

**THE ECONOMIC EFFECTS OF AN OESTRUS
SYNCHRONISATION PROTOCOL USING PROSTAGLANDIN
AND REPRODUCTIVE TRACT SCORING IN BEEF HEIFERS IN
SOUTH AFRICA**

D E HOLM

2006

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AND REPRODUCTIVE TRACT SCORING IN BEEF HEIFERS IN
SOUTH AFRICA**

by

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Submitted to the Department of Production Animal Studies,

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in partial fulfilment of the requirements for the degree

MSc (Veterinary Science)

Pretoria, September 2006

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SUMMARY

In this study 272 beef heifers were studied from just prior to their first breeding season (15 October 2003), through their second breeding season and until just after they had weaned their first calves in March 2005. The study consisted of two main parts: in the first part, heifers were randomly allocated to either a synchronised TEST group or an unsynchronised CONTROL group. The TEST group received artificial insemination (AI) for 6 days followed by prostaglandin $F_{2\alpha}$ (PGF) treatment on day 6 (PGF/6) and further AI for a total of 50 days, which was followed after a 6 day break by a 42 day bull breeding season. The CONTROL group were bred for the same period without PGF treatment. Synchronisation resulted in a reduction in days to first insemination ($P < 0.01$) and days to calving ($P = 0.04$). No significant difference could be demonstrated in pregnancy rate to the 50 day AI season (60.0% vs. 51.8%, TEST and CONTROL groups respectively, $P = 0.18$), final pregnancy rate (82.2% vs. 83.2%, $P = 0.87$) or pregnancy rate to the subsequent breeding season (96.0% vs. 95.0%, $P = 1.00$). A significant increase in mean weaning mass of the calves due to synchronisation could not be demonstrated (207.0 kg vs. 201.4 kg, TEST and CONTROL groups respectively, $P = 0.32$). However, data from this study were used to calculate the benefit:cost ratio, and a value of 2.8 was reached, representing the return on investment for the synchronisation protocol under these circumstances. It was concluded from this study that a PGF/6 protocol may lead to a change in the total mass of calves weaned by changing days to calving and thus weaning mass, birth mass of calves, weaning rate and/or the ratio of male:female calves born. It was further concluded that a practical way to predict the cost effectiveness of an oestrus synchronisation protocol is to determine the ratio between the total cost of the programme and the price of weaner calves per kg live mass. This ratio represents the minimum increase in mean weaning mass that has to be achieved for the programme to be cost effective if no increase in weaning rate is achieved.

In the second part of this study, reproductive tract scoring (RTS) was performed on the same group of heifers one day before the onset of their first breeding season. The effect of RTS on several reproduction and production outcomes was tested, and the association of RTS with the outcomes was compared to the associations of other input variables such as mass, age, body condition score (BCS) and Kleiber ratio using multiple or univariable linear or logistic regression. RTS was associated with pregnancy rate to the 50 day AI season ($P < 0.01$), days to calving ($r = 0.28$, $P < 0.01$), calf weaning mass ($r = 0.22$, $P < 0.01$) and pregnancy rate to the subsequent breeding season ($P < 0.01$). These associations were mostly independent of associations with mass, age and BCS before the onset of the first breeding season. RTS was a better predictor of fertility than was Kleiber ratio, and similar in its prediction of calf weaning mass. It was concluded from this study that RTS is a unique predictor of heifer fertility, compares well with (but is independent of) other traits used as a predictor of production outcomes and is likely to be a good predictor of life production of the cow.

1. ACKNOWLEDGEMENTS

My wife Katrien has supported me through the entire time of this study, and without her motivation and trust this would never have been possible.

My promoter Professor Peter Thompson guided me in a very professional way through this study, and by doing that taught me a lot about research and epidemiology techniques and logical reasoning, which I will be able to apply in my career in future.

My co-promoter Dr Pete Irons, with his contagious enthusiasm, stimulated my interest in this particular field of study, and guided me in my practical development during this time. He also made valuable contributions regarding interpretation of the results.

My friend and colleague, Dr Anton Stone, convinced me that post-graduate studies was the way to ensure long-lasting job satisfaction, for which I am very grateful.

Dr Danie Odendaal, formerly of Pfizer Animal Health (South Africa), initiated both aspects of this study, and played an important leadership role without which this study would never have started.

Pfizer Animal Health (South Africa) provided unconditional financial support for this study.

Johannesburg Water's Northern Farm, and all the management and staff, provided the animals, facilities and practical help for this study. Their excellent management and good record keeping system made my task a lot easier.

Drs Rupert and Tanya Baber of Triple 'B' Ranch Bonsmaras near Vaalwater in Limpopo Province provided animals to do a pilot study, and also helped to shape the practical approach to this study.

This trial was performed under protocol number 36-5-620, as approved by the Animal Use and Care Committee of the University of Pretoria.

2. LIST OF ABBREVIATIONS

AI	Artificial Insemination
ADG	Average daily gain
AP	Age at puberty
AUC	Area under the curve
BCS	Body condition score
CI	Confidence Interval
CL	Corpus luteum
CPI	Consumer Price Index
CPIX	Consumer Price Index excluding mortgages
FAO	Food and Agricultural Organisation of the United Nations
FV	Future value
GnRH	Gonadotropin Releasing Hormone
GRF	Growth Hormone Releasing Factor
IGF-1	Insulin-like growth factor 1
IRR	Internal Rate of Return
LH	Luteinising Hormone

Lut	Lutalyse
NPV	Net present value
OR	Odds Ratio
PD	Pregnancy diagnosis by rectal palpation
PGF	Prostaglandin F _{2α}
PGF/6	Synchronisation protocol where PGF is administered on the morning of day six of the breeding season to all female animals that had not been inseminated by that time.
PV	Present value
ROC	Receiver-operating characteristic (analysis)
RTS	Reproductive tract score
SA	South Africa (The Republic of South Africa)
SAPROR	South African Prime Overdraft Rate
USA	The United States of America
WM	Mean weaning mass
WR	Weaning rate
WP	Price for weaned calves per kg live mass

3. LITERATURE REVIEW

Puberty in heifers

Age at puberty (AP) in heifers is conveniently defined as the age when a heifer displays visual signs of oestrus for the first time (Pineda, 2003). Other authors prefer to use age at first ovulation for this definition, but because this is not easily noticed, and because it precedes first oestrus only by a few days, it does not have significant practical implication (Foster, 1994). The hormonal onset of puberty, and factors leading to this event, is still not fully understood, but some studies have clarified the basic principles.

In the normal oestrus cycle, oestrogen produced by the growing follicle has a stimulatory effect on the pulse frequency of gonadotropin releasing hormone (GnRH) secretion by the hypothalamus, which in turn leads to the secretion of gonadotropins by the pituitary gland and ovulation of the dominant follicle (Foster, 1994). It seems that estradiol-17 *beta* produced by the prepuberal ovaries has a negative feedback effect on the hypothalamus and/or pituitary, which prevents the surge of gonadotropin release from the pituitary gland (Day et al, 1984). This was confirmed more recently by Gasser (2006) who demonstrated that luteinising hormone (LH) pulse frequency was higher in ovariectomised heifers than in intact heifers or ovariectomised heifers that received an oestradiol implant. This negative feedback seems to occur until a suitable stage of somatic development has been reached, after which a decline in the concentration of inhibitory binding sites for estradiol occurs at the hypothalamus and/or pituitary, leading to the release of gonadotropins by the pituitary (Day et al, 1987). This hypothesis is further supported by the fact that a genetic association has been demonstrated between age at puberty and growth traits (Brinks, 1994), making it seem that a critical body mass has to be reached for puberty to be induced (Stevenson, 1997).

Leptin, a hormone produced by adipocytes, has been demonstrated to have a stimulatory effect on the hypothalamus-pituitary axis and secretion of gonadotropins, and although serum leptin levels are higher in pubertal than pre-pubertal heifers, researchers have not been able to induce puberty by administration of exogenous leptin (Barb and

Kraeling, 2004). Barb and Kraeling suggest that leptin, as a link between metabolic status and the neuroendocrine axis, is a permissive rather than a triggering signal for puberty, indicating that if a certain body condition has not been reached, puberty will be delayed. Armstrong et al (1992) demonstrated that insulin-like growth factor 1 (IGF-1) possibly plays a role in the onset of puberty. In their experiment age at puberty was delayed by active immunisation against growth hormone releasing factor (GRF), which also led to a decrease in IGF-1.

Age at puberty (AP) is a moderately heritable trait ($h^2 = 0.43$) with favourable association with weaning mass and yearling mass of the offspring, and also with lifetime production of the cow (Brinks, 1994). Other factors affecting the onset of puberty in heifers include nutrition, seasonal effects, climate, biostimulation (presence of bull) and breed (Pineda, 2003). Seasonal differences, although not so important in cattle (Pineda, 2003), will be caused by the fact that heifers were at different stages of their development at varying times of the season, and this variation will be relatively small in a group of heifers that were born during a short calving season. Genetic and seasonal differences must account for most of the variation in AP amongst uniform heifers that are managed together as a group. King (1983) reports that heifers born later in the calving season had younger ages at puberty than those born early, although their actual dates of onset of puberty were later. The flow diagram in Figure 3.1 below demonstrates the factors affecting AP, and also the pathways through which AP and other factors affect production outcome when artificial insemination is used, although its effect on lifetime production of the cow through repeated early calving dates (Anderson, 1991) is not included in this diagram.

The effect of nutrition on the onset of puberty was studied in more detail recently. Gasser (2006) found that so-called precocious puberty (puberty before the age of 300 days) could be achieved in heifers by early weaning and feeding of a high-concentrate (maize) diet. This early onset of puberty is achieved by advancing the reduction in oestradiol negative feedback on the secretion of gonadotropins to an earlier age.

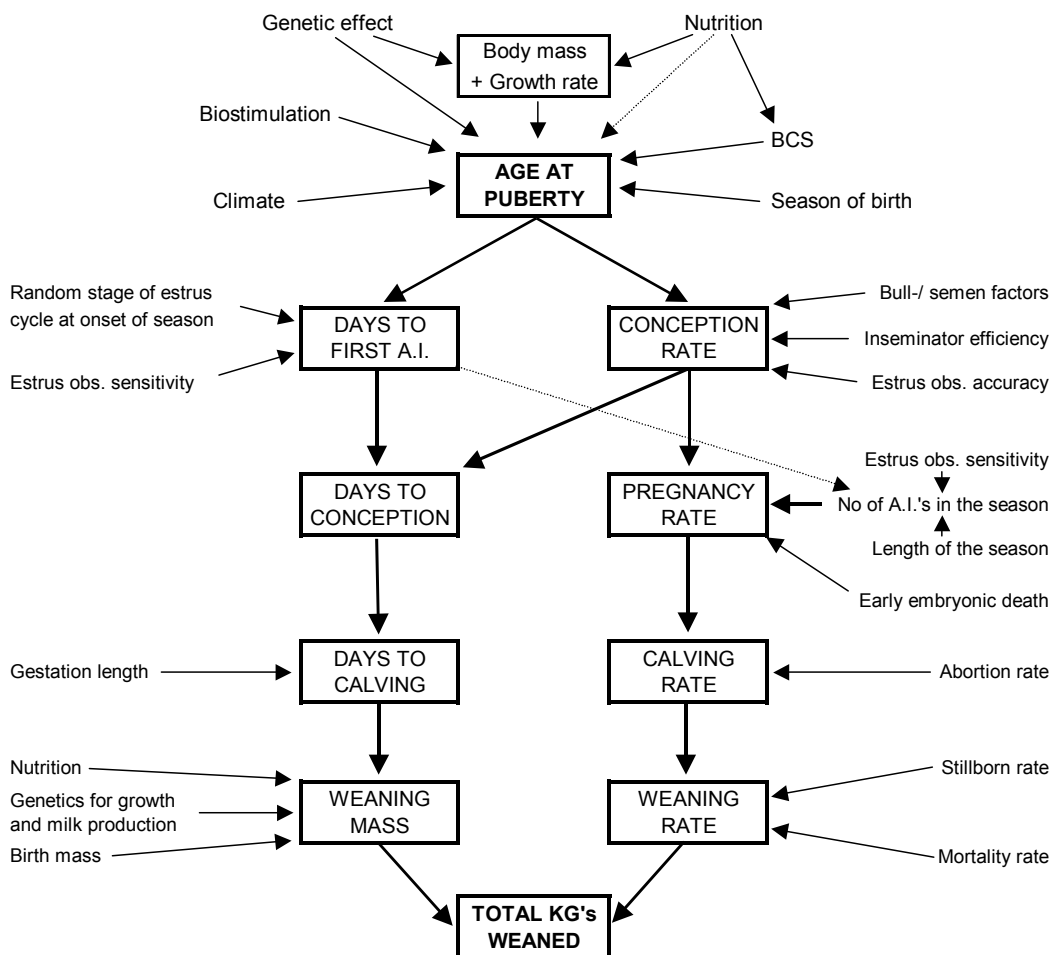


Figure 3.1: Diagram illustrating factors affecting age at puberty, and the pathways through which age at puberty and other factors affect production outcome.

Reproductive tract scoring

Although AP can be determined for individual animals by observing for visual signs of oestrus, it is impractical to apply this method in a large group of heifers (Anderson et al 1990). In the past, visual appraisal of the animals, together with weighing, body condition scoring and calculated indices such as the Kleiber ratio (Scholtz and Roux, 1988) have been used to select heifers for breeding in South Africa (SA) and elsewhere. Anderson et al. (1990) developed a standardised reproductive tract scoring (RTS) method to measure age at puberty of heifers directly. This method involves rectal palpation of the reproductive tracts and ovarian structures and is scored from 1 to 5, where heifers with scores 1 and 2 are not cycling, those with score 3 are on the verge of puberty, and those with scores 4 and 5 are

cycling (see Table 3.1 below). Anderson et al (1990) recommends three possible applications of the RTS system: firstly as a screening test to determine the pubertal status of heifers before the breeding season, secondly as an indication of the nutritional requirements of heifers when sufficient time is allowed before the breeding season, or thirdly as a selection tool for AP. For the latter application it is important to do the examination at a strategic time, when approximately 50% of the heifers are cycling. The importance of timing of this procedure is highlighted by Spire and Holtz (1995), who found that RTS may be of limited use in properly developed replacement heifers, due to the fact that only 8 out of 1,489 heifers in their trial had immature reproductive tracts. The RTS score as a method of selection, has been found to be correlated to AP, response to synchronisation and pregnancy rate to synchronised oestrus, and has an estimated heritability (h^2) of 0.32 (Anderson et al., 1990). Brinks (1994) also mentions that RTS has similar genetic correlations with birth mass, weaning mass and yearling mass than age at puberty, making it a valid method to measure AP. Interestingly enough, the correlation between RTS and birth mass has been reported to be negative, indicating that selection for this trait, is likely to lead to lower birth mass of calves (Brinks, 1994), which is desirable. Anderson et al. (1990) reported that age did not account for a significant portion of the variation in RTS, while group, condition score and mass were highly significant sources of variation. Pence and Bredahl (1998) evaluated RTS as a predictive measure of pregnancy outcome, and demonstrated a strong association between RTS and pregnancy rate to AI as well as final pregnancy rate (including a bull breeding season). Heifers with RTS of 1 were culled before the breeding season during their experiment, but a difference in pregnancy rate to AI of 12% between RTS of 2 and 5, and 18% in the case of the final pregnancy rate was shown. Rosenkrans and Hardin (2002) evaluated the accuracy and repeatability of the RTS system and found it to be a repeatable (within veterinarian and between veterinarians) method to estimate pubertal status. Multicategory (5 by 5 Table) Kappa values (a measure of degree of agreement beyond chance) were 0.64 and 0.46 for agreement within veterinarian and between veterinarians respectively. These Kappa values represent moderate to substantial agreement beyond chance.

Table 3.1: Reproductive Tract Scoring system (Anderson, 1991)

Reproductive Tract Score	Uterine horns	Ovaries			
		Length (mm)	Height (mm)	Width (mm)	Ovarian structures
1	Immature < 20mm diameter, no tone	15	10	8	No palpable structures
2	20 - 25mm diameter, no tone	18	12	10	8mm follicles
3	25 - 30mm diameter, slight tone	22	15	10	8 - 10mm follicles
4	30mm diameter, good tone	30	16	12	> 10mm follicles, Corpus Luteum possible
5	>30mm diameter, good tone, erect	>32	20	15	> 10mm follicles, Corpus Luteum present

Schwalbach (1999) developed a RTS system for post-partum beef cows, with good association with pregnancy outcome. This system, although indicating the level of cyclicity is different from the system used in heifers, because it is an indication of the level of uterine involution and recovery from post-partum anoestrus in cows. It is also a 5-point system and based on the system used for heifers (Anderson et al, 1990).

Donovan et al (2003) found a significant correlation between diagonal pelvimetry measurement and first service conception rate (OR = 1.85, $P < 0.01$), this correlation only existed in summer in their study, where first AI conception rate was significantly lower than in winter. On the other hand, according to Chenoweth and Sanderson (2001) pelvic area is not an effective predictor of future calving difficulty, and has the added disadvantage of selecting for larger cows and higher birth mass of calves.

Oestrus synchronisation and artificial insemination

Artificial Insemination in the beef industry in South Africa

The use of AI as a tool in enhancing production efficiency in beef cattle is underutilised in SA. A survey done by the FAO in 1993 showed that SA had an AI coverage (number of first AI's/total number of eligible females) of 1% in beef cattle, which was the lowest of the countries in the same category included in the study (Chupin and Thibier, 1995). Calculations based on the size of the national herd and the number of semen doses sold reveals that AI accounts for less than 0.5% of beef calves in SA. This is surprising considering the benefits to be gained by the use of AI, of which accelerating genetic progress is the most important (Chenoweth and Sanderson, 2001).

Reasons for the low uptake of AI include the costs of the labour and skill and infrastructure requirements. Oestrus synchronisation has been proposed as a means of reducing these costs, by concentrating the labour utilisation into brief periods of the year (Gaines et al, 1993). Potential additional benefits of synchronisation include the increased weaning mass due to the earlier calving dates of synchronised animals (Gaines et al, 1993). However, no studies have been done under South African conditions to quantify these benefits.

Prostaglandin

Prostaglandin $F_{2\alpha}$, a derivative of linolenic and arachidonic acids, was only discovered in 1969 to be the substance responsible for luteolysis (Noakes, 2001). The name prostaglandin was given to the substance due to the fact that it was first discovered in fresh semen, and assumed to be produced by the prostate gland (Noakes, 2001). Different types of prostaglandin exist, and the F prostaglandin was so named due to its solubility in phosphate ("*fosfat*"), while PGE was found to be soluble in ether. The uterine wall produces PGF under natural circumstances when an embryo is not detected in the uterus. This release of PGF is stimulated by oxytocin release from the ovary (Noakes, 2001), after which PGF is

directly transported to the ovary via the counter current mechanism caused by the close integration of the utero-ovarian vein and the ovarian artery (Bearden et al, 2001). The release of PGF takes place at approximately 6-hourly intervals, and it further stimulates the release of oxytocin from the ovaries (Noakes, 2001). This means that the ovary, although indirectly (via oxytocin) responsible for luteolysis, is dependant on PGF release from the uterus for this function. During the postpartum period in cows, PGF is released in high doses, and is not only responsible for luteolysis, but also plays an important role in the contraction of the uterus and reduction in size as well as return to normal function of the uterus (involution) (Lindell and Kindahl, 1983). Other uses of PGF in cows include the induction of abortion and parturition (Wright and Malmo, 1992), and treatment of chronic post partum endometritis in dairy cows (Jackson, 1977).

