intravaginal electrical resistance, cervicovaginal mucus characteristics and blood progesterone and LH. *Anim. Reprod. Sci.* 5:259-273.
- Alliston, C. W., T. B. Patterson, and L. C. Ulberg. 1958. Crystallisation patterns of cervical mucus as related to estrus in beef cattle. *J. Anim. Sci.* 17:322-325.
- Appa Rao, K. B. C., S. M. Totey, and C. H. Pawshe. 1994. Biopsy of Indian Zebu and crossbred cattle preimplantation embryos and sexing by polymerase chain reaction. *Anim. Reprod. Sci.* 34:209-216.
- Ballinger, H. J. 1970. The effect of inseminations carried out early or late in oestrus on the sex ratio of calves born. *Vet. Rec.* 86:631.
- Baxter, S. J., and W. R. Ward. 1997. Incidence of fetal loss in dairy cattle after pregnancy diagnosis using an ultrasound scanner. *Vet. Rec.* 140:287-288.
- Beal, W. E., R. C. Perry, and L. R. Corah. 1992. The use of ultrasound in monitoring reproductive physiology of beef cattle. *J. Anim. Sci.* 70:924-929.
- Bearden, H. J., and J. W. Fuquay. 2000. *Applied Animal Reproduction*, 5th ed. Prentice-Hall, New Jersey.
- Bellows, R. A., R. E. Short, G. P. Kitto, R. B. Staigmiller, and M. D. MacNeil. 1990. Influence of sire, sex of fetus and type of pregnancy on conceptus development. *Theriogenology* 34:941-954.

- Bernard, C., J. P. Valet, R. Beland, and R. D. Lambert. 1983. Prediction of bovine ovulation by a rapid radioimmunoassay for plasma LH. *J. Repr. Fertil.* 68:425-436.
- Bongso, T. A., and P. K. Basrur. 1975. Prenatal diagnosis of sex in cattle by amniocentesis. *Vet. Rec.* 96:124-127.
- Booman, P., L. Kruijt, R. Veerhuis, A. M. Hengst, M. Tieman, and F. E. Ruch. 1989. Sexing bovine embryos with monoclonal antibodies against the H-Y antigen. *Livest. Prod. Sci.* 23:1-16.
- Bull, J. J. 1983. Evolution of sex determining mechanisms. The Benjamin Cummings Publishing Company, Menlo Park, Calif.
- Cameron, E. Z., W. L. Linklater, K. J. Stafford, and C. J. Veltman. 1999. Birth sex ratios relate to mare condition at conception in kaimanawa horses. *Behav. Ecol.* 10:472-475.
- Canfield, R. W., and W. R. Butler. 1989. Accuracy of predicting the LH surge and optimal insemination time in Holstein heifers using a vaginal resistance probe. *Theriogenology* 31:835-842.
- Chandler, J. E., H. C. Steinholt-Chenevert, R. W. Adkinson, and E. B. Moser. 1998. Sex ratio variation between ejaculates within sire evaluated by polymerase chain reaction, calving and farrowing records. *J. Dairy Sci.* 81:1855-1867.
- Chapman, A. B., and J. L. Lush. 1932. Twinning, sex ratios, and genetic variability in birth weights in sheep. *J. Hered.* 23:473-478.
- Chew, B. P., L. C. Maier, J. K. Hillers, and A. S. Hodgson. 1981. Relationships between calf birth weight and dam's subsequent 200- and 305-day yields of milk, fat, and total solids in Holsteins. *J. Dairy Sci.* 64:2401-2408.

- Christenson, R. K., S. E. Echtenkamp, and D. B. Laster. 1975. Oestrus, LH, ovulation and fertility in beef heifers. *J. Reprod. Fertil.* 43:543-546.
- Clapper, J. A., J. S. Ottobre, A. C. Ottobre, and D. L. Zartman. 1990. Estrual rise in body temperature in the bovine I. Temporal relationships with serum patterns of reproductive hormones. *Anim. Reprod. Sci.* 23:89-98.
- Clutton-Brock, T. H., and G. R. Iason. 1986. Sex ratio variation on mammals. *Q. Rev. Bio.* 61:339-374.
- Collier, R. J., S. G. Doelger, H. H. Head, W. W. Thatcher, and C. J. Wilcox. 1982. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight and postpartum milk yield of Holstein cows. *J. Anim. Sci.* 54:309-319.
- Côté, S. D., and M. Festa-Bianchet. 2001. Offspring sex ratio in relation to maternal age and social rank in mountain goats (*Oreamnos americanus*). *Behav. Ecol. Sociobiol.* 49:260-265.
- Cran, D. G., L. A. Johnson, N. G. A. Miller, D. Cochrane, and C. Polge. 1993. Production of bovine calves following separation of X- and Y-chromosome bearing sperm and in vitro fertilisation. *Vet. Rec.* 132:40-41.
- Curran, S., and O. J. Ginther. 1991. Ultrasonic determination of foetal gender in horses and cattle under farm conditions. *Theriogenology* 36:809-814.
- Curran, S., J. P. Kastelic, and O. J. Ginther. 1989. Determining sex of the bovine fetus by ultrasonic assessment of the relative location of the genital tubercle. *Anim. Reprod. Sci.* 19:217-227.