Oestrus synchronisation using PGF

Dinoprost (Lutalyse, Pfizer Animal Health, PO Box 783720, Sandton, 2146, South Africa), a natural prostaglandin, causes breakdown of the corpus luteum (CL) in bovines from day 5 until day 17 of the oestrus cycle, and can be used to synchronise any female's oestrus cycle from day 7 onwards (Wright and Malmo, 1992). The time from treatment to induced oestrus is inconsistent, but generally varies from 2 to 5 days in heifers (Wenzel, 1997), depending mainly on the stage of the follicular wave at the time of treatment (Kastelic and Ginther, 1991)(Macmillan et al, 2003). Jackson (Peters and Benboulaid, 1998) showed that heifers treated on day 12 to day 14 of the oestrus cycle show oestrus later than those treated on day 7 to 8 or 15 to 16. This fits with a oestrus cycle with 3 follicular waves, which occurs most commonly in cattle (Sirois and Fortune, 1988). Coulson et al (1979) performed a study where LH peaks were determined after dinoprost treatment, and it was found that there were two groups of responses: those that had a LH peak around 70 hours after treatment, and those that had a LH peak around 81 hours after treatment. These findings are consistent with the above hypothesis.

PGF is a safe drug, causing only slight transitory increase in heart rate as side effect at normal dosages (Goyings et al, 1978), with no known long term negative effects, although the effect on delayed puberty when administered to prepubertal heifers is not quite clear (Crowe et al, 1994). Prostaglandins are metabolised rapidly, by oxidation in the lungs, and have a short half-life (Colazo et al, 2002). Colazo et al (2002) demonstrated that dose, but not route of administration, affected the response to PGF (cloprostenol) treatment and the time from treatment to ovulation.

Several different strategies exist to synchronise cows (or heifers) with PGF. These include the following (Wright and Malmo, 1992):

1. Administration of PGF twice to all cows, 11 to 12 (or up to 14) days apart, with insemination only after the second treatment. This method allows for the best synchrony of all the PGF strategies, and although it is usually used with oestrus observation and AI, fixed time AI (without oestrus observation) can be attempted with this method at 72 and 96 hours after the second treatment. All cows have to be treated twice making this the most expensive strategy.
2. Administration of PGF twice, 6 to 12 days apart, with oestrus observation and AI starting immediately after the first injection, and the second treatment being given only to animals that have not been inseminated by that time. This leads to a reduction in the number of cows that need two treatments, but also to poorer synchrony of the whole group.
3. Oestrus observation and AI for 6 days, followed by PGF treatment to all cows that have not been inseminated by that time (PGF/6). This leads to further reduction in cost due to the fact that only those animals that have not shown oestrus by day 6 will be treated, and will only receive one treatment. It also allows assessment of the degree of cyclicity in the herd before any expense is made. All cycling animals that have not shown oestrus before day 6 would be between days 7 and 20 of their oestrus cycles at the time of treatment, and are likely to respond to PGF treatment.

4. One treatment only, leading to 70 to 75% response to treatment in all cycling animals.
5. Oestrus observation precedes the mating season by 11 days. Cows in oestrus between days –11 and –6 receive PGF treatment on the first day, and those in oestrus between days –6 and the first day of the breeding season are treated on day 6. The aim of this system is to inseminate as many cows in the first 10 days of the breeding season as possible. Those cycling cows that were not seen in oestrus between days –11 and the first day of breeding are likely to be between days 12 and 21 of their oestrus cycles at the onset of the breeding season, and should show natural oestrus within the first 10 days of the breeding season.

Fertility after PGF synchronisation

McIntosh et al (1984) reviewed 17 trials on the effect of synchronisation using PGF on conception rate, and concluded that synchronisation significantly improved first insemination conception rate (58 vs. 51%, $P < 0.01$). Wright and Malmo (1992) report on similar findings, and hypothesise that the reason for this may be associated with overall better quality of ova associated with a period of luteolysis shorter than that which occurs naturally.

Morrel et al (1991) report on an apparent decline in fertility in heifers after repeated oestrus synchronisation with PGF (cloprostenol). This was an incidental finding in a study of pre-conceptual sex selection, and many factors may have been responsible for this decline in fertility, including seasonal differences, and changes in the procedures applied to the semen. Donovan et al (2002) found that dairy heifers were 33% less likely to conceive after PGF (dinoprost) treatment than after naturally occurring oestrus ($P = 0.03$) in a multi variate study using 601 heifers. In their study, PGF was used on all heifers that had not shown oestrus by day 5 of being entered into the breeding herd, and again if a palpable CL was present 10 days after the first PGF injection where no oestrus was detected.

Gaines et al. (1993) and Gaines (1994) report in their study of the economic benefits of a PGF/6 protocol, that a pregnancy rate of induced oestrus was higher than that of a naturally occurring oestrus (79% vs. 70%). The *P*-value is not given by them, but can be calculated as 0.34 using their data, making the difference statistically insignificant. This data was obtained from heifers that were inseminated at different times of the breeding season, where those inseminated at induced oestrus were only inseminated early in the season (days 6 – 10) while those inseminated at naturally occurring oestrus were inseminated either before day 6 or after day 10.

Jackson et al (1984) report that there is no effect on the subsequent oestrus cycle or on later conception rate, if conception did not occur at the oestrus induced by PGF (cloprostenol).

LeBlanc et al (2002) found that treatment of endometritis with PGF in dairy cows between 20 and 26 days in milk was associated with a significant reduction in pregnancy rate.

Uterine massage

Dementsova (1986) reported faster return to normal function of the uterus and ovaries, as well as a decreased service period in a group of cows that received 5 minutes of uterine massage every 2 days from 3-5 days post partum, compared to untreated controls. It is hypothesised that this effect is caused by PGF release from the uterus. However, various researchers (Resende et al, 1991, Andrade et al, 1991) have not been able to demonstrate any effect of shorter periods of uterine massage (30 seconds) on outcome of (time to) return to oestrus and pregnancy rates.

Factors affecting gender proportion when AI is used

The effect of timing of insemination on gender proportion

Rorie (1999) reviewed studies on the effect of timing of insemination on gender proportion of the offspring. Studies as early as 1891 indicated an increased proportion of male offspring conceived by inseminations (natural or artificial) late in oestrus, when compared to inseminations early in oestrus. This has been demonstrated in various species (Rorie, 1999), and Verme and Ozoga (1981), in their study on white-tailed deer, suggested that this was a mechanism for the species to balance the numbers of male and female animals: if male animals are scarce, mating will take place later in oestrus, leading to more male offspring to restore the normal ratio of male:female animals, and vice versa. However, Rorie found contradicting evidence, where later studies on larger numbers of animals showed no difference in gender proportion. Rorie further reports that in a more recent study using an Ovatec probe that measures electrical resistance of cervical mucous in the vagina (which is inversely related to oestrogen levels), a clear difference was shown in gender proportions between early and late inseminations. However, this could not be repeated using electronic mounting detector technology (Rorie, 1999). Rorie also reports on studies performed on early and late in vitro fertilisation leading to decreased, and increased male:female offspring ratios respectively.

The effect of semen batch on gender proportion

Chandler et al (1998) reported significant variation in proportions of X- and Y-bearing sperm in different semen batches within bull, suggesting that one batch of semen can lead to more male, or more female offspring.

The effect of synchronisation on gender proportion

Rorie (1999) reports on various studies where prostaglandin synchronisation led to increased proportion of male offspring, while synchronisation protocols using progesterone, seems to have led to increased proportion of female offspring. However it was uncertain whether change in gender proportion was caused by synchronisation protocol, or by the timing of insemination in relation to onset of oestrus as a result of synchronisation. This therefore needs further investigation.

The economic effects of a PGF/6 synchronisation protocol

Gaines et al. (1993) and Gaines (1994) performed the only study to our knowledge that studied the economic effects of a synchronisation protocol using prostaglandin. Their study, on a group of 129 13-month-old Holstein cross Hereford heifers, used the entire group of heifers as the test group, and simulated a control group using a random number generator to assign those heifers that conceived between days 7 and 10 a new conception date between days 7 and 21. In their study group, 96/129 (74.4%) heifers received a prostaglandin injection on day 6. First service conception rate was high (99/129 or 76.7%), and only those heifers that conceived on first service were used for the simulation model. Days to calving was calculated for the simulation model using the known gestation period of that animal. Median days to pregnancy was given as 8 vs. 15.6 for the test group and simulation model respectively, and median days to calving was 10.5 vs. 18 (P - values not given). Calves were weighed at approximately 150 days of age, and a mass was calculated for the simulated model using the known ADG of the calves at the time of weaning, and subtracting that (times the number of days that the calf was born later) from the real weaning mass. This resulted in a significantly higher weaning mass for the test group than for the simulation group (176 kg vs. 172 kg, $P < 0.01$). Using a cost of \$4.00 per prostaglandin injection (including labour) and a value of \$1.76 per kg of calf, a return on investment of 1.92 was reported by Gaines et al. (1993) and an average cost-benefit of 2.16 (on a number of simulations, using the same raw data) by Gaines (1994).

Gottschall (1999) performed a study on beef heifers where AI was performed for 7 days, at which time RTS was performed on all heifers that had not been inseminated by then. After the heifers with RTS 1 were culled, all remaining heifers were treated with PGF and AI was continued until day 12. They had a control group that was not synchronised, and mated by a bull. Heifers that were inseminated during the first 7 days were included in the group of heifers with RTS 5. A monotonic increase in pregnancy rate for RTS 2,3,4 and 5 was demonstrated, and there was no difference in the mean mass before breeding of the pregnant and the non-pregnant animals. Gottschall concluded from this that the protocol used (PGF on day 7 + RTS) was an efficient way of concentrating the calving season.

4. RESEARCH QUESTIONS

1. Does synchronisation of oestrus with artificial insemination (AI) lead to significant benefits in beef heifers in South Africa?
2. Can the cost effectiveness of oestrus synchronisation be determined?
3. Is reproductive tract scoring (RTS) a valid predictor of performance in South African beef heifers?

5. HYPOTHESES

1. A PGF/6 protocol has no effect on reproduction and production outcomes in beef heifers.
2. The cost effectiveness of oestrus synchronisation cannot be determined.
3. Reproductive tract scoring (RTS) is not an accurate predictor of reproduction and production performance in beef heifers.

6. OBJECTIVES

1. To compare a PGF/6 protocol to the control (no synchronisation) with respect to various reproduction, production and economic outcomes.
2. To perform a cost benefit analysis for a PGF/6 protocol, using an unsynchronised group as control.
3. To compare RTS to other methods of predicting beef heifer performance.

7. MATERIALS AND METHODS

Model system

This was a prospective field trial, in which two groups of heifers were studied in parallel: The TEST group in which the PGF/6 protocol was applied, and the CONTROL group of unsynchronised heifers. Simultaneously, a prospective study was performed to determine the association of RTS and other predictors of heifer performance with production and reproduction outcomes.

Sample size

A difference in conception rate of 15% between the two study groups was expected. To compare the study groups, when $\alpha = 0.1$ with a power of 80%, it was calculated that at least 110 animals in would have been needed per group (whereas if $\alpha = 0.05$, then $n = 147$). Thus, a total heifer group of at least 220 was required.

Experimental design

A group of 272 Bovelder heifers, at Johannesburg Water's Northern Farm was selected for this trial in October 2003. The Bovelder breed is a synthetic beef breed developed at Northern Farm, consisting of many different Bos Taurus and Bos Indicus breeds (Angus, Hereford, Bonsmara, Simmentaler, Charolais, Afrikaner, Brown Swiss, Brahman and Friesland amongst others), and selected by a strict ongoing progeny testing programme. The heifers originated from two different farms: about half of them were born on Northern Farm, while the other half were born on Olifantsvlei Farm, and later moved to the nearby Northern Farm. Northern Farm is at Diepsloot, 30 km North of Johannesburg, SA, and is located next to the Northern Wastewater Works of Johannesburg Water, with its Northern border being the Jukskei river (latitude 25°50'S). This farm is located on the highveld of Gauteng, approximately 1380m above sea level, and annual rainfall averages 690mm per year, falling between September and May (summer rainfall area) (De Villiers, 2006). Temperatures in this region range from 14.9°C (mean minimum) to 27.0°C (mean maximum) in January, and from 2.7°C (mean minimum) to 18.2°C (mean maximum) in June (De Villiers, 2006). Processed water from the water works is used to irrigate kikuyu, clover and ryegrass pastures on this farm, and supplemental to these pastures, animals are fed grass hay, silage and mixed ration diets at times when increased levels of nutrition are required. Three weeks before the onset of the trial, the mass of Olifantsvlei heifers was lower than that of Northern Farm heifers, and the Olifantsvlei heifers were managed separately, and put on an increased level of nutrition for those last three weeks.

The heifers' ages at the start of the breeding season ranged from 364 to 486 (median 431) days. Two days before the onset of the insemination season (day -1), all heifers were weighed, body condition scored (BCS) using the 5-point scale with half points (Edmonson et al, 1989) and reproductive tract scored (RTS) using the system described by Anderson et al (1990). Mass ranged from 261 to 407 (mean 314.4) kg, while RTS ranged from 1 to 5 and BCS from 3.0 to 4.5.

In order to avoid bias caused by farm of origin, RTS or mass, heifers were ranked firstly by farm of origin, secondly by RTS and thirdly by mass, and then block randomised in pairs to either the TEST or the CONTROL group. Because of the correlation between mass and age ($r = 0.24$, $P < 0.01$), age was not included in the block randomisation process. Mean RTS, mean BCS, and mean mass were compared between the two groups, to confirm that the groups were comparable. The TEST group consisted of 136, and the CONTROL group of 137 heifers.

One day before the start of inseminations (day 0), *Estrus Alert* oestrus detection stickers (CRI, PO Box 717, Howick, 3290, South Africa) were applied to the sacrum of all heifers. *Estrus Alert* stickers are brightly coloured stickers covered with a thin layer of silver paint that is brushed off when a heifer (or cow) is mounted repeatedly by herd mates. An activated sticker is seen easily in a group of animals. These stickers were only used for the first oestrus, and were removed when the heifer was inseminated for the first time. Apart from using this oestrus observation aid, visual observation was performed from dusk until dawn every night, and heifers that were seen in behavioural oestrus were marked with paint and then separated for AI in the morning.

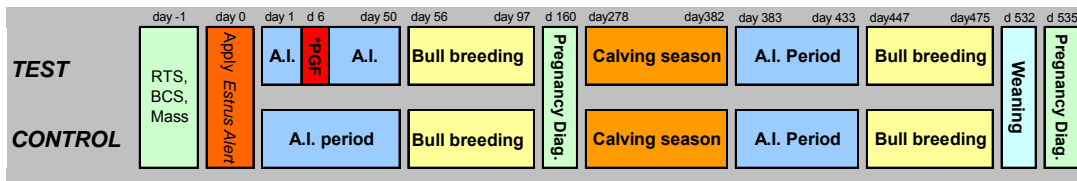
The normal farming practice was otherwise followed for these heifers, and the two groups (TEST and CONTROL) were managed together. Frozen semen of 11 different Bovelder bulls was allocated to heifers according to the normal practice on this farm. Farm management, the AI technician and workers that performed oestrus observation were blinded to study group, by keeping the records for the purpose of this trial separate from the farm records. The insemination season started on 15 October 2003 (day 1). Heifers were inseminated once a day by one experienced AI technician. One insemination per detected oestrus was normal practice on this farm, and this was also done during the study. In the TEST group, all heifers that had not shown oestrus by the morning of day 6, received an intramuscular injection of 25mg dinoprost (Lutalyse, Pfizer Animal Health) on that morning. All heifers, including those of the CONTROL group, were moved through the crush on that morning. Oestrus detection and AI continued for a period of 50 days (days 1 – 50). After the end of the AI period, there was a window period of 5 days (days 51 – 55), followed by a

period of natural breeding with bulls of 42 days (days 56 – 97). The reason for the window period between AI and natural breeding was to allow easy determination of parentage by calving date.

Days into the AI season, and bull, were recorded for all inseminations during the breeding season, as part of the normal farm record system. A veterinarian performed pregnancy diagnoses (PDs) by rectal palpation 90 days after the removal of bulls. Because PDs were done at a time when stage of pregnancy is difficult to determine accurately, AI dates were used to assist the veterinarian in deciding whether a pregnancy was likely to be conceived from artificial insemination (AI) or natural mating.

After PDs, heifers were further monitored, and farm management collected the following data: abortions, calving date, mass and gender of calf, dystocia, stillbirths, calf mortality, cow mortality and weaning mass. All calves were weaned on the same day (29 March 2005), and either kept as replacement heifers, or as potential breeding bulls, or sold. Before the onset of the subsequent breeding season, researchers recorded BCS again, and those first calf cows that were still on the farm were prepared for their second breeding season.

A similar breeding season (AI, then bull) was used in this subsequent breeding season, but it started 2 weeks later than the previous year (1 November 2004), and there was a period of 14 days between AI and bull-breeding seasons. The bull-breeding season in the second year lasted for 30 days. Similar records were collected during this subsequent breeding season, but the trial was terminated at the time of PDs, 60 days after the removal of bulls.



*PGF: Inject all heifers that are not inseminated by day 6, with 5ml Lutalyse (Dinoprost)

Figure 7.1: Graphic illustration of protocol

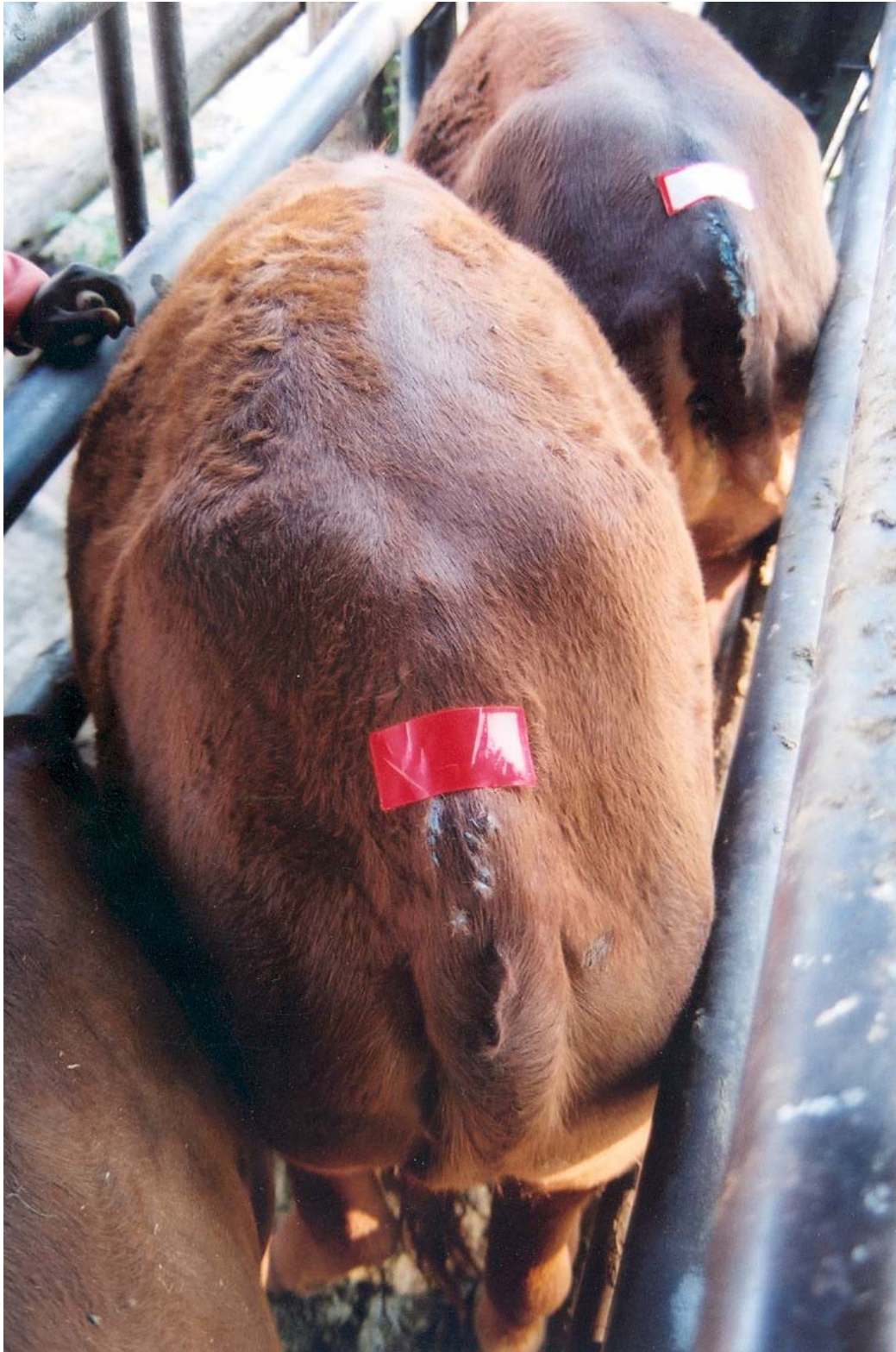


Figure 7.1: The heifer in the foreground shows a positive test result to the Estrus Alert oestrus detection aid, while the heifer in the background still has a negative result

Analytical procedures

All data were entered into a spreadsheet for analysis. Proportions were compared (TEST vs. CONTROL group) using the Fisher exact test for 2x2 tables, or the Chi-square test for 2xk tables, while means and medians were compared using the Student's *t*-test and Wilcoxon rank-sum test respectively. For data that had a normal distribution, means were compared between the groups (such as weaning mass of calves etc), while medians were used for data with a non-normal distribution (such as age), and the log-rank test was used for data of time-to-an-event (such as days to calving).

Numerical (pre-breeding mass and age) and categorical (pre-breeding BCS and RTS) input variables were compared with respect to their effects on production and reproduction outcomes. Associations between these input variables and outcomes were tested using either logistic regression (where the outcome was binary e.g. pregnant vs. not pregnant), multiple regression (where the outcome was a continuous numerical scale e.g. weaning mass) or Cox proportional hazards regression (where the outcome was time to an event such as days to calving). Receiver-operating characteristic (ROC) curves were generated for logistic regression models on pregnancy outcome, and areas under the curve (AUC) were calculated for ROC curves.

Statistical analyses were done using NCSS 2004 (NCSS, Kaysville, UT, USA) and Epicalc 2000 (<http://www.brixtonhealth.com/epicalc.html>).

Cost benefit analysis

All costs were recorded, as well as time spent to perform procedures. Cost benefit analysis was performed on the data, using basic accounting to calculate cost and return (benefit) per animal. A discount rate was included to allow for time difference between costs incurred and returns. Bank interest rate was estimated at 15%, and inflation rate at 5%. The discount rate was calculated using the following equation (Noordhuizen, 2001):

$$PV = FV \div (1+r/100)^n$$

where PV = present value, FV = future value, r = real annual interest rate (interest rate – inflation rate) and n = number of years.

After costs and returns have been valued and discounted to a common time base, the following value criteria were calculated:

Net Present Value

Net present value (NPV) of return is simply the difference between return and cost (i.e. the profit), including the discount rate. It does not take into account the relative volume of costs incurred (Noordhuizen, 2001).

$$NPV(\text{return}) = PV(\text{gross return}) - PV(\text{cost})$$

Benefit Cost Ratio

This ratio compares the Present Value of return to the cost, and reflects the relative volume of costs and benefits (Noordhuizen, 2001).

$$\text{Benefit Cost Ratio} = PV(\text{gross return}) \div PV(\text{cost})$$

Break-even point

The break-even point was calculated for different factors that affected the benefit, and gives an indication of the minimum effect of the synchronisation protocol necessary for it to make economic sense.

Internal Rate of Return

While the discount rate allows for the time difference between early costs and later returns (benefit), it is calculated by using present market rates, which are not stable. It changes with time due to macro-economic factors and interest rate prediction is beyond the scope of this study. For that reason, we can also look at Internal Rate of Return (IRR), which indicates the highest level of interest rate at which the action taken will still make economic sense

$$\text{IRR} = [\text{FV}(\text{net return}) \div \text{PV}(\text{cost})] \div n$$

where PV = present value, FV = future value and n = number of years (Huirne and Dijkhuizen, 1997).

Definitions

Days to first insemination was defined as the number of days into the breeding season when a heifer was inseminated for the first time. When a heifer did not show oestrus and consequently did not get inseminated during the 50 day AI season, a value of 50 days to first insemination was given to her, but the value was censored in a separate column for the purpose of Cox regression.

Days to pregnancy was defined as the number of days into the breeding season that it took for a heifer to become pregnant. These data were firstly obtained from rectal pregnancy diagnoses, but some difficulty occurred in determining the origin (AI vs. natural mating) of the pregnancy for those heifers that were inseminated during the last few days of the AI season. The reason for this difficulty was that there was only a short break between AI and the bull-breeding season, and no oestrus data was available for the bull-breeding season. Therefore, this data was corrected in some instances according to calving date. When a heifer did not become pregnant during the 50 day AI season, a value of 50 days to pregnancy was given to her, but the value was censored in a separate column for the purpose of Cox regression.

All abortions was defined as those heifers that were seen to have aborted (***confirmed abortion***), plus those that were recorded to be pregnant at rectal pregnancy diagnosis, but failed to calve (***suspected abortion***). This last group was examined again at the end of the calving season and found to be not pregnant.

Days to calving was defined as the number of days into the calving season when a heifer calved. When a heifer did not calve during the 105 day calving season, a value of 105 days to calving was given to her, but the value was censored in a separate column for the purpose of Cox regression. Those heifers that died or were sold before the calving season were recorded as missing values.

Days to birth was defined as the number of days into the calving season when a calf was born (same value as days to calving, but relates to the calf).

Day 6 not inseminated rate was defined as the proportion of heifers that did not show oestrus (and were thus not inseminated) during days 1 to 6 of the breeding season. In the TEST group this was the proportion of heifers that were treated with PGF.

Kleiber Ratio was defined as the corrected ADG (205 or 365 days) divided by the metabolic mass of the animal at that time ($\text{mass}^{0.75}$). It is a trait with moderate heritability used to select efficient replacement heifers and is highly correlated with feed conversion rate (Scholtz and Roux, 1988).

The **rate of normal inter-oestrus periods** was defined as the number of inter-oestrus periods that fell in the range 16-25 days, as a proportion of the total number of inter-oestrus periods recorded.

Twenty-five day insemination rate was defined as the rate of heifers that received at least one insemination during the first 25 days of the AI season, as a proportion of the number of heifers submitted for breeding.

Weaning rate (WR) was defined as the number of calves weaned as a proportion of the total number of female animals at the beginning of the breeding season.

Sensitivity was defined as the proportion of positive test results in the group of animals that are positive (or the probability of a positive test outcome in a positive individual).

Specificity was defined as the proportion of negative test results in the group of animals that are negative (or the probability of a negative test outcome in a negative individual).

Odds Ratio (OR) was defined as the ratio of the odds of a certain outcome in the presence of a factor, to the odds of that outcome in the absence of that factor.

The **Consumer Price Index (CPI)** was defined as the percentage increase in consumer prices over a certain time period (usually 1 year).

The **South African Prime Overdraft Rate (SAPROR)** was defined as the lending rate of the South African Reserve Bank to commercial banks.

8. RESULTS

Comparison of the TEST and CONTROL groups before the AI season

There were no differences in any of the parameters between the TEST and CONTROL groups (Table 8.1), apart from age, where median age was 4 days more in the TEST group than in the CONTROL group ($P = 0.02$). Bull allocation was similar between groups ($P = 0.79$). Heifers from each farm of origin were exactly equally allocated to the TEST and CONTROL groups.

Table 8.1: Comparison of TEST and CONTROL groups

	TEST		CONTROL		P
	n = 135		n = 137		
	Mean	Median	Mean	Median	
Reproductive Tract Score (RTS)	3.12	3.00	3.12	3.00	1.00
Mass before onset of breeding (kg)	314.4	316.0	314.4	315.0	1.00
Age at onset of breeding season (days)	433.6	433	426.9	429	0.02
Body Condition Score (BCS)	3.75	4.00	3.73	4.00	0.70

The researcher had no control over the sale and death of heifers, 3 heifers in the control group died before calving, and 5/111 and 17/114 pregnant heifers were sold from the TEST and CONTROL groups respectively ($P = 0.01$).

One heifer was removed from the group after study groups were allocated because of a fractured leg, and the final numbers were 135 in the TEST, and 137 in the CONTROL groups.

RTS was not recorded for one heifer, and it was dealt with as a missing sample.

The effects of the PGF/6 synchronisation protocol

The effects of PGF treatment on the following outcomes:

Proportion of heifers inseminated from days 7-11 of the breeding season

Of all the heifers, 93/272 (34%) showed oestrus and were inseminated on or before day 6 of the breeding season. In the TEST group, 87/135 heifers had not shown oestrus by day 6, and were hence treated with PGF, while the corresponding proportion was similar in the CONTROL group (92/137, $P = 0.70$). Following treatment, 62/87 (71%) heifers in the TEST group were inseminated in the period ranging from days 7 to 11, while 24/92 (26%) in the CONTROL group were inseminated during that same period ($P < 0.01$). Of the 25 heifers in the TEST group that did not show oestrus within 5 days of PGF treatment, 13 showed their first oestrus between days 12 and 21 of the trial, 5 showed first oestrus after day 21 and 6 never showed oestrus during the 50 day AI period.

The time needed to treat the heifers that had not shown oestrus by day 6, was 1 hour for the group of 135 heifers, and five farm labourers, one AI technician and one veterinarian were involved.

Days to first insemination

Because of the non-Normal distribution of days to first insemination (Figure 8.1), the medians were used to compare the two groups, and were day 8 and day 11 for the TEST and CONTROL groups respectively ($P < 0.01$). Survival analysis demonstrated a significant difference in the days to first insemination ($P < 0.01$), with a Cox-Mantel hazard ratio of 1.39 (95% CI 1.09 – 1.78). Figure 8.2 is a plot of the survival curves for days to first insemination (TEST vs. CONTROL groups).

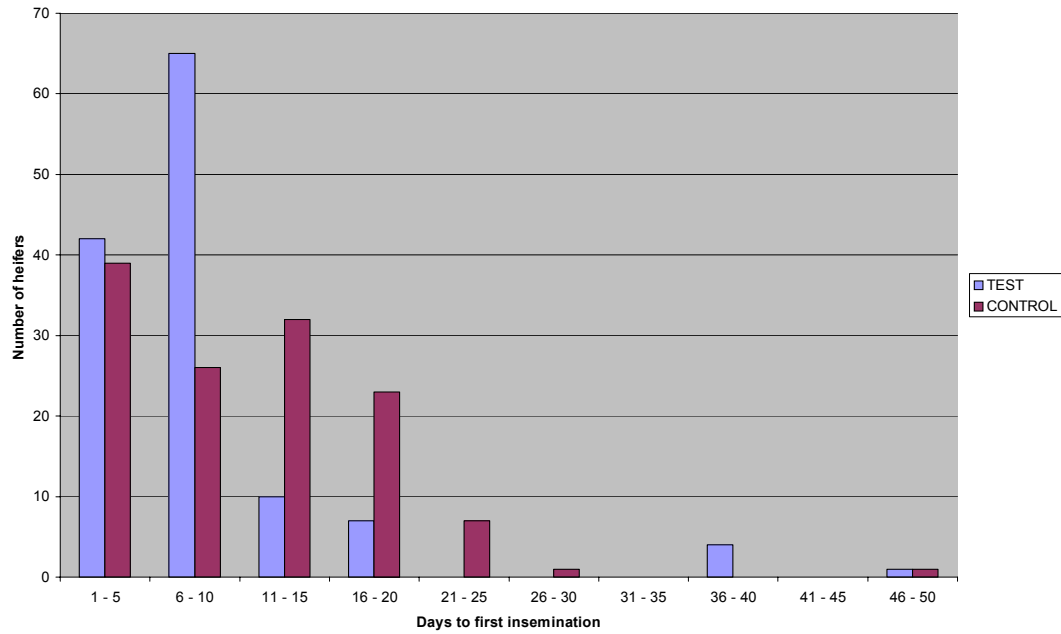


Figure 8.1: Distribution of days to first insemination

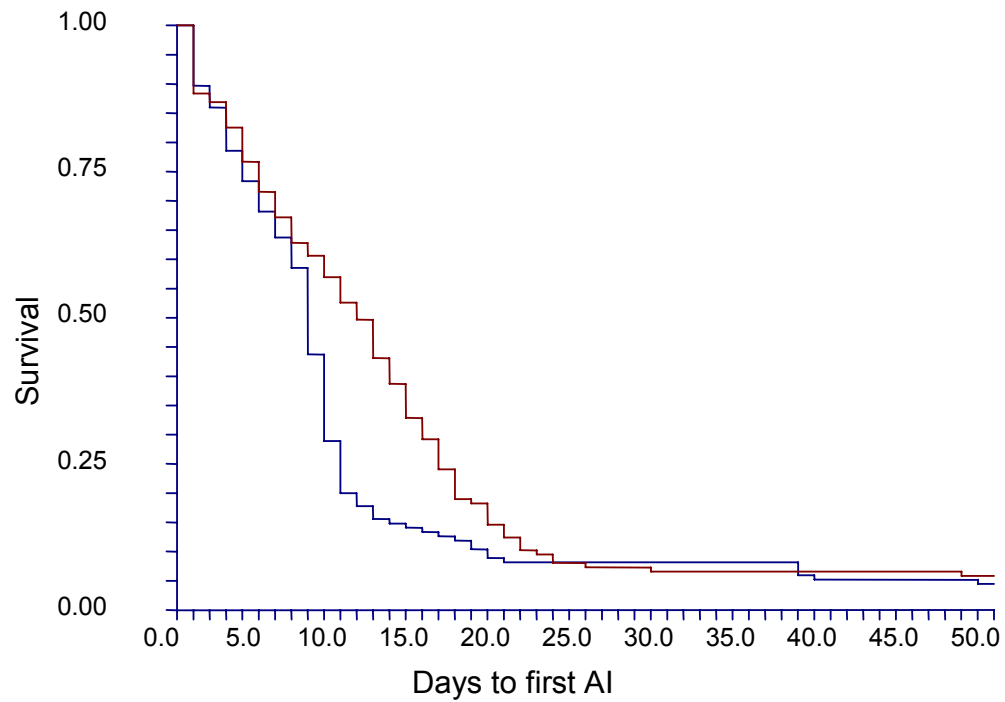


Figure 8.2: Kaplan-Meier survival curve for days to first insemination. The blue line represents the TEST group, and the red line the CONTROL group.



Figure 8.3: The increased level of sexual activity was quite obvious on the morning of day 8

Repeat breeding

Heifers that were inseminated twice or more during the breeding season represented 45% (58/128) of the TEST group, and 40% (52/129) of the CONTROL group, the difference being not significant ($P = 0.45$). Heifers that were inseminated for the first time after day 6 repeated once at a similar rate between the TEST and CONTROL groups (37% (31/83), and 35% (30/87) respectively, $P = 0.75$), but TEST heifers tended to have a higher rate of repeating twice, than those in the CONTROL group (9/83 = 11% vs. 4/87 = 5%, $P = 0.16$).

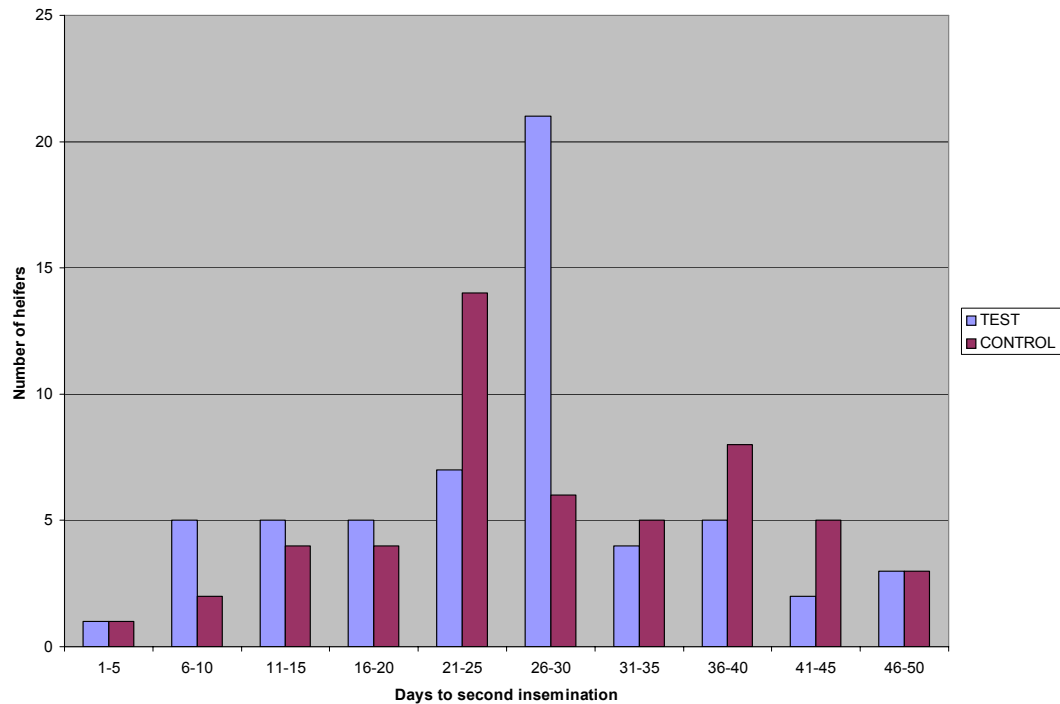


Figure 8.4: Distribution of days to second insemination (or repeat breeding)

Totals of 209 and 196 inseminations were performed in the TEST and CONTROL groups respectively (Table 8.2).

Pregnancy rates

Pregnancy rates between the TEST and CONTROL groups were not significantly different, although there was a tendency in the TEST group to have a higher pregnancy rate to artificial insemination. Results are summarised in Table 8.2 below.