- Darwin, C. 1894. *The Descent of Man and Selection in Relation to Sex*. 2nd ed rev. John Murray, London.
- Davis, K. C., D. D. Kress, D. E. Doornbos, and D. C. Anderson. 1998. Heterosis and breed additive effects for Hereford, Tarentaise, and the reciprocal crosses for calf traits. *J. Anim. Sci.* 76:701-705.
- Dominko, T. and N. L. First. 1997. Relationship between the maturational state of oocytes at the time of insemination and sex ratio of subsequent early bovine embryos. *Theriogenology* 47:1041-1050.
- Echternkamp, S. E. 1993. Relationship between placental development and calf birth weight in beef cattle. *Anim. Reprod. Sci.* 32:1-13.
- Edmondson, A. J., R. A. Fissore, R. L. Pashen, and R. H. Bondurant. 1986. The use of ultrasonography for the study of the bovine reproductive tract. I. Normal and pathological ovarian structures. *Anim. Reprod. Sci.* 12:157-165.
- Erb, R. E., B. P. Chew, P. V. Malven, M. F. D'Amico, C. N. Zamet, and V. F. Colenbrander. 1980. Variables associated with peripartum traits in dairy cows. VII. Hormones, calf traits and subsequent milk yield. *J. Anim. Sci.* 51:143-152.
- Esmay, M. L. 1978. *Principles of animal environment*. AVI Publishing Company, Westport, Connecticut.
- Feldmann, F., E. Aizinbud, H. Schindler, and H. Broda. 1978. The electrical conductivity inside the bovine vaginal wall. *Anim. Prod.* 26:61-65.
- Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

- Fissore, R. A., A. J. Edmondston, R. L. Pashen, and R. H. Bondurant. 1986. The use of ultrasonography for the study of the bovine reproductive tract II. Non-pregnant, pregnant and pathological conditions of the uterus. *Anim. Reprod. Sci.* 12:167-177.
- Fugger, E. F. 1999. Clinical experience with flow cytometric separation of human X-and Y-chromosome bearing sperm. *Theriogenology* 52:1435-1440.
- Garcia, A. and M. Salaheddine. 1997. Bovine ultrasound-guided transvaginal amniocentesis. *Theriogenology* 47:1003-1008.
- Gardner, K. E. 1950. The sex ratio in calves resulting from artificial insemination. *J. Dairy Sci.* 33:391 (abs).
- Gray, E. and K. Hurt. 1979. Distribution of sexes in cattle. *J. Hered.* 70:273-274.
- Genstat, 2002. Release 6.1, 6th ed. VSN International, Oxford.
- Gonzalez, L. V., J. W. Fuquay, and H. J. Bearden. 1985. Insemination management for a one-injection prostaglandin $F_{2\alpha}$ synchronization regimen. I. One daily insemination period versus use of the A.M./P.M. rule. *Theriogenology* 24:495-500.
- Gordon, M. J. 1958. The control of sex. *New Scientist* 4:87-94.
- Guerrero, R. 1974. Association of the type and time of insemination within the menstrual cycle with the human sex ratio at birth. *N. Engl. J. Med.* 291:1056-1059.
- Gutiérrez-Adán, A., E. Behboodi, G. B., Anderson, J. F. Medrano, and J. D. Murray. 1996. Relationships between stage of development and sex of