Table 8.2: Summary of pregnancy rate results

	TEST	CONTROL	P
First insemination pregnancy rate	51/128 (39.8%)	54/129 (41.9%)	0.80
Pregnancy Rate to 50 day AI period	81/135 (60.0%)	71/137 (51.8%)	0.18
Final pregnancy rate	111/135 (82.2%)	114/137 (83.2%)	0.87
Number of AI's per conception in the group	209/81 (2.58)	196/71 (2.76)	0.76

There was a tendency for first insemination pregnancy rate to be higher in those inseminations that followed within 5 days of PGF treatment (in the TEST group), when compared to all first inseminations that did not follow within 5 days of treatment in the TEST and CONTROL groups combined (31/62 (50%) vs. 73/197 (37%), $P = 0.08$). Figure 8.5 demonstrates the cumulative conception rates of the TEST and CONTROL groups over time.

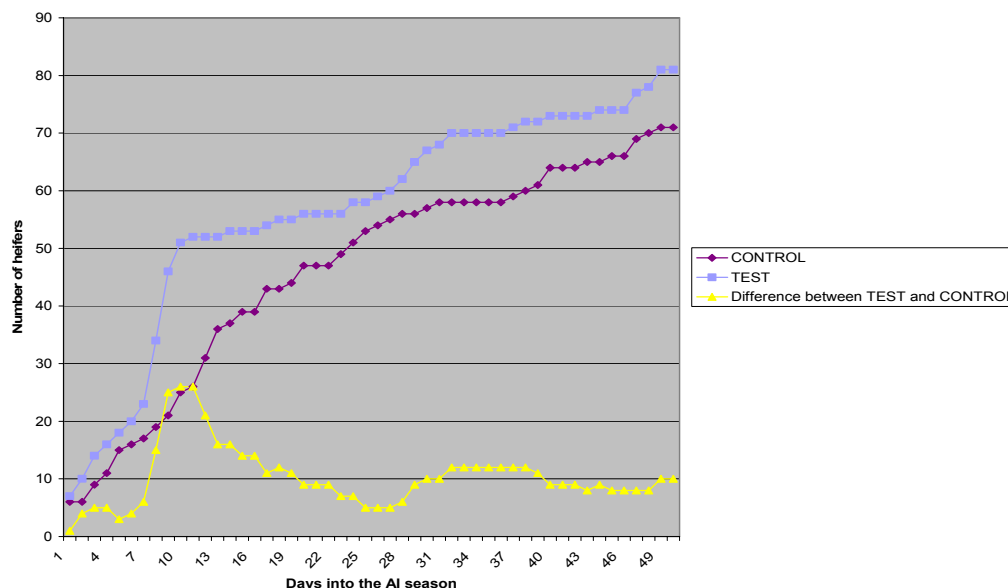


Figure 8.5: Cumulative pregnancy rates for the TEST and CONTROL groups during the 50 day AI season.

Days to pregnancy

When days to pregnancy was corrected according to calving date, it appeared that 8 heifers were inseminated once after they had conceived, of which 3 were inseminated within a normal inter-oestrus period (17-25 days).

Median days to pregnancy tended to be different between the groups (31 and 47 days for the TEST and CONTROL groups respectively, $P = 0.06$). Survival analysis revealed no significant difference in days to pregnancy, with a Cox-Mantel hazard ratio of 1.11 (95% CI 0.81 – 1.52, $P = 0.53$).

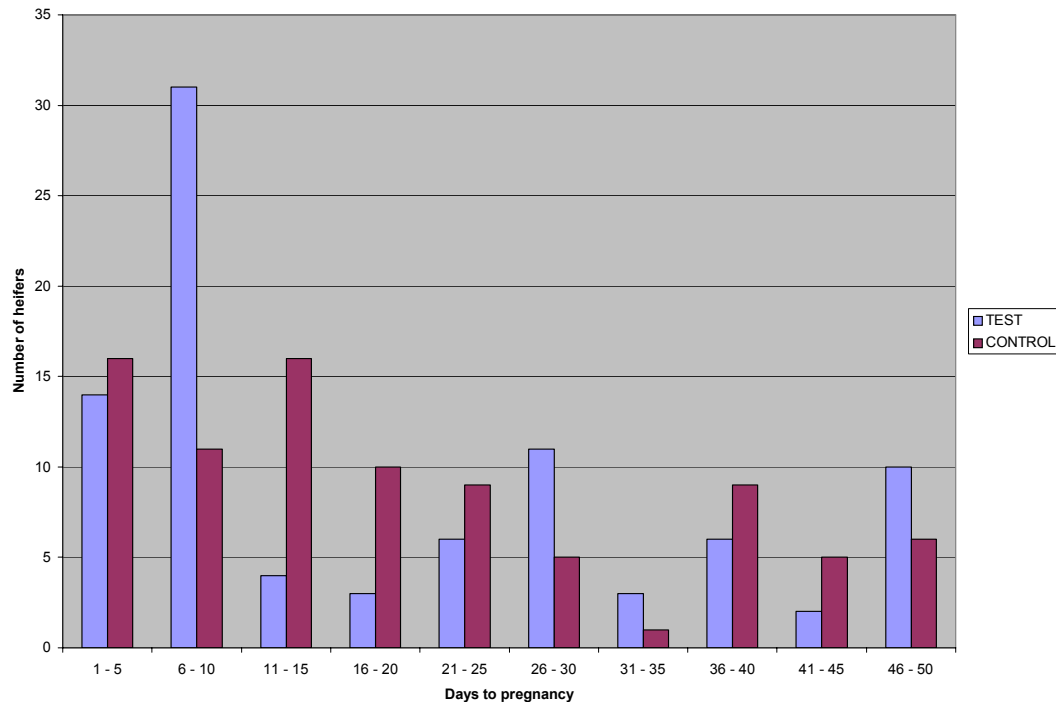


Figure 8.6: Distribution of days to pregnancy

Abortion, still birth and dystocia rates

All “suspected abortions” occurred in pregnancies that were conceived during the bull-breeding period, apart from one in the TEST group that was conceived on day 47 of the AI period.

There was no significant difference in all abortions between the TEST and CONTROL groups (5/111 vs. 11/114, $P = 0.19$). Confirmed abortions tended to be lower in the TEST than in the CONTROL group (1/111 vs. 7/114, $P = 0.07$). All abortions of AI foetuses that were conceived after day 6 (Lutalyse treatment) tended to be, but were not significantly less in the TEST than the CONTROL groups (1/60 vs. 4/54, $P = 0.19$). The one abortion of an AI pregnancy that occurred after day 6 in the TEST group was conceived on day 47 of the breeding season, while those of the CONTROL group were conceived on days 7,8,25 and 49.

Stillbirths tended to be higher in the TEST than in the CONTROL group, but the difference was not significant (9/106 vs. 3/105, $P = 0.13$). A similar tendency occurred for

stillbirth of AI calves (6/79 vs. 1/64, $P = 0.13$). Table 8.3 summarises the findings of abortion, still birth and dystocia rates.

Table 8.3: Summary of abortion, still birth and dystocia rates.

	TEST	CONTROL	P
All abortions	5/111 (4.5%)	11/114 (9.7%)	0.19
Confirmed abortions	1/111 (0.9%)	7/114 (6.1%)	0.07
Abortion of foetuses that were conceived after day 6	1/60 (1.7%)	4/54 (7.4%)	0.12
Still births	9/106 (8.5%)	3/105 (2.9%)	0.13
Still births AI calves	6/79 (7.6%)	1/64 (1.6%)	0.13
Dystocia cases	4/106 (3.8%)	3/105 (2.9%)	0.99

Days to Calving and gestation length

Median days to calving for the TEST group was 21 days, while for the CONTROL group it was 29 days ($P = 0.06$). This is presented graphically in Figure 8.7. There was a significant difference between median days to calving of AI calves between the groups (TEST = 14 days and CONTROL = 20 days, $P = 0.04$). The Kaplan-Meier survival curve for days to calving is shown in Figure 8.7 below. Survival analysis demonstrated a significant difference in days to calving ($P = 0.04$), with a Cox-Mantel hazard ratio of 1.35 (95% CI 1.01 – 1.80).

The mean gestation length in the TEST group was 282.3 days, while in the CONTROL group it was 283.1 days ($P = 0.33$). Gestation length could only be calculated for AI calves.

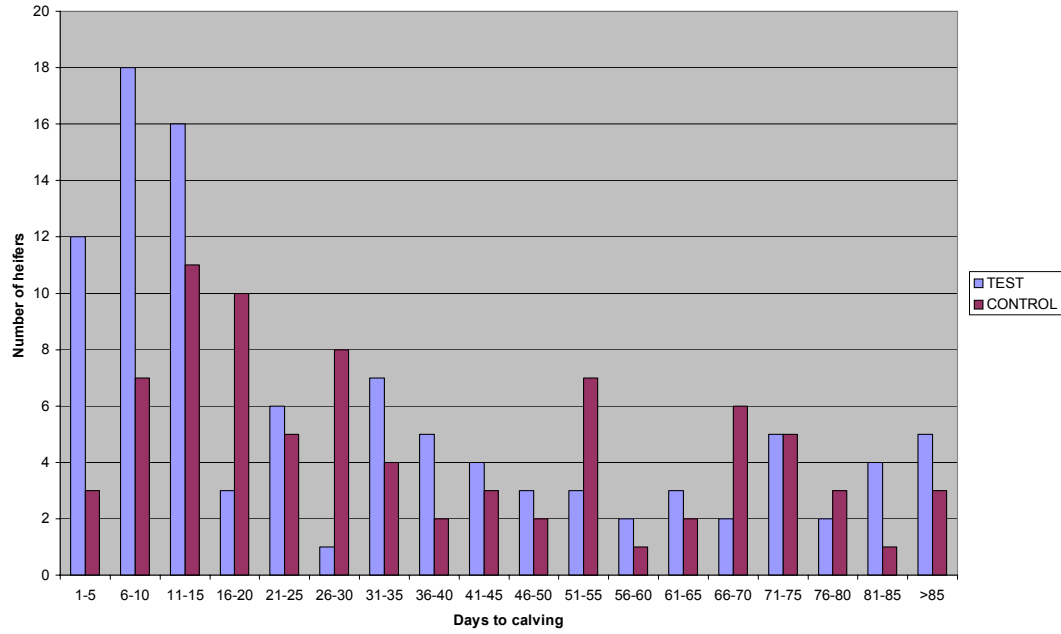


Figure 8.7: Distribution of days to calving for all calves born during the study.

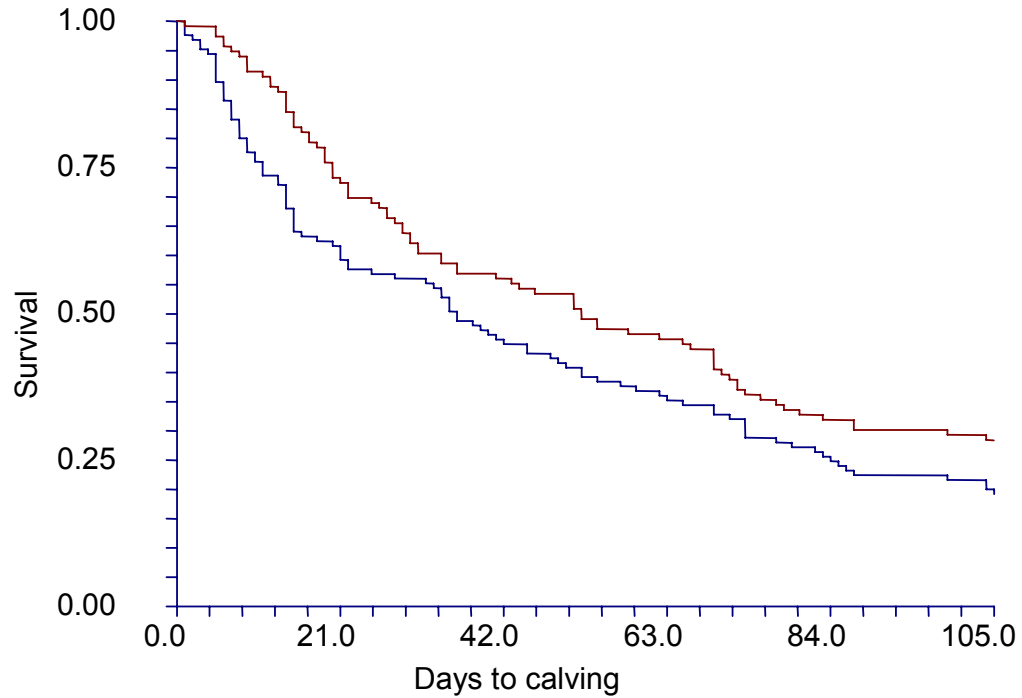


Figure 8.8: Kaplan-Meier survival curve for days to calving. The blue line indicates the TEST group.

Gender proportions

There were 113 male (61%), and 71 female calves born during this study. In the TEST group 64% (65/101) calves were male, compared to 58% (48/83) male calves in the CONTROL group ($P = 0.45$). During the first 40 days of the calving season, more male calves were born in the TEST than in the CONTROL groups (48/68 or 71% vs. 26/50 or 52%, $P = 0.05$). The ratio of male calves born during the first 40 days of the calving season in the TEST group, compared to all other births, once again demonstrates that there was a tendency for more male calves to be born after synchronisation (48/68 or 71% vs. 65/116 or 56%, $P = 0.06$).

Pregnancy rates and gender proportions, per bull

Only one bull (D171) had significantly more pregnancies in the TEST than the CONTROL group (10/13 = 77% vs. 5/15 = 33%, $P = 0.03$). No one bull produced a significantly higher proportion of male calves when compared to the rest of the bulls. The effects of the Lutalyse treatment on conception rate and gender proportions, per bull, are summarised in Table 8.4. Abortion rate did not differ significantly between bulls ($P = 0.16$).

Table 8.4: Summary of pregnancy rates, calf gender proportions and abortion rates per bull

Bull I.D.	TEST				CONTROL			
	Number of heifers	Pregnancies from AI	Male:Female calves born	Abortions	Number of heifers	Pregnancies from AI	Male:Female calves born	Abortions
B026	13	10	8:2	0	16	11	2:8	1
B044	12	6	3:3	0	11	6	2:2	2
D055	12	10	7:3	0	14	8	5:1	2
D063	13	9	5:4	0	17	9*	7:2**	0
D069	14	10	5:4	1	9	6	2:4	0
D083	0	0	N.A.	N.A.	2	1	1:0	0
D089	2	0	N.A.	N.A.	0	0	N.A.	N.A.
D095	13	4	2:2	0	10	7	6:2**	0
D171	13	10	7:3	0	15	5*	2:2	0
F260	10	6	4:2	0	9	4	2:1	1
L134	14	8	6:2	0	12	4	2:2	0
VB09	19	8	5:2	1	22	10	4:4	2

* One heifer in this group died before calving

** One heifer in this group produced twins

Calf birth mass

The mean birth mass for calves in the TEST group was 30.3 kg, while in the CONTROL group it was 30.7 kg ($P = 0.60$). Mean birth mass of the male calves was significantly higher than that of the female calves (31.1 vs. 29.5 kg, $P = 0.04$). However, multiple regression revealed that calf birth mass was associated less significantly with calf gender ($P = 0.20$) than with days to pregnancy ($P = 0.02$) and gestation length ($P < 0.01$). The difference between mean gestation length of the male and female calves was not statistically significant (283.2 vs. 281.9 days, $P = 0.13$).

Calf weaning mass, weaning rate and average daily gain (ADG)

Although calf weaning mass in the TEST group appeared higher, the difference in mean weaning mass between the TEST and CONTROL groups was not significant (207.0 kg vs. 201.4 kg, $P = 0.32$). Similarly, mean weaning mass of all calves born to AI was not significantly different between TEST and CONTROL groups (216.8 kg vs. 211.8 kg, $P = 0.37$). Mean average daily gain (ADG) was also similar between groups (0.742 kg vs. 0.748 kg, $P = 0.85$).

Weaning rate of AI calves was not significantly different between the TEST and the CONTROL groups ($P = 0.17$) (Table 8.6).

Table 8.6: Summary of weaning rates compared between groups.

	TEST	CONTROL	P
Total calves weaned from original group bred	72/135 (53.3%)	65/137 (47.4%)	0.34
A.I. calves weaned from original group bred	58/135 (43.0%)	47/137 (34.3%)	0.17

Table 8.7 summarises the interaction between gender and study group on mean weaning mass. In the TEST group, there was a significant difference between mean weaning mass of the male and female calves (216.6 kg vs. 189.4 kg $P < 0.01$), while in the CONTROL group this difference was not significant (205.6 kg vs. 196.1 kg, $P = 0.23$). Table 8.7 also shows that no significant difference could be demonstrated within either of the two genders between the TEST and CONTROL group ($P = 0.15$ and 0.38 for male and female calves respectively) and also not for the total group of calves weaned ($P = 0.32$).

Table 8.7: Effect of study group and gender on mean weaning mass of the calves.

	TEST	CONTROL	P
Mean weaning mass (kg) of male calves (n)	216.5 (46)	205.6 (36)	0.15
Mean weaning mass (kg) of female calves (n)	189.4 (25)	196.1 (29)	0.38
Mean weaning mass (kg) of all calves (n)	207.0 (71)	201.4 (65)	0.32
	$P < 0.01$	$P = 0.23$	

Effects of the PGF/6 protocol that could lead to long term benefits:***Body Condition Score at the start of the subsequent breeding season***

Ninety-one and 80 heifers from the original TEST and CONTROL groups respectively, remained in the herd until their second breeding season. Mean BCS at the onset of the subsequent breeding season tended to be higher in the TEST than in the CONTROL group (3.33 vs. 3.22, $P = 0.06$).

Days to first insemination in the subsequent breeding season, and calving to conception interval

The median days to first insemination in the subsequent breeding season was similar between the TEST and CONTROL groups (14 and 12.5 days respectively, $P = 0.73$). The median calving to conception interval in the TEST group was significantly longer than that in the CONTROL group (109 vs. 102 days, $P = 0.04$).

Pregnancy rate of the subsequent breeding season

Pregnancy rates in the subsequent breeding season were similar between groups, and are given in Table 8.8.

Table 8.8: Summary of pregnancy rates in the subsequent breeding season.

	TEST	CONTROL	P
Pregnancy rate to subsequent AI season	68/91 (75%)	62/80 (78%)	0.77
Final pregnancy rate to subsequent season	87/91 (96%)	76/80 (95%)	1.00

Other relevant findings

Association between days to calving and subsequent pregnancy rate

Univariable logistic regression demonstrated a negative association between days to calving and subsequent pregnancy of -0.03 for both pregnancy to AI and to AI plus bull season ($P < 0.01$, and $P = 0.02$ respectively). Figure 8.9 demonstrates the relationship between days to calving and pregnancy to the subsequent AI season. When the data is examined retrospectively, the median days to calving of those cows that became pregnant during the subsequent AI season was significantly lower than that of the cows that were not pregnant to AI (20 vs. 54, $P < 0.01$). For final pregnancy a similar difference was demonstrated between median days to calving (22 vs. 72.5, $P = 0.03$).

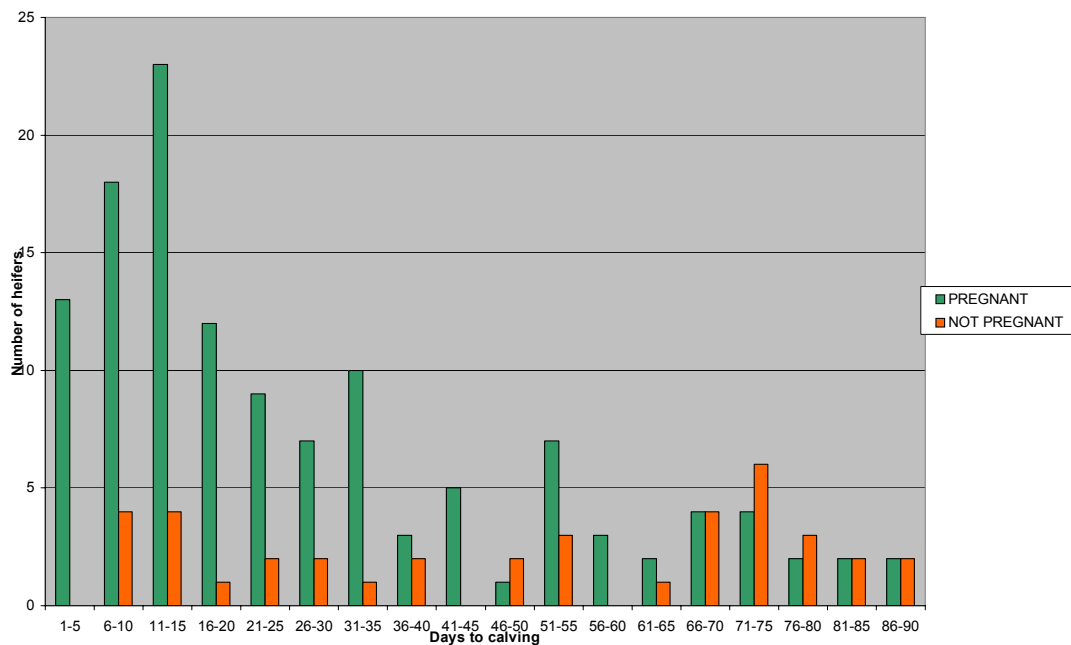


Figure 8.9: Distribution of days to calving for heifers that did and did not conceive in the subsequent 50 day AI season

Association between days to calving and calf weaning mass

There was a linear association between days to calving and calf weaning mass ($P < 0.01$) as is demonstrated in Figure 8.10.

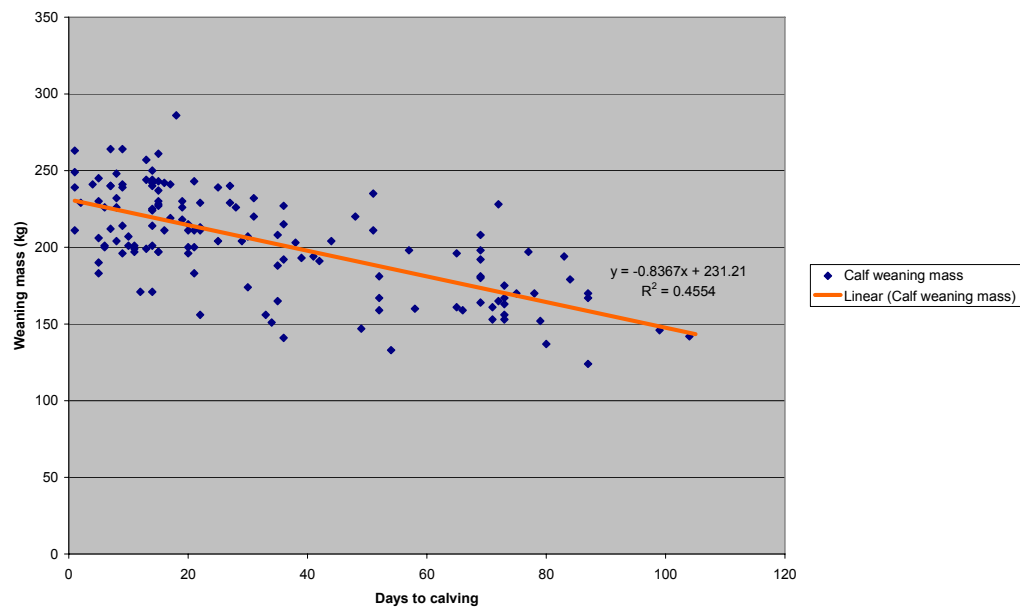


Figure 8.10: Association between days to calving and calf weaning mass

Temporal pattern of growth (ADG)

There was a positive association between date of birth of the heifers and their ADG at weaning, as can be seen in Figure 8.11 ($P < 0.01$). There was a tendency towards a negative association between days to calving and ADG in the calves born during this trial ($P = 0.10$) (Figure 8.12). Multiple regression of ADG on days to calving and calf gender as input variables shows that both variables are independently associated with ADG ($P < 0.01$).

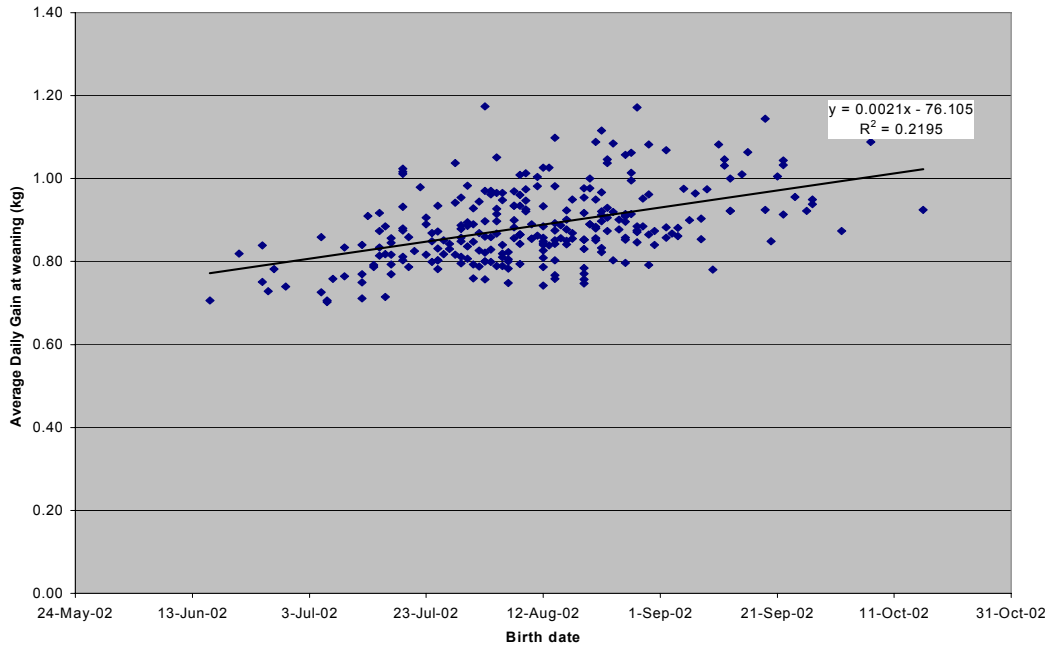


Figure 8.11: Association between birth date and ADG (at weaning) of the breeding heifers.

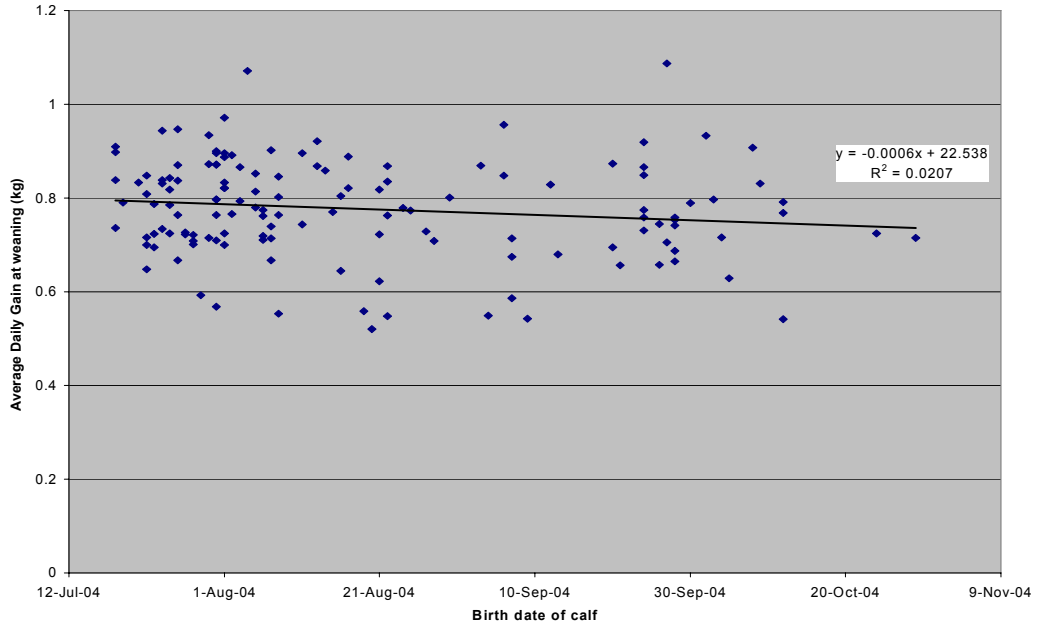


Figure 8.12: Association between birth date and ADG (at weaning) of the calves

Calf mortality

There was a high incidence of calf mortality during this study. In total, of the 171 calves that were born alive, 34 (20%) died in the period from birth to weaning. Cause of death was not recorded for each case, but the perception was that most deaths occurred as a result of diarrhoea. Mortality rates were similar for study group (21% and 19% for TEST and CONTROL groups respectively, $P = 0.84$) and also for gender (21% and 18% for male and female respectively, $P = 0.70$). Calves with birth mass below 26 kg had a significantly higher mortality rate than those with birth mass above 25 kg (38% or 10/26 vs. 17% or 24/145, $P = 0.02$). Calves that were born during the first 30 days of the calving season, had a mortality rate of 18% (18/99), which was significantly lower than the rate for calves born after day 30 of the calving season (34% or 29/85, $P = 0.02$). The effects of calf birth mass and days to calving on calf mortality are demonstrated through multiple logistic regression (Table 8.9), which shows that both variables are independently associated with the outcome.

Table 8.9: Effects of calf birth mass and days to calving on calf mortality (multiple logistic regression)

Variable	Coef	SE	95% CI		P
Calf birth mass	-0.096	0.040	-0.174	-0.018	0.02
Days to calving	0.015	0.007	0.001	0.029	0.03

Inter-oestrus periods

The inter-oestrus periods showed a roughly normal distribution within the range of 16 to 25 days (see Figure 8.13), but there was a high proportion of inter-oestrus periods that fell outside this range, especially short periods (<16 days). These short periods were mainly recorded at the beginning of the breeding season (Table 8.10). The rate of normal inter-oestrus periods is defined as the number of inter-oestrus periods that fell in the range 16-25 days, as a proportion of the total number of inter-oestrus periods recorded, and was 55%,

62% and 67% for first, second and third inter-oestrus periods respectively (Table 8.10). The total rate of normal inter-oestrus periods was 55% for both the TEST and CONTROL groups, and the mean inter-oestrus period was 19.0 for both the TEST and CONTROL groups ($P = 0.99$). Of the 22 inter-oestrus periods longer than 25 days, 10 fell in the range 34 – 48 days.

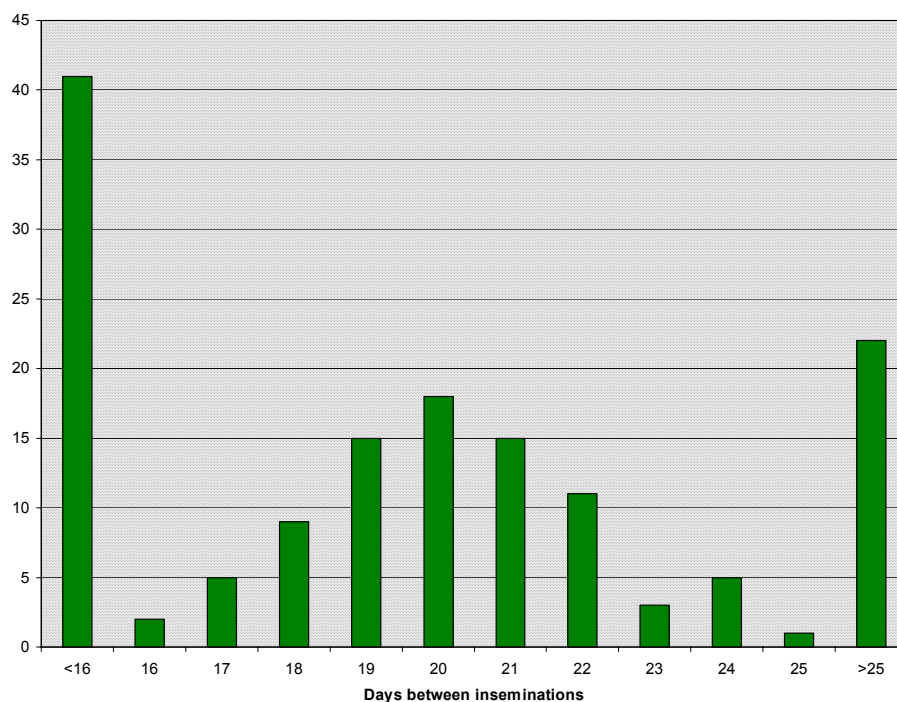


Figure 8.13: Graph illustrating the spread of inter-oestrus periods

Table 8.10: Summary of the inter-oestrus intervals recorded per first, second or third interval

Number of days	Inter-oestrus period			Total
	First	Second	Third	
<16	33	9	1	41
16-25	61	21	2	84
>25	18	5	0	22
Total	110	34	3	147

Cost effectiveness of the PGF/6 synchronisation protocol

Cost of PGF/6 protocol per animal

Variable costs in the treatment group consisted of the cost of treatment and the cost of additional inseminations performed. The cost of Lutalyse treatment was equal to the purchase price of Lutalyse, plus that of a needle and syringe, and the cost of labour. The cost of one dose of Lutalyse (5ml) including 14% VAT and a 60% mark-up fee (L) was calculated at R40.00, while the same cost of a disposable 5ml syringe and 18G 1.5" needle (NS) was R1.75. Labour cost was minimal, as the only addition to normal farming routine in the case of this experiment, was that all the heifers had to be moved through the crush on day 6, where only the heifers that were to be inseminated on that day, would normally have been moved through the crush. This procedure took 1 hour, and the additional cost of labour for 135 animals was estimated to be R800.00, including the cost of farm labour and the veterinarian's fee. The cost of labour (Labour) can therefore be estimated as $R800.00/135 = R5.93$ per heifer.

The Day 6 Not Inseminated Rate (D6NIR) is defined as the number of heifers that had not been inseminated by day 6 of the breeding season divided by the total population of heifers, and in the case of the TEST group the rate was 0.64.

We can now calculate the cost of Lutalyse treatment per heifer in the group with the following equation:

Cost of Lutalyse treatment per heifer	= (D6NIR x (L+NS))+Labour
In the current trial	= (0.64 x (R40.00+R1.75))+R5.93
	= (0.64 x R41.75) + R5.93
	= R32.65

The number of additional inseminations performed per heifer (AddAI), was defined as the difference between the total number of inseminations performed in the TEST and CONTROL groups, corrected for the size of the TEST group, and divided by the total number of heifers in the TEST group. In the CONTROL group, $196/137 = 1.43$ inseminations were performed per animal over the entire 50-day breeding season. In the TEST group, $209/135 = 1.55$ inseminations were performed per heifer in the original group. Therefore, 0.12 additional inseminations per heifer were performed in the TEST group. The cost of one insemination during the breeding season was estimated at R60.00, which included the cost of the semen straw, labour and all other disposable items.

We can now calculate the cost of additional inseminations performed per heifer in the group as follows:

$$\begin{aligned}
 \text{Cost of additional inseminations per heifer} &= \text{AddAI} \times (\text{cost of insemination}) \\
 \text{In the current trial} &= 0.12 \times \text{R}60.00 \\
 &= \text{R}7.20
 \end{aligned}$$

The total cost of the Lutalyse protocol was therefore R39.85 per heifer.

Benefits from Lutalyse treatment protocol per animal

The direct benefit of such a synchronisation protocol is the fact that a higher total mass of calves can be weaned. It is therefore a function of weaning rate (WR), mean weaning mass (WM) and price per kg live mass for a weaned calf (WP). The price for weaner calves at the time of weaning was R8.50/kg live mass (Van Schalkwyk, 2005). Thus the benefit can be calculated as follows:

$$\begin{aligned}
 \text{Direct benefit per heifer in the group} &= \text{WP} [(\text{WM}(\text{TEST}) \times \text{WR}(\text{TEST})) \\
 &\quad - (\text{WM}(\text{CONTROL}) \times \text{WR}(\text{CONTROL}))] \\
 \text{In the current trial} &= \text{R}8.50/\text{kg} [(207 \text{ kg} \times 0.53) \\
 &\quad - (201.4 \text{ kg} \times 0.47)] \\
 &= \text{R}127.93
 \end{aligned}$$

Discounting for time

The year-on-year CPIX for SA for the period October 2003 to April 2005 averaged 3.5% (Bloomberg Network, 2006). The SAPROR for the period of this study averaged 11.5% (Bloomberg Network, 2006), and the lending rate of commercial banks could typically be fixed at 2% above prime. Calculating a discount rate for benefit per heifer in the group, using the formula for present value (PV) (Noordhuizen, 2001), gives the following (time period was 532 days or 18 months):

$$\begin{aligned} \text{Present Value (return)} &= \text{FV} \div (1 + r/100)^n \\ &= \text{R}127.93 \div (1 + (13.5 - 3.5)/100)^{1.5} \\ &= \text{R}111.25 \end{aligned}$$

Net Present Value

The net present value can be calculated as follows:

$$\begin{aligned} \text{Net Present Value (return)} &= \text{PV}(\text{gross return}) - \text{PV}(\text{cost}) \\ &= \text{R}111.25 - \text{R}39.85 \\ &= \text{R}71.40 \end{aligned}$$

Benefit:cost ratio

The benefit:cost ratio can be calculated as follows:

$$\begin{aligned} \text{Benefit:cost Ratio} &= \text{PV}(\text{gross return}) \div \text{PV}(\text{cost}) \\ &= \text{R}111.25 \div \text{R}39.85 \\ &= 2.8 \end{aligned}$$

Break-even point

Given the performance during the synchronisation programme in the study, the break-even point for the weaner price can be calculated as follows:

$$\begin{aligned} \text{Break even point (weaner price/kg live mass)} &= \text{PV}(\text{cost}) \div [(\text{WM}(\text{TEST}) \times \text{WR}(\text{TEST})) \\ &\quad - (\text{WM}(\text{CONTROL}) \times \text{WR}(\text{CONTROL}))] \\ &= (\text{R}39.85 \times (1 + 10/100)^{1.5}) \div [(207 \text{ kg} \times 0.53) \\ &\quad - (201.4 \text{ kg} \times 0.47)] \\ &= \text{R}3.05 \end{aligned}$$

One can also calculate the break-even point for the increase in the average mass of the weaned calf, if we assume that there is no increase in weaning rate, as follows:

$$\begin{aligned}\text{Break even point (increase in weaning mass)} &= \text{PV}(\text{cost}) \div \text{WP} \\ &= (\text{R}39.85 \times (1+10/100)^{1.5}) \div \text{R}8.50 \\ &= 5.4 \text{ kg}\end{aligned}$$

Internal rate of return

The internal rate of return can be calculated as follows:

$$\begin{aligned}\text{Internal Rate of Return (IRR)} &= [\text{FV}(\text{net return}) \div \text{PV}(\text{cost})] \div n \\ &= ((127.93 - 39.85) \div 39.85) \div 1.5 \\ &= 2.21 \div 1.5 \\ &= 1.47 \text{ or } 147\%\end{aligned}$$

Associations of pre-breeding RTS, mass, BCS, age and Kleiber ratio with reproduction and production outcomes

Associations between RTS, and other pre-breeding indices

Using univariable linear regression, age, mass and BCS before the onset of the breeding season were associated with RTS, with R^2 values of 0.07, 0.02 and 0.02 ($P = 0.03$, $P < 0.01$ and $P < 0.01$ respectively). Table 8.11 is a summary of the multiple regression model for RTS, where the pre-breeding age, mass and BCS are given as independent variables. It shows pre-breeding age as being the only variable with significant independent association with RTS ($P < 0.01$).