- bovine IVM-IVF embryos cultured in vitro versus in the sheep oviduct. *Theriogenology* 46:515-525.
- Hair, J. F. Jr., R. E., Anderson, R. L. Tatham and W. C. Black. 1998. *Multivariate Data Analysis*, 5th ed. Prentice Hall, New Jersey.
- Herr, C. M. and K. C. Reed. 1991. Micromanipulation of bovine embryos for sex determination. *Theriogenology* 35:45-54.
- Hoefs, M., and U. Nowlan. 1994. Distorted sex ratios in young ungulates: the role of nutrition. *J. Mammalogy* 75:631-636.
- Horrell, R. I., R. Kilgour, K. L. MacMillan, and K. Bremmer. 1984. Evaluation of fluctuations in milk yield and parlour behaviour as indicators of oestrus in dairy cows. *Vet. Rec.* 114:36-39.
- Jafar, S. I. and A. P. F. Flint. 1996. Sex selection in mammals: a review. *Theriogenology* 46:191-200.
- Johnson, L. A., D. G. Cran, and C. Polge. 1994. Recent advances in sex preselection of cattle: flow cytometric sorting of X- and Y- chromosome bearing sperm based on DNA to produce progeny. *Theriogenology* 41:51-56.
- Kamimura, S., N. Nishiyama, S. Ookutsu, K. Goto, and K. Hamana. 1997. Determination of bovine fetal sex by PCR using fetal fluid aspirated by transvaginal ultrasound-guided amniocentesis. *Theriogenology* 47:1563-1569.
- Karam, H. A. 1957. Multiple birth and sex ratio in Rahmani sheep. *J. Anim. Sci.* 16:990-997.

- Kars, A. A., G. J. Erasmus, and J. van der Westhuizen. 1994. Factors influencing growth traits in the Nguni cattle stud at Bartlow Combine. *S. Afr. J. Anim. Sci.* 24:18-21.
- Kent, J. P. 1995. Birth sex ratios in sheep over nine lambing seasons: years 7-9 and the effects of ageing. *Behav. Ecol. Sociobiol.* 36:101-104.
- Kertz, A. F., L. F. Reutzel, B. A. Barton, and R. L. Ely. 1997. Body weight, body condition score, and wither height of prepartum Holstein cows and birth weight and sex of calves by parity: A database and summary. *J. Dairy Sci.* 80:525-529.
- Kiddy, C. A. 1977. Variation in physical activity as an indication of oestrus in dairy cows. *J. Dairy Sci.* 60:235-243.
- Kohlmann, S. G. 1999. Adaptive fetal sex allocation in elk: evidence and implications. *J. Wildl. Manage.* 63:1109-1117.
- Kyle, B. L., A. D. Kennedy, and J. A. Small. 1998. Measurement of vaginal temperature by radiotelemetry for the prediction of oestrus in beef cows. *Theriogenology* 49:1437-1449.
- Leibo, S. P. and W. F. Rall. 1987. Determination of prenatal sex in cattle by amniocentesis. *Theriogenology* 27:246 (abs).
- Leibo, S. P. and W. F. Rall. 1990. Prenatal diagnosis of sex in bovine fetuses by amniocentesis. *Theriogenology* 33:531-552.
- Lewis, G. S., E. Aizinbud, and A. R. Lehrer. 1989. Changes in electrical resistance of vulvar tissue in Holstein cows during ovarian cycles and after treatment with prostaglandin $F_{2\alpha}$. *Anim. Reprod. Sci.* 18:183-197.

- Lewis, G. S. and S. K. Newman. 1984. Changes throughout the estrous cycles of variables that might indicate estrus in dairy cows. *J. Dairy Sci.* 67:146-152.
- Longergan, P., H. Khatir, F. Piumi, D. Rieger, P. Humblot, and M. P. Boland. 1999. Effect of time interval from insemination to first cleavage on the developmental characteristics, sex ratio and pregnancy rate after transfer of bovine embryos. *J. Reprod. Fertil.* 117:159-167.
- Lopez, H., T. D. Bunch, and M. P. Shipka. 2002. Estrogen concentrations in milk at estrus and ovulation in dairy cows. *Anim. Reprod. Sci.* 72:37-46.
- Lummaa, V., J. Merila, and A. Kause. 1998. Adaptive sex ratio variation in pre industrial human (*Homo sapiens*) populations? *Proc. R. Soc. Lond. B Biol. Sci.* 265: 563-568.
- McElhenney, W. H., C. R. Long, J. F. Bakerand, and T. C. Cartwright. 1986. Production characteristics of first generation cows of a five-breed diallel: reproduction of mature cows and preweaning performance of calves by two third-breed sires. *J. Anim. Sci.* 63:59-67.
- Meikle, D. B., L. C. Drickamer, S. H. Vessey, T. L. Rosenthal, and K. S. Fitzgerald. 1993. Maternal dominance rank and secondary sex ratio in domestic swine. *Anim. Behav.* 46: 79-85.
- Mendle, M., A. J. Zanella, D. M. Broom, and C. T. Whittemore. 1995. Maternal social status and birth sex ratio in domestic pigs: an analysis of mechanisms. *Anim. Behav.* 50: 1361-1370.
- Merilan, C. P. 1983. Evaluation of bovine cervical mucus during estrus cycle by nuclear magnetic resonance *J. Dairy Sci.* 66:1184-1188.
- Mikeska, J. C., and G. L. Williams. 1988. Timing of preovulatory endocrine events, estrus and ovulation in Brahman x Hereford females

synchronized with norgestomet and estradiol valerate. *J. Anim. Sci.* 66:939-946.

Mittwoch, U. 1973. *Genetics of Sex Differentiation*. Academic Press, New York.

Mittwoch, U. 1996. Sex-determining mechanisms in animals. *TREE* 11:63-67.

Monard, A-M., P. Duncan, H. Fritz, and C. Feh. 1997. Variations in the birth sex ratio and neonatal mortality in a natural herd of horses. *Behav. Ecol. Sociobiol.* 41:243-249.

Morrell, J. M., K. D. Keeler, D. E. Noakes, N. M. Mackenzie, and D. W. Dresser. 1988. Sexing of sperm by flow cytometry. *Vet. Rec.* 122:322-324.

Mortimer, R. G., M. D. Salman, M. Gutierrez, and J. D. Olson. 1990. Effects of androgenizing dairy heifers with ear implants containing testosterone and estrogen on detection of oestrus. *J. Dairy. Sci.* 73:1773-1778.

Mosher, M. D., J. S. Ottobre, G. K. Haibel, and D. L. Zartman. 1990. Estrual rise in body temperature in the bovine II. The temporal relationship with ovulation. *Anim. Reprod. Sci.* 23:99-107.

Müller, E. and G. Wittkowski. 1986. Visualization of male and female characteristics of bovine fetuses by real-time ultrasonics. *Theriogenology* 25:571-574.

Napier, K. M. and P. D. Mullaney. 1974. Sex ratio in sheep. *J. Reprod. Fertil.* 39:391-392.

Nkuuhe, J. R. and J. G. Manns. 1985. Relationship between time of prostaglandin injection and ovulation in beef cattle. *Can. J. Anim. Sci.* 65:405-409.

- Paul, A. and J. Kuester. 1987. Sex ratio adjustment in a seasonally breeding primate species: Evidence from the Barbary Macaque population at Affenberg Salem. *Ethology* 74:117-132.
- Peters, A. R. 1989. Changes in the electrical resistance of the vaginal mucosa in prostaglandin-treated cows. *Vet. Rec.* 124:505-507.
- Pierson, R. A., and O. J. Ginther. 1984. Ultrasonography of the bovine ovary. *Theriogenology* 21:495-504.
- Pinheiro, O. L., C. M. Barrows, R. A. Figueiredo, E. R. do Valle, R. O. Encarnacao, and C. R. Padovani. 1998. Estrous behaviour and the estrus-to-ovulation interval in Nelore cattle (*Bos indicus*) with natural estrus or estrus induced with prostaglandin F2 alpha or norgestomet and estradiol valerate. *Theriogenology* 49:667-691.
- Powell, R. L., H. D. Norman, and F. N. Dickenson. 1975. Sire differences in sex ratio of progeny. *J. Dairy Sci.* 58:1723-1726.
- Prior, R. L., and D. B. Laster. 1979. Development of the bovine fetus. *J. Anim. Sci.* 48:1546-1533.
- Pursley, R. J., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss and gender ratio after synchronization of ovulation in lactating dairy cows. *J. Dairy Sci.* 81:2139-2144.
- Rajamahendran, R., J. Robinson, S. Desbottes, and J. S. Walton. 1989. Temporal relationships among estrus, body temperature, milk yield, progesterone and luteinizing hormone levels, and ovulation in dairy cows. *Theriogenology* 31:1173-1182.