Table 8.11: Effects of pre-breeding age, mass and BCS on RTS (multiple regression)

Variable	Coef	SE	95% CI		P
Age	0.013	0.003	0.006	0.019	<0.01
Mass	0.002	0.003	-0.003	0.007	0.40
BCS	0.219	0.195	-0.163	0.601	0.26

Associations with 25 day insemination rate and days to first AI

The 25 day insemination rate was defined as the proportion of heifers that received at least one insemination during the first 25 days of the AI season. Twenty-five day insemination rates were 75% (12/16), 86% (60/70), 94% (76/81), 99% (73/74) and 97% (29/30) for heifers with RTS 1,2,3,4 and 5 respectively. Insemination rate for heifers with RTS 1 and 2 was significantly lower than for those with RTS 3 to 5 (84% vs. 96%, $P < 0.01$).

Heifers with RTS 2 had a median days to first AI of 11 (90% CI 9-14), which was significantly higher than heifers in all other RTS categories including RTS of 1 ($P = 0.04$). Heifers with RTS 3 had a median days to first AI significantly lower than those with RTS 2, and significantly higher than those with RTS 5, but similar to those with RTS 4. There was no significant difference in median days to first AI between those heifers with RTS 4 and those

with RTS 5. Heifers with RTS 1 had a median days to first AI similar to those with RTS 3,4 and 5. Table 8.12 summarises these results.

Table 8.12: Median days to first AI by RTS category

Median Days to first AI (90% C.I.)	n	RTS	P-values for differences in median Days to first AI (Wilcoxon rank-sum test)			
6 (1-10)	12	1	1			
11 (9-14)	65	2	0.04	2		
8 (7-9)	76	3	0.37	0.03	3	
8 (6-9)	74	4	0.72	<0.01	0.35	4
6 (3-8)	30	5	0.62	<0.01	0.05	0.16

Table 8.13 gives a summary of the multiple regression report for days to first AI, where RTS is compared to mass, BCS and age before the breeding season as independent (input) variables.

Table 8.13: Effect of pre-breeding RTS, mass, BCS and age on days to first AI (multiple regression)

Variable	Coef	SE	95% CI		P
RTS	-1.051	0.444	-1.921	-0.182	0.02
Mass	0.027	0.019	-0.010	0.063	0.15
BCS	-4.428	1.404	-7.180	-1.675	<0.01
Age	-0.014	0.024	-0.061	0.034	0.57

Associations with Pregnancy Rates

Pregnancy rates for the 50 day AI season were 31% (5/16), 40% (28/70), 53% (43/81), 70% (52/74) and 80% (24/30) for heifers with RTS of 1,2,3,4 and 5 respectively. Pregnancy rate to the AI season did not differ between heifers with RTS of 1 to 3 ($P = 0.14$), but those with RTS of 4 or 5 had a higher pregnancy rate to AI than those with scores of 1 to 3 ($P = 0.03$ and $P = 0.02$ for RTS 4 and 5 respectively). There was no difference in pregnancy rate between heifers with a RTS of 4 and those with RTS 5 ($P = 0.34$). These results are summarised in Table 8.14.

Table 8.14: Pregnancy rate to the 50 day AI season by RTS category

Pregnancy rate to AI	n	RTS	P-values for differences in pregnancy rates (Fisher's exact)			
31%	16	1	1			
40%	70	2	0.58	2		
53%	81	3	0.17	0.14	3	
70%	74	4	<0.01	<0.01	0.03	4
80%	30	5	<0.01	<0.01	0.02	0.34

Pregnancy rates for the entire breeding season (including the period of bull breeding) were 56%, 76%, 81%, 92% and 93% for heifers with RTS of 1,2,3,4 and 5 respectively. Significance of these differences was similar to those differences seen in the pregnancy rates to the AI season, except that there was a significant difference in final pregnancy rate between heifers with RTS 1 and 3 ($P = 0.05$), and there was no significant difference in pregnancy rate between those with RTS of 3 and 5 ($P = 0.15$). These results are summarised in Table 8.15.

Table 8.15: Final pregnancy rate (including bull season) by RTS category

Pregnancy rate final	n	RTS	P-values for differences in pregnancy rates (Fisher's exact)			
56%	16	1	1			
76%	70	2	0.13	2		
81%	81	3	0.05	0.43	3	
92%	74	4	<0.01	0.01	0.07	4
93%	30	5	<0.01	0.05	0.15	1

When RTS is compared to mass, BCS and age before the breeding season with regards to their independent associations with pregnancy to the 50 day AI season (PD to AI) using multiple logistic regression, only RTS has an independent association that is statistically significant ($P < 0.01$). Table 8.16 is an extract from the multiple logistic regression model for pregnancy to the 50-day AI season.

Table 8.16: Effect of pre-breeding RTS, mass, BCS and age on pregnancy to AI (logistic regression)

Predictor	Coef	SE	95% CI		P
RTS	0.572	0.129	0.319	0.825	<0.01
Mass	0.008	0.005	-0.003	0.018	0.15
BCS	0.211	0.385	-0.545	0.966	0.58
Age	-0.002	0.006	-0.016	0.011	0.74

Similarly, when RTS is compared to mass, BCS and age before the breeding season with regards to their associations with final pregnancy (including the period of bull breeding) using multiple logistic regression, all variables apart from mass before the breeding season have positive associations with final pregnancy, but once again only RTS has a significant independent association ($P < 0.01$). These results are shown in Table 8.17.

Table 8.17: Effect of pre-breeding RTS, mass, BCS and age on final pregnancy (logistic regression)

Predictor	Coef	SE	95% CI		P
RTS	0.580	0.170	0.247	0.914	<0.01
Mass	0.006	0.007	-0.008	0.019	0.41
BCS	0.186	0.483	-0.761	1.133	0.70
Age	0.010	0.009	-0.006	0.027	0.23

Univariable logistic regression for pregnancy to the 50 day AI season showed an R^2 value of 0.33 for RTS ($P < 0.01$) and < 0.01 for Kleiber ratio ($P = 0.05$).

Associations with days to calving

Medians of days to calving were 53.5, 52, 28, 15 and 18 for heifers with RTS 1,2,3,4 and 5 respectively. There was a significant difference in median days to calving between heifers with RTS 2, and those with RTS 3,4 or 5 ($P = 0.02$), but no difference in median days to calving between those with RTS of 1 and 2 ($P = 0.46$). Similarly there was no significant difference between medians of days to calving for heifers with RTS of 3, 4 or 5 ($P = 0.23$). There was a near-significant difference in median days to calving between heifers with RTS 1, and those with RTS 3 ($P = 0.08$). These results are summarised in Table 8.18.

Table 8.18: Median days to calving by RTS category

Median Days to calving (90% C.I.)	n	RTS	P-values for differences in median Days to calving (Wilcoxon rank-sum test)			
53.5 (16-82)	8	1	1			
52 (31-59)	41	2	0.46	2		
28 (15-43)	49	3	0.08	0.02	3	
15 (14-21)	61	4	0.01	<0.01	0.28	4
18 (9-39)	23	5	0.02	<0.01	0.23	0.81

Table 8.19 gives a summary of the multiple regression report of days to calving, with RTS, mass, BCS and age before the breeding season as independent variables. RTS has the most significant (negative) independent association with days to calving indicating that an increase in RTS leads to an earlier calving date.

The 79 heifers that had a BCS of 3 or 3.5 before the breeding season had a median days to calving of 32, which was significantly higher than the median days to calving of 20, for the 103 heifers with pre-breeding BCS of 4 or 4.5 ($P < 0.01$).

Table 8.19: Effects of pre-breeding RTS, mass, BCS and age on days to calving (multiple regression)

Variable	Coef	SE	95% CI		P
RTS	-7.018	1.856	-10.680	-3.356	<0.01
Mass	0.156	0.082	-0.006	0.319	0.06
BCS	-11.794	6.102	-23.837	0.025	0.05
Age	-0.114	0.103	-0.318	0.010	0.27

Univariable linear regression showed that RTS has a significant association with days to calving ($R^2 = 0.08$, $P < 0.01$) and Kleiber ratio tended to correlate with days to calving ($R^2 = 0.02$, $P = 0.10$).

Associations with calf mortality

Associations between study group, calf gender, days to calving and calf birth mass on the one hand, and calf mortality on the other hand, have been discussed before (Table 8.9). Mortality rates of 50%, 29%, 35%, 13% and 26% were recorded for calves born from heifers with RTS 1,2,3,4 and 5 respectively. Calves born from heifers with RTS 1 to 3 had a significantly higher mortality rate than calves born from heifers with RTS 4 or 5 (34% or 33/98, vs. 16% or 14/85, $P = 0.01$).

A significant difference in mortality rate could be demonstrated between calves born to heifers that weighed more than 330 kg before the onset of breeding (31% or 15/49) and calves born to heifers that weighed up to 330 kg (16% or 19/122) ($P = 0.03$).

Associations with dystocia

There were a total of 7 dystocia cases out of 183 births during this trial. Dystocia was strongly associated with birth mass of the calf. All the dystocia cases were in calves that weighed more than 35 kg at birth (7/31) while there were no cases in calves weighing up to 35 kg at birth (0/152). Dystocia was not associated with RTS: amongst heifers with RTS 1 or 2, there was a dystocia incidence of 1/49 (2.0%), while amongst heifers with RTS 3 to 5 the incidence was 6/134 (4.5%) ($P = 0.68$).

Associations with birth mass of the calf

Birth mass of the calf had a temporal pattern. Days to birth was positively associated with birth mass of the calf. The calves with birth mass greater than 35 kg ($n = 31$) had a median days to birth of 52 days (95% CI 43 – 69) while the calves with birth mass up to 35 kg had a median days to birth of 20.5 days (95% CI 16 – 28, $P < 0.01$). Birth mass was also associated with mass of the heifer at the start of the breeding season (Table 8.20).

Table 8.20: Effects of pre-breeding RTS, mass, BCS and age on birth mass of the calves born (multiple regression)

Variable (before breeding)	Coef	SE	95% CI		P
RTS	-0.42	0.36	-1.14	0.30	0.25
Mass	0.05	0.02	0.03	0.09	<0.01
BCS	-1.74	1.20	-4.10	0.63	0.15
Age	-0.01	0.02	-0.05	0.03	0.58

Associations with calf weaning mass

Calves of heifers with RTS 1 and 2 ($n = 33$) had a mean weaning mass of 186.7 kg (90% CI 176.0 – 197.4 kg) while calves of heifers with RTS 3, 4 and 5 ($n = 102$) had a mean weaning mass of 210.1 kg (90% CI 203.8 – 216.4 kg) ($P < 0.01$). The only group of heifers with a single RTS that weaned calves with a mean weaning mass significantly different to the mean weaning mass of calves from other RTS category heifers, were those with RTS 2. The mean weaning mass of this group of calves was 185.8 kg, which was significantly lower than those with RTS 3, 4 and 5 ($P < 0.01$), but did not differ from those with RTS 1 ($P = 0.64$). Table 8.21 is a summary of the multiple regression report for weaning mass of the calves, where pre-breeding RTS, mass, BCS and age were given as independent input variables.

Table 8.21: Effects of pre-breeding RTS, mass, BCS and age on weaning mass of the calves (multiple regression)

Variable	Coef	SE	95% CI		P
RTS	6.296	2.728	0.898	11.693	0.02
Mass	-0.073	0.131	-0.331	0.185	0.58
BCS	5.528	8.637	-11.559	22.616	0.52
Age	0.250	0.146	-0.038	0.538	0.09

Table 8.22 is a similar multiple regression model as that in Table 8.21, but this time days to calving is included as an input variable. Table 8.22 shows that only age and days to calving have significant independent associations with calf weaning mass.

Table 8.22: Effects of pre-breeding RTS, mass, BCS and age, and days to calving on weaning mass of the calves (multiple regression)

Variable	Coef	SE	95% CI		P
RTS	-0.341	2.290	-4.880	4.198	0.88
Mass	0.123	0.105	-0.086	0.331	0.25
BCS	-0.456	6.633	-13.578	12.666	0.95
Age	0.449	0.074	0.304	0.595	<0.01
Days to calving	-0.796	0.092	-0.977	-0.612	<0.01

Univariable linear regression showed a significant association between RTS and calf weaning mass ($R^2 = 0.05$, $P < 0.01$), and also between Kleiber ratio before the onset of breeding and weaning mass of the calf ($R^2 = 0.03$, $P = 0.05$). Figure 8.14 below shows the association between RTS and calf weaning mass, as well as the linear trend line.

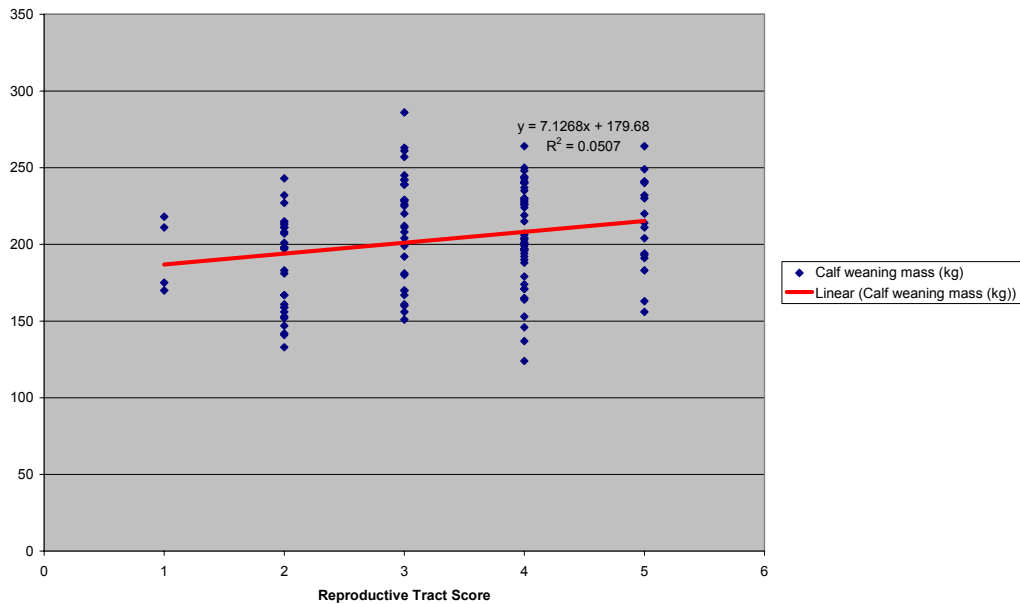


Figure 8.14: Association between RTS and calf weaning mass

Associations with pregnancy rates after the subsequent breeding

season

Of those heifers that were still on the farm for the subsequent breeding season, 63% (5/8), 61% (22/36), 72% (33/46), 85% (50/59) and 90% (19/21) with original RTS of 1,2,3,4 and 5 respectively, became pregnant during the subsequent 50 day AI season. Those with original RTS of 2 had a significantly lower pregnancy rate to the subsequent AI season than those with RTS of 4 and 5 ($P = 0.03$), but all other differences were not significant ($P = 0.11$), as shown in Table 8.23.

Table 8.23: Pregnancy rates to the 50 day AI season in the subsequent breeding season, by original RTS category

Pregnancy rate to AI	n	RTS	P-values for differences in pregnancy rates (Fisher's exact)			
63%	8	1	1			
61%	36	2	1	2		
72%	46	3	0.68	0.35	3	
85%	59	4	0.15	0.01	0.15	4
90%	21	5	0.11	0.03	0.12	0.72

Univariable logistic regression showed a significant association between RTS (before the first breeding season) and pregnancy outcome after the second AI season ($R^2 = 0.84$, $P < 0.01$), while hardly any variation in pregnancy rate after the second AI season could be explained by variation in Kleiber ratio (before the first breeding season) ($R^2 < 0.01$).

Receiver-operating characteristic (ROC) analyses of pregnancy

outcomes

ROC analysis for RTS, yielded an area under the curve (AUC) of 0.65 for its prediction of pregnancy outcome after the 50 day AI season (Figure 8.15A), and an AUC of 0.66 for its prediction of pregnancy outcome after the subsequent AI season. For both BCS and Kleiber ratio, ROC analysis of pregnancy outcome after the 50 day AI season yielded an AUC of 0.50. Combining BCS and Kleiber ratio with RTS in a model of pregnancy to the 50 day AI season yielded an AUC of 0.67 (Figure 8.15B).

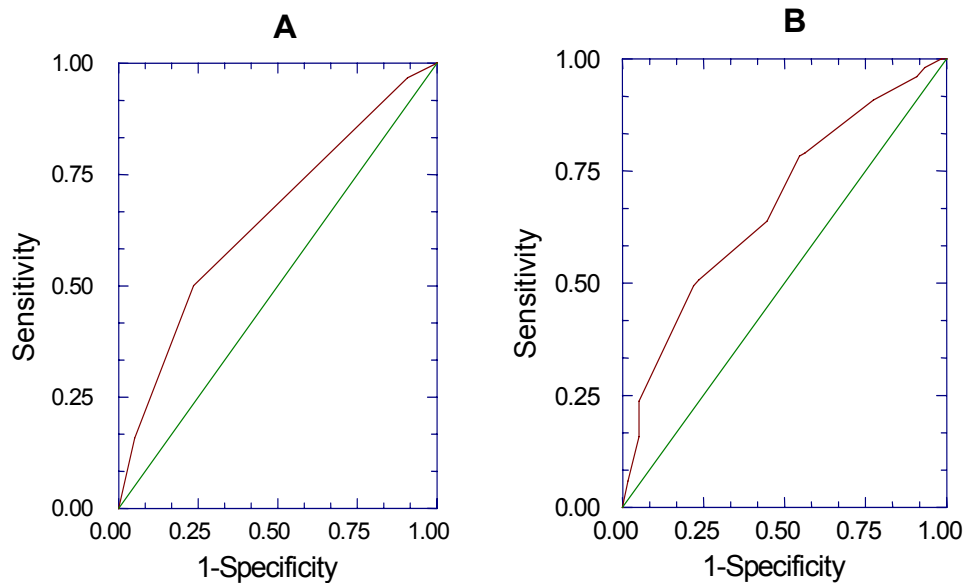


Figure 8.15: ROC curves for RTS (A) and RTS, BCS and Kleiber ratio (B) on pregnancy outcome after the 50 day AI season

The influence of synchronisation on the effects of RTS

The influence of the PGF/6 synchronisation protocol was examined for the effects of RTS on the pregnancy rate to the 50 day AI season and days to calving. Table 8.24 demonstrates that study group did not have any significant effect on pregnancy rate to the 50 day AI season within RTS categories.

Table 8.24: Pregnancy rate to the 50 day AI season by study group and RTS category

RTS	Pregnancy rate to AI		Pregnancy rate to AI		P
	n		n		
	CONTROL		TEST		
1	8	38%	8	25%	1.00
2	35	40%	35	40%	1.00
3	41	44%	40	63%	0.12
4	37	65%	37	76%	0.45
5	15	80%	15	80%	1.00

Table 8.25 indicates a significant difference in median days to calving between the TEST and CONTROL groups in this study for heifers with RTS 3 ($P = 0.03$), but not for any of the other RTS categories. For median days to calving within the CONTROL group there was a significant difference between heifers with RTS 3 and those with RTS 4 ($P=0.01$), but no difference between heifers with RTS 2 and those with RTS 3 ($P = 0.81$). For median days to calving within the TEST group though, there was a significant difference between heifers with RTS 2 and those with RTS 3 ($P = 0.05$), but no difference between heifers with RTS 3 and those with RTS 4 ($P = 0.34$).

Table 8.25: Median days to calving by study group and RTS category

RTS	Median Days to calving		Median Days to calving		P
	n		n		
	CONTROL		TEST		
1	2	105	6	79.5	0.26
2	19	69	22	61	0.56
3	22	69	27	34	0.03
4	28	27	33	15	0.11
5	11	18	12	39	0.57

9. DISCUSSION

Potential for bias in study group allocation

The way that the TEST and CONTROL groups were allocated, was based on evidence that RTS and mass were likely to have the most significant effects on outcomes of heifer performance (Brinks, 1994). Heifers were ranked within each farm of origin according to RTS and mass, and then randomly allocated to study group (see Experimental design). Because of the numerical values of mass and age before breeding, the researcher had to choose between these two variables for the secondary ranking. After several attempts at setting up two study groups with similar mean and median RTS and mass values, it turned out during later analysis that there was a difference in the age before breeding caused by this allocation (see Table 8.1). However, age, but not mass before breeding, was significantly associated with RTS (Table 8.11). One may therefore reason that if the effect of age on heifer performance was due to its effect on RTS, age would have been unlikely to have had any effect on study group due to the fact that RTS was similar for the study groups.

The only independent effect (not associated with the effects of the other variables: mass, BCS and RTS) of age on heifer performance that could be demonstrated in this study was its effect on calf weaning mass (Tables 8.21 and 8.22). It seems from Table 8.22 that this effect was a truly unique association, independent of pre-breeding age's association with days to calving (or any of the other input variables). This tends to contradict the above reasoning, suggesting that a difference in median age between groups could have enhanced the effect of synchronisation on calf weaning mass.

Sample size calculation

In retrospect, it became obvious that the likelihood of demonstrating a difference in pregnancy rate between the two study groups was in fact very small. In a normal population of cycling cows, an average of 1/21 or 4.8% should be inseminated every day for the first 21 days of the breeding season, and the proportion of inseminated cows that do not conceive with their first insemination are likely to return to oestrus 21 (+/-2) days later. Therefore, the number inseminated each day for the second 21 days of the breeding season should be a factor of the conception rate during the first 21 days ($1/21 \times (1 - \text{conception rate})$). This calculation can also be applied to the third and fourth 21-day oestrus cycles. The above calculations were used to set up a simulation model of the expected number of inseminations to be performed on every day. From previous farm records it seemed likely that a pregnancy rate between 60 and 70% could be expected after 50 days of AI in the study group, and that 90% of heifers could be expected to cycle during the 50 day AI season. Using the simulation model, it could then be extrapolated that the conception rate must be 40% if a 70% pregnancy rate amongst cycling heifers is to be achieved after a 50 day breeding season. The simulation model assumes a constant conception rate throughout the breeding season, inter-oestrus period of exactly 21 days and a negligible early embryonic death rate. If one assumes that the number of heifers inseminated on days 1,2,3 and 4 after PGF injection in the TEST group is expected to be 3/21, 7/21, 3/21 and 2/21 respectively, the expected cumulative pregnancy rate during a 50 day breeding season can be calculated using the same simulation model, and can then be compared with that of the unsynchronised CONTROL group. This theoretical model assumes that conception rate to natural, and to synchronised oestrus will be the same.

Figure 9.1 demonstrates the expected cumulative pregnancy rates calculated by this model for the PGF/6 protocol (TEST group), and for the unsynchronised CONTROL group. Figure 9.1 also plots the difference between the expected pregnancy rates at different stages of the breeding season.

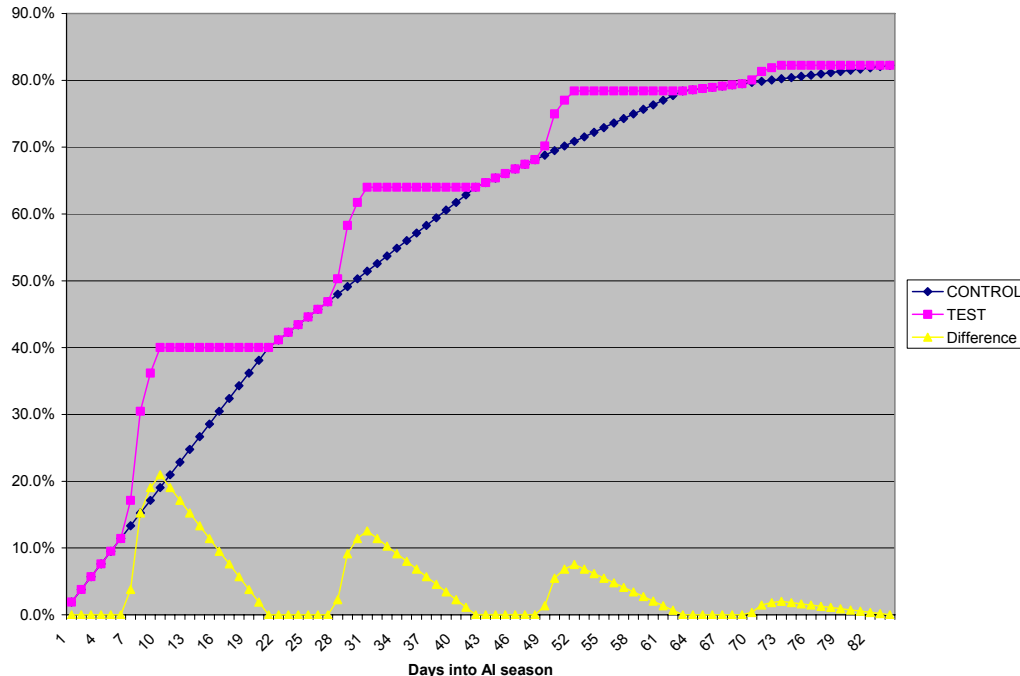


Figure 9.1: Simulated model of the cumulative pregnancy rates for TEST and CONTROL groups if the conception rate remains constant at 40% for every insemination.

It is clear that the most significant difference in cumulative pregnancy rate will occur at day 10 (21% difference), after which the difference in pregnancy rate is expected to decrease to zero again between days 21 and 27. It is then expected to rise again with a peak at day 31, but as the cumulative pregnancy rate increases over time, the peak in difference between pregnancy rates of the two groups is likely to decrease. It seems, according to this model, that a difference in pregnancy rate can be demonstrated at day 50 of the breeding season, but this difference is likely to be only 5.5%. To demonstrate a significant difference between the two groups, when $\alpha = 0.1$ with a power of 80%, it was calculated that at least 849 animals would have been needed per group. This was not practically feasible, and looking at the model in Figure 9.1, it becomes clear that being able to demonstrate a difference in pregnancy rate between the synchronised and unsynchronised groups is quite unlikely for a 50 day breeding season when the variation in oestrus cycle length and other biological factors are to be taken into consideration. It also becomes clear that the longer the

breeding season, the more diluted the effect of synchronisation on the pregnancy rate becomes, and the less likely to demonstrate a difference in pregnancy rate between the two study groups.

On the other hand, when the same model used for Figure 9.1 is used to simulate a dataset of 1,000 heifers per study group, it can be calculated that the median days to pregnancy for the two groups in a 50 day breeding season is 19 (mean = 21.1) and 10 (mean = 17.4) for the CONTROL (unsynchronised) and TEST (synchronised) groups respectively. Using these results from the simulated data, a minimum sample size could be calculated that would be required to demonstrate a difference in median days to pregnancy between the study groups. For $\alpha = 0.1$, and with a power of 80%, this sample size needed was 166 per group (i.e. 332 animals in total), which is less than that needed to demonstrate a difference in pregnancy rate, meaning that it will be more likely to demonstrate a significant difference in median days to pregnancy, than in pregnancy rate.

External factors that affected the results of this study

Synchronisation of unknown cause in the study group

When a normally asynchronous group of cycling heifers is considered, with a mean oestrus cycle length of 21 days, one would expect to have 28.6% (6/21) heifers having shown oestrus by the sixth day. In the group under investigation, this proportion was higher: 93 out of the 257 (36.2%) cycling heifers had shown their first oestrus by the end of the sixth day. This is significantly higher than the expected 28.6% ($P < 0.01$), and thus a significant synchronisation occurred. This phenomenon could possibly have been caused by the preceding rectal palpation when heifers were examined for RTS on day -1. Demenstova (1986) reported that uterine massage could induce the onset of cyclicity in cows, especially in combination with biostimulation. Biostimulation, or an increased level of nutrition could also have been responsible for this phenomenon. It can be followed in the results from days to first insemination (Figure 8.1), through repeat breeding (days to second insemination) (Figure 8.4) and days to pregnancy (Figure 8.5) up to days to calving (Figure 8.7). It seems to become more diluted towards days to calving, which can be explained by variation in gestation length, but which could also point to the possibility that this phenomenon was at least partially caused by inaccurate (non-specific) oestrus observation during the first 5 days of the breeding season. This synchronisation of unknown cause would have decreased the significance of any further attempts to synchronise the animals, by artificially decreasing days to calving in the CONTROL group. On the other hand, it would also have reduced the number of animals that needed PGF treatment, and thus the cost per animal in the TEST group.

Oestrus observation specificity and sensitivity

From Figure 8.13 it is clear that the median inter-oestrus period was in fact 20 and not 21 days, which is in agreement with Wright and Malmo (1992). Figure 8.13 and Table 8.10 indicate that specificity (accuracy) of oestrus observation was not optimal. The large proportion of inter-oestrus intervals that fell outside of the normal range, suggests false

positive observations. The fact that the *Estrus Alert* oestrus detection aid was used for the first time on the farm during this trial can be a possible explanation for this over-sensitive oestrus observation, seeing that the signal given by this aid can be over-interpreted by inexperienced users (Holm, unpublished data). It was also noted that the rate of normal inter-oestrus intervals increased from the first to the second inter-oestrus intervals, indicating that the specificity of oestrus detection improved during the trial, supporting the above hypothesis. Sensitivity (or efficiency) of oestrus detection on the other hand, seems to have been reasonably good, as only 10 out of 147 inter-oestrus intervals fell in the range 34 – 48 days, indicating that one oestrus could have been missed. It has to be kept in mind that short inter-oestrus intervals in the beginning of the breeding season, could also have been true positives, if a lot of heifers had only just reached puberty at the beginning of the breeding season, when short and irregular oestrus cycles are possible (Foster, 1994).

The phenomenon of poor oestrus detection accuracy could easily have affected results, by being a factor contributing to the synchronisation of unknown origin seen at the beginning of the oestrus cycle (see above), as well as reasonably low first AI conception rate and the fact that some heifers were inseminated again after conception (see Results).

Poor oestrus observation specificity would have caused a false increase in the day 6 not inseminated rate (D6NIR), and thus denied some heifers the opportunity to be synchronised. Apart from that, it would have decreased the difference in response to synchronisation between the TEST and CONTROL groups due to the fact that some heifers could have been falsely recorded as being in oestrus during the period day 7 to day 11. This would have had a more significant effect on the CONTROL group than the TEST group, as a higher proportion of heifers in the TEST group were truly in oestrus. Further, it would have led to a discrepancy between days to first AI and days to conception (as well as days to calving). These effects have to be kept in mind when interpreting the results of this study, and it is once again most likely that poor oestrus observation specificity would have diluted any differences in outcome between the groups rather than enhanced them.

Calf mortality

The high mortality rate of calves before weaning certainly indicates a severe problem that warrants investigation. Unfortunately, the researcher was not aware of the problem at the time, and no records were available that could have been analysed. It seems from the data available though, that there was a temporal pattern of calf mortality, and together with the observation that most calves that died were suffering from diarrhoea, this makes an infectious cause the most likely reason for this problem. Date of onset of disease was not recorded, but a temporal pattern is seen in the fact that there was a strong association between days to calving and mortality. This implies that calves born later during the calving season were more likely to die than those born early. It is hypothesised that some infectious agent caused diarrhoea and led to death of smaller calves. This is supported by the fact that calves with a lower birth mass were more likely to die (Table 8.9). It seems thus that whatever caused death had a predilection for small calves, and it is hypothesised that diarrhoea led to more severe dehydration and acute onset of metabolic acidosis in calves of lower body mass.

The association between RTS and calf mortality is probably not a unique association, but rather confounding that occurred due to the fact that calves of those heifers with high RTS were born early in the calving season. Interestingly enough, heifer mass before the onset of breeding had a positive association with calf mortality, which further supports the above hypothesis because heifer mass also had a positive association with days to calving (Table 8.19).

Although due to the temporal occurrence of disease it seems that calves were protected against mortality by a high RTS of their dams in this experiment, it has to be noted that the opposite could have been true had the risk factor that led to mortality occurred at a different (i.e. earlier) time after the onset of the calving season.

Study group was not significantly associated with calf mortality in this experiment, but this demonstrates the fact that synchronisation of oestrus in general leads to an increase in the risk of a high incidence of seasonally occurring disease.

The effects of the PGF/6 synchronisation protocol

Response to PGF treatment

Response to PGF treatment is defined as those heifers that showed oestrus within 5 days of PGF treatment. The proportion of heifers that responded to PGF treatment (71%) was low compared to other studies (Gaines, 1994). This poor response was not predictable by RTS, and almost 50% of those heifers that did not respond to treatment, showed their first oestrus at random stages between days 12 and 21. There was a slight concentration of first oestrus incidence between days 18 and 20 (5 heifers), possibly indicating that a better response to treatment could have been achieved if PGF was only administered on day 7 or later. Another possibility is that these heifers did not have susceptible CL's at the time of PGF treatment, and that the oestrus seen after day 12 was their first oestrus. This is a less likely possibility, as there was a random occurrence of first oestrus between days 12 and 21, and no heifers showing first oestrus after day 21 until day 38 in the TEST group.

Synchronisation achieved by the PGF/6 protocol and its effect on weaning mass

Despite the above, significant synchronisation was achieved in the period day 7 to day 11 of the breeding season in the TEST group, leading to a significant reduction in days to first insemination. This eventually led to a significant reduction in days to calving in the TEST group. Days to calving can theoretically be affected by days to pregnancy, gestation length and abortion rate (if seasonal) (see Figure 3.1). There was no difference in gestation length, and a significant difference in days to pregnancy and abortion rates could not be demonstrated, but if the higher number of abortions in the CONTROL group was from heifers that would have calved early in the season, this and the (albeit insignificant) difference in days to pregnancy could additively have led to the significant difference in days to calving.

In their study, Gaines et al (1993) extrapolate from the difference in days to pregnancy that there will be a difference in weaning mass. In the current experiment though,

it is seen that a significant difference in median days to calving does not necessarily lead to a significant difference in weaning mass, despite the strong association between days to calving and weaning mass seen in Figure 8.11. Several reasons for this exist, and one has to look at ADG and birth mass to explain this phenomenon. If there is a significant difference in days to calving, and ADG and birth mass is the same for all calves, it should naturally lead to a significant increase in weaning mass (Gaines et al, 1993). If however, ADG or birth mass is less for calves that were born earlier due to seasonal differences in the level of nutrition, heat stress or other factors, this will dilute the effect of earlier born calves. During this experiment, a significant association between birth date and ADG did not exist, but it seems to have occurred in the group of heifers, indicating that such a scenario, although not repeatable, is a possibility (see Figures 8.11 and 8.12). This statement is made with caution though, because the reason for the temporal pattern of ADG of the heifers before this study is not known, and it may well have occurred artificially due to heifers being selected for a minimum mass. This would have put extra selection pressure for growth (ADG) on those heifers that were born later.

Calf birth mass, however, showed a strong association with days to calving: calves born later were heavier at birth than early born calves (see later for reasons). This would have been a confounding factor that diluted the association between days to calving and weaning mass of the calf. Of course, if ADG and birth mass is higher for calves born earlier, this will on the other hand artificially enhance the effect of synchronisation on weaning mass. In this experiment the tendency for ADG to be higher for early born calves did most likely not compensate for the effect of days to calving on birth mass.

It is quite likely that in heifers these temporal patterns occur mainly due to the fact that the heifers are still growing, and ADG and birth mass of their calves increase as they get older, in which case it will be a repeatable finding for heifers, causing a reduced effect of synchronisation on weaning mass in these young animals.

Another factor that can affect ADG as well as calf birth mass is the proportion of male:female calves, as it is generally accepted that male calves have higher birth mass and

grow faster than female calves. In this experiment, more male calves were born early in the season in the TEST group ($P = 0.05$), but it was not sufficient to cause a temporal pattern in ADG or counteract the temporal pattern seen in birth mass. However, the effect this had on weaning mass can be seen in Table 8.7: there was a bigger difference in mean weaning mass of the male calves than of the female calves between the TEST and CONTROL groups, although not significant. Further, there was a significant difference between male and female calves within the TEST group ($P < 0.01$), but this was not the case within the CONTROL group ($P = 0.23$).

Previous researchers have ignored these potentially confounding factors affecting calf weaning mass, and assumed that birth mass and ADG will be the same for all calves, despite differences in time of birth (Gaines et al, 1993).

Although the whole article of Gottschall (1999) was not available to the researcher, it seems from the abstract that the conclusion made is not supported by the study design. It was concluded by Gottschall that the 12-day AI programme (with PGF treatment on day 7) was effective in concentrating the calving season, but there seems to have been too many potential confounders to come to that conclusion. The control group was not synchronised and natural mating was used, while the test group was synchronised and AI was used, making it impossible to distinguish between the effect of synchronisation and the effect of natural mating vs. AI.