- Rajamahendran, R., and C. Taylor. 1991. Follicular dynamics and temporal relationships among body temperature, oestrus, the surge of luteinizing hormone and ovulation in Holstein heifers treated with norgestomet. *J. Reprod. Fertil.* 92:461-467.
- Reeves, J. J., N. W. Rantanen, and M. Hauser. 1984. Transrectal real-time ultrasound scanning of the cow reproductive tract. *Theriogenology* 21:485-494.
- Rendel, J. M. and A. Robertson. 1950. Estimation of genetic gain in milk yield by selection in a closed herd of dairy cattle. *J. Genetics* 50:1-9.
- Rorie, R. W. 1999. Effect of timing of artificial insemination on sex ratio. *Theriogenology* 52:1273-1280.
- Rorie, R. W., T. D. Lester, B. R. Lindsey, and R. W. McNew. 1999. Effect of timing of artificial insemination on gender ratio in beef cattle. *Theriogenology* 52:1035-1041.
- Rust, J. M., D. J. Opperman, P. L. Brand, F. C. d W. Scheepers, and J. E. Venter. 1996. Assessment of the effectiveness of a heat detecting probe after oestrus synchronization in Friesian heifers. In: Proc. 2nd All Africa Conference on Animal Agriculture, 1-4 April, Pretoria.
- Sawyer, G. J., I. D. Russell-Brown, and J. K. Silcock. 1986. A comparison of three methods of oestrus detection in commercial dairy herds verified by serum progesterone analysis. *Anim. Reprod. Sci.* 10:1-10.
- Schilling, E., and J. Zust. 1968. Diagnosis of oestrus and ovulation in cows by pH measurements *intra vaginam* and by apparent viscosity of vaginal mucous. *J. Reprod. Fertil.* 15:307-311.

- Schofield, S. A., C. J. C. Phillips, and A. R. Owens. 1991. Variation in the milk production, activity rate and electrical impedance of cervical mucus over the oestrous period of dairy cows. *Anim. Reprod. Sci.* 24:231-248.
- Scipioni, R. L., R. H. Foote, S. V. Lamb, C. E. Hall, D. H. Lein, and S. J. Shin. 1982. Electric probe measurements of cervico-vaginal mucus for detection of ovulation in dairy cows. Sanitation, clinical observations and microflora. *Cornell Vet.* 72:269-278.
- Seidel, G. E. and L. A. Johnson. 1999. Sexing mammalian sperm-overview. *Theriogenology* 52:1267-1272.
- Seidel, G. E., J. L. Schenk, L. A. Herickhoff, S. P. Doyle, Z. Brink, R. D. Green, and D. G. Cran. 1999. Inseminations of heifers with sexed semen. *Theriogenology* 52:1407-1420.
- Smith, J. W., S. L. Spahr, and H. B. Puckett. 1989. Electrical conductivity of reproductive tissue for detection of oestrus in dairy cows. *J. Dairy Sci.* 72:693-701.
- Sprecher, D. J. and J. B. Kaneene. 1992. Diagnostic techniques for transvaginal-transuterine aspiration of bovine fetal fluid during the early fetal period. *Theriogenology* 38:581-587.
- Stevenson, J. S., M. W. Smith, J. R. Jaeger, L. R. Corah, and D. G. LeFever. 1996. Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beef heifers. *J. Anim. Sci.* 74:729-735.
- Swanson, L. V. and H. D. Hafs. 1971. LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. *J. Anim. Sci.* 33:1038-1041.

- Thatcher, W. W., C. J., Wilcox, R. J. Collier, D. S. Eley, and H. H. Head 1980. Bovine conceptus-maternal interactions during the pre- and postpartum periods. *J. Dairy Sci.* 63:1530-1540.
- Trivers, R. L., and D. E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90-92
- Verme, L. J. and J. J. Ozoga. 1981. Sex ratio of white-tailed deer and the estrus cycle. *J. Wildl. Manage.* 45:710-714.
- Walker, W. L., R. L. Nebel, and M. L. McGillard. 1996. Time of ovulation relative to mounting activity in dairy cattle. *J. Dairy Sci.* 79:1555-1561.
- Walton, J. S., and G. J. King 1986. Indicators of estrus in Holstein cows housed in tie stalls. *J. Dairy Sci.* 69:2966-2973.
- Wehner, G. R., C. Wood, A. Tague, D. Barker, and H. Hubert. 1997. Efficiency of the OVATEC unit for oestrous detection and calf sex control in beef cows. *Anim. Reprod. Sci.* 46:27-34.
- Williams, T. J. 1986. A technique for sexing mouse embryos by a visual colorimetric assay of the X-linked enzyme, glucose-6-phosphate dehydrogenase. *Theriogenology* 25:733-739.
- Williamson, N. B., R. S. Morris, D. C. Blood, and C. M. Cannon. 1972. A study of oestrous behaviour and oestrus detection methods in a large commercial dairy herd. *Vet. Rec.* 91:50-58.
- Wrenn, T. R., J. Bitman, and J. F. Sykes. 1958. Body temperature variations in dairy cattle during the estrous cycle and pregnancy. *J. Dairy Sci.* 41:1071-1076.

Xu, K. P., B. R. Yadav, W. A. King, and K. J. Betteridge. 1992. Sex-related differences in developmental rates of bovine embryos produced and cultured in vitro. *Mol. Reprod. Dev.* 31:249-252.