Total mass of calf weaned per group

The total mass of calf weaned per group is a function of the weaning rate and the weaning mass. In this study, the mass of all weaners totaled 14,843 kg in the TEST group and 13,060 kg in the CONTROL group. It is important to keep in mind that these values cannot be compared statistically, and that these values were biased by the fact that more heifers in the CONTROL group died or were sold before calving. All heifers sold conceived to the bull-breeding season. This has to be kept in mind, as fewer heifers that would have calved late stayed on in the CONTROL group than in the TEST group. This is a confounding

factor that would have decreased days to calving, increased mean weaning mass and decreased weaning rate in the CONTROL group (relative to the TEST group).

Repeat breeding and fertility after PGF treatment

The tendency for improved first AI conception rate after PGF treatment seen in this experiment is in disagreement with Donovan et al (2002), but in agreement with the findings of Gaines et al (1993) and McIntosh et al (1984). It seems contradictory in this study, that the number of heifers that repeated twice (i.e. had 3 inseminations) tended to be higher in the TEST than the CONTROL group, but this simply occurred due to the fact that more heifers in the TEST group had a third opportunity to show oestrus and be inseminated during the 50 day AI season.

Several possible explanations exist for the phenomenon seen in this experiment that first AI conception rate was higher after synchronisation than after unsynchronised oestrus. Bias by the AI technicians could not be ruled out completely, as the sudden increase in the number of heifers presented for AI per day was quite obvious (Figure 8.3). It is also possible that the higher number of inseminations performed per day could have affected the skill of the technician. Oestrus observation can also be affected by synchronisation, because a higher number of heifers in oestrus at any time will lead to increased sexual activity such as mounting of herd mates, that will make the recognition of oestrus signs easier. The theory proposed by Wright and Malmo (1992), of more fertile ovulations following PGF treatment, cannot be excluded by this study though.

The hypothesis of a more fertile oestrus after PGF treatment can thus neither be accepted nor rejected by this study, and more specific research is required for this.

Effect of PGF treatment on abortion rate

The tendency for a reduced abortion rate in the TEST group of this study was an unexpected finding. Suspected abortions occurred only in foetuses conceived after day 46, which means that at the time of PD these foetuses were between 2 and 4 months of gestation, indicating that it was possible for these foetuses to have been resorbed after PD. Poor specificity of PD in early pregnancies could also be a possible explanation. Although the total abortions was not different between the groups, those that occurred in foetuses conceived after day 6 tended to be different. The only abortion of an AI foetus in the TEST group that was conceived after day 6, was a suspected abortion of a foetus conceived on day 47 (41 days after PGF treatment), while in the CONTROL group abortions occurred in foetuses conceived randomly over the 50 day AI season. No reference could be found supporting this phenomenon, and the data of this study were not sufficient to come to any conclusions.

Long-term benefits of synchronisation

In this study, a significant difference in days to calving could be demonstrated between the TEST and CONTROL groups. One would expect that heifers calving earlier in the calving season will have more time to recover from the stress of calving before the onset of the subsequent breeding season (Chenoweth and Sanderson, 2001), and that this may benefit them in their future production. This association between days to calving and pregnancy rate in the subsequent breeding season was well demonstrated in this study (Figure 8.9). A significant effect of synchronisation could be demonstrated on the BCS of first calf cows at the beginning of the subsequent season, supporting the above statement, but no significant effect could be demonstrated on days to first insemination or pregnancy rate of the subsequent season. This led to the fact that calving to conception interval in synchronised heifers was in fact longer than in unsynchronised CONTROL animals. This scenario will have to be kept in mind if calving to conception interval is to be used as a selection tool for cows or their offspring in future, as synchronised heifers may be unfairly discriminated against.

Cost effectiveness of synchronisation

Net present value

The net present value indicates the profit per animal unit in the initial group that was synchronised, corrected for time. The value was R71.40 per heifer. This value does not reflect the (relatively small) initial cost per heifer.

Break-even point

The break even point for the weaner price was R3.05. This means that in the current study, the weaner calf price had to be at least R3.05/kg live mass in order for the PGF/6 protocol to have been cost effective. The weaner price was more than twice this amount at the time, and would have been unlikely to drop to such a low level.

The break-even point for the increase in mean weaning mass (if we assume that there was no increase in weaning rate) was an increase of 5.4 kg. Although the actual increase in mean weaning mass in this trial was 5.6 kg, this difference was not a statistically significant finding (see Table 8.7), and therefore not necessarily repeatable. It is fair to assume that there could well have been no increase in weaning rate, as the actual increase in weaning rate in this trial was partly biased by the higher number of heifers in the CONTROL group that died or that were sold before calving down, making this break-even point a more reliable measure of cost effectiveness of this study. In their study, Gaines et al. (1993) demonstrated an increase in mean weaning mass of 3.8 kg, lower than the break-even point in this study.

Internal rate of return (IRR)

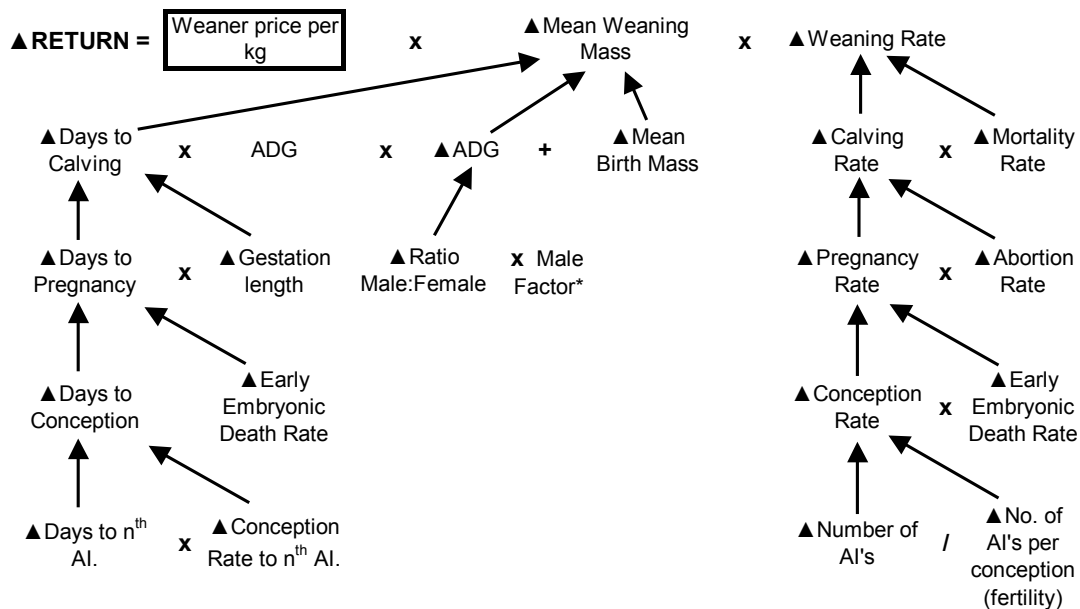
The internal rate of return calculated in this trial was 147%, which in simple terms implies that as long as interest rates were to remain below that level, the current trial would

have been cost effective. In the current favourable economic environment in South Africa such a high interest rate is extremely unlikely.

Benefit:cost ratio

In their experiment, Gaines et al. (1993) showed a return on investment of 1.92. This means that for every \$1 spent on synchronisation, \$1.92 was returned at the time of weaning. In their study they did not include interest calculations, and they made the assumption that the only factor affecting the total mass of calves weaned would be days to calving. They demonstrated an increase in average weaning mass of 3.8 kg, and from this increase only, calculated the return on investment of 1.92. In the current study, an increase in average weaning mass of 5.6 kg was shown, but this was not a statistically significant finding. However, cost benefit was determined on the given data, and a benefit:cost ratio of 2.8 was shown in the current study, which means that R2.80 was returned at the time of weaning for every R1 spent on synchronisation. For a simple scenario like this, benefit:cost ratio equals return on investment, and one can see that the current study demonstrated a better return on investment than the study by Gaines et al, due to the difference in gender proportion as well as the difference in the number of calves weaned between the TEST and CONTROL groups. This was seen despite the fact that the cost of treatment was relatively higher, and that interest was included in this study.

It becomes clear from analysing these data, that there are several factors that will affect the cost effectiveness of a synchronisation programme. On the one hand, cost will be determined by the cost of drugs and disposables, cost of labour and cost of financing (interest). On the other hand, the increased income will be determined by the effect of the synchronisation protocol on total mass of the calves weaned (via its effect on weaning rate and average weaning mass), and by the value of weaner calves at the time of weaning. The different factors affecting the change in return due to synchronisation in a weaner operation are summarised in Figure 9.2, and this diagram also shows the relationship between these factors. Because the effect of synchronisation on the weaning rate is less likely to be a repeatable finding, the leg on the right hand side of this flow diagram is less significant.



* Male Factor = ADG (male calves) / ADG (all calves)

\blacktriangle = "Change in", expressed as a rate (synchronised / unsynchronised), except for days to an event and gestation length where it is expressed as mean number of days (unsynchronised - synchronised), as well as mean weaning mass where it is expressed in kg difference (synchronised - unsynchronised).

Figure 9.2: Simplified schematic representation of pathways through which cost effectiveness of synchronisation can be affected in a weaner operation.

The effect of fertility (or age at puberty) on the cost effectiveness of synchronisation

Synchronisation with PGF requires a corpus luteum (CL) of certain age to be present. In order for a CL to be present, the heifers should have ovulated at least once before the PGF treatment. Heifers that are pre-pubertal will not respond to PGF, and because all heifers that have not shown oestrus by day 6 were treated in this protocol, it means that pre-pubertal heifers were also treated, with no effect. The higher the proportion of pre-pubertal heifers in the group, the lower the effect (and cost effectiveness) of synchronisation. Relatively low fertility of natural pasture raised South African beef heifers could be a factor affecting the decision of South African beef farmers not to use synchronisation.

Pre-selecting the heifers by RTS can help overcome this potential problem, by selecting a group that is more likely to respond to synchronisation, and improve the cost effectiveness. In that case, of course, the cost of RTS will have to be included in the calculation.

The ratio between cost of PGF treatment and the weaner price (break-even point for increase in weaning mass)

If we include the cost of labour and disposable items in the cost of PGF treatment, this (including interest), and the price for weaned calves (per kg) are the only two market factors that will determine the cost effectiveness of synchronisation. All the other factors are biological factors. Because one is on the cost side, and the other on the income (benefit) side, one can look at a ratio between the two to determine the likelihood of cost effectiveness. In the case of this study, the ratio was 5.4:1 ($(R40.00 \times 1.15)/R8.50$). In the case of Gaines et al (1993), the ratio was 2.3:1 ($\$4.00/\1.76), making it much more likely to be cost effective. It has to be noted, that Gaines et al (1993) did not discount the benefit for time (which means that they ignored the cost of initial capital outlay). Discounting for time in a country with low interest rates (such as the USA at the time) will have less effect than in SA, but should still be included.

This ratio is also the break-even point for the increase in weaning mass (see results), and has therefore practical implication. It can be applied to different synchronisation protocols using the total cost of synchronisation and the price of weaned calves as fixed costs to determine the break-even point of increase in weaning mass that has to be achieved, as well as to compare the likely cost effectiveness of different synchronisation protocols. This ratio will change with time as interest rate, cost of treatment and value of weaned calves change, and needs to be re-assessed before each breeding season. Although cost of treatment can be determined before synchronisation is carried out, the other two factors are variable. In the current South African market interest rates can be estimated for the future, and can be fixed for a certain time period. On the other hand, there is no future market for the beef industry in South Africa at this stage to fix the price of weaned calves. Due to fluctuations in the value of weaned calves, there is a need for such a market to predict the cost effectiveness of synchronisation before the onset of breeding.

Gaines et al (1993) showed a mean increase in weaning mass of 3.8 kg after 10 simulations using their model. Comparing this to the results of the current experiment, indicates that the PGF/6 protocol given the results obtained in their experiment would not be cost effective under current South African conditions, as the increase in WM (3.8 kg) was less than the break-even point in this experiment (5.4 kg). Three obvious factors leading to this discrepancy between cost effectiveness in the USA and that in SA, are relatively high cost of treatment, relatively low price for weaned calves and relatively high interest rates in South Africa. These factors could possibly influence the decision of South African farmers not to use synchronisation.

Effect of synchronisation on risk

Apart from all these variables, it is also clear that the environment (climate, nutrition and management) can play very significant roles in the cost effectiveness of synchronisation. The effect of season and year on ADG is an example of this (Figures 8.11 and 8.12), and is in agreement with Stevenson (1997), as is the finding of King (1983) where seasonality in age of puberty was demonstrated. Although it is possible that this temporal pattern of ADG (Figure 8.11) was artificially caused by the fact that heifers were selected by mass (and the younger heifers had to have higher ADG to be selected), such a phenomenon can significantly dilute the benefit of synchronisation. It can be speculated that nutrition, weather or any management influences could have led to this, but it is difficult to prove this in retrospect. During the season of study, this phenomenon did not occur again, indicating that it is not a repeatable phenomenon that could be included in future planning (unless the exact cause can be determined). During this study, there was also a significant association between calf birth date and calf mortality, where calves born early in the season were less likely to die than those born later (see earlier). This would have had a positive effect on the cost effectiveness of the synchronisation protocol under study, as more calves were born in the early part of the season in the TEST group, leading to lower mortality compared to the CONTROL group. Apart from direct losses due to mortality, indirect production losses due to disease (poor growth), could have a similar effect.

The potential effect of such temporal difference in growth (ADG), as well as the possible seasonal occurrence of mortality, could cause a synchronisation programme to be highly cost effective one year, but not at all during another year. In fact, although synchronisation can improve income significantly, it also causes an increased risk of severe production loss due to the potential for high incidence of seasonally occurring poor growth or mortality. If the bulk of calves are born during a time not favourable for growth or survival, due to synchronisation, it can lead to a lower total mass of calves weaned. This risk should ideally be calculated and included in the cost benefit analysis, to give a true reflection of the cost effectiveness of synchronisation. However, risk analysis is beyond the scope of this study, and it will require further work.

A summary of factors affecting the cost effectiveness of synchronisation

Several different factors that can influence the cost effectiveness of synchronisation have been discussed so far. These factors can broadly be divided into market factors (not controllable by farm management) and management factors (Figure 9.3). A certain margin of safety also needs to be included to buffer the increased risk of severe production loss due to a very concentrated calving season (environment factors), which can lead to severe losses if a certain disease or other disaster hits at a time that affects calves of a certain age more severely.

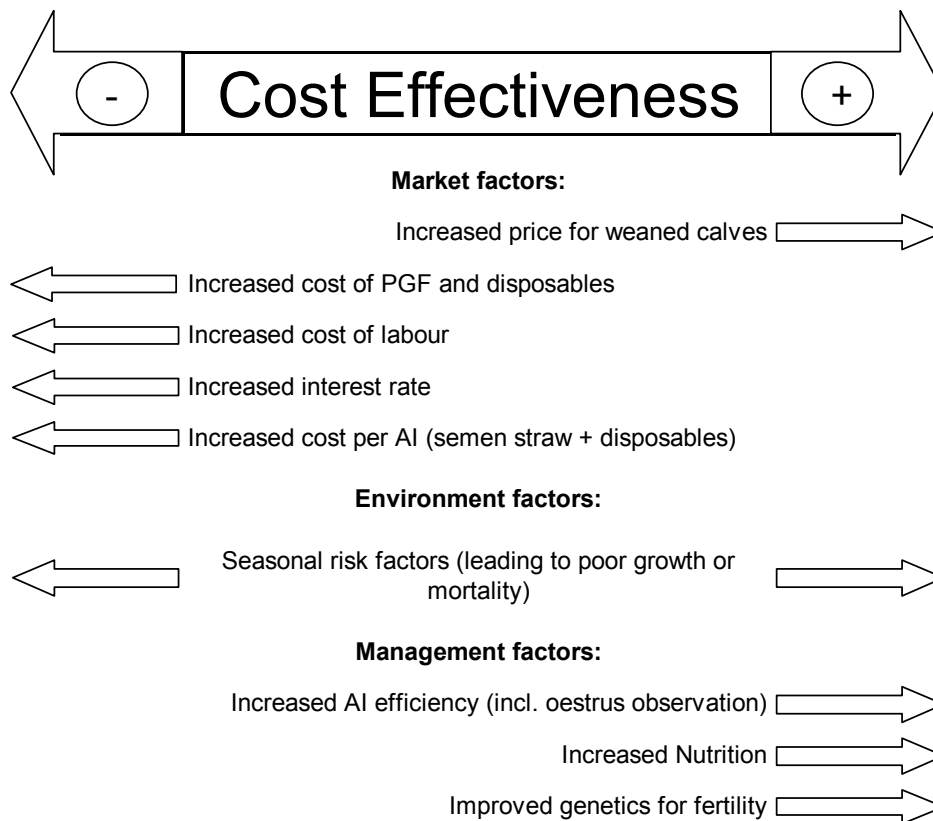


Figure 9.3: Factors influencing the cost effectiveness of an oestrus synchronisation programme

Pre-breeding RTS, mass, BCS, age and Kleiber ratio as predictors of heifer performance

RTS and BCS are ordinal variables, which strictly speaking should be included into multiple regression models as categorical predictors. In this study, both RTS and BCS were included as continuous numerical variables. Both of them are subjective scoring systems reflecting underlying continuous biological variation, therefore intermediate values can also make biological sense. Including them as numerical variables assumes that the difference in effect between categories is the same (i.e. linear). This is not always the case, however a monotonic increase or decrease in outcome is almost always seen for the effects of RTS and BCS on reproduction and production outcomes.

From the literature review one would expect that RTS, if it is a true indicator of pubertal status, would be associated mostly with mass, but possibly also with BCS and age (Stevenson, 1997 and Hall et al, 1997). In the case of this study however, RTS was strongest associated with age before breeding (Table 8.11). One of the possible reasons may be that the heifers used in this study were relatively young at the time of the onset of breeding (± 15 months), and that they were previously raised in a semi-intensive system, where nutrition was optimal, resulting in optimal growth of most of the heifers. This would mean that age became a more important factor determining the onset of puberty than mass. Another possibility could be that the variation in mass was relatively small due to pre-selection for mass, either through culling of lighter heifers, or through selection for growth over many generations. It seems from the results in Table 8.11, that RTS as an indicator of AP, was in the case of this study not determined by mass of the animal as was expected, but was independently associated with AP.

The researcher was inexperienced in the use of the RTS system, and found it difficult in some heifers to allocate a score, as all the different measurements taken were not always consistent with one score (see Table 3.1). The size of the ovaries as well as presence of any ovarian structures was interpreted as the most important measurements in such cases.

Some other measurements such as tone of the uterine horns are subjective, and interpretation thereof is difficult without the necessary experience. It may be that better results could be obtained if a system can be developed that takes only one set of objective measurements. Pelvimetry (Donovan et al, 2003) is an example of such a scoring system, but it was designed to predict dystocia, and not primarily as a way to select for age at puberty. Further research in this field is necessary to determine the most significant measurement or set of measurements in order to simplify and improve the current RTS system.

Most systems used to select beef heifers before the breeding season are tools to select for growth (or production), and most use mass of the animal as the main part of the selection calculation, whether mass is used on its own, or as part of an indicator for growth or feed efficiency such as Kleiber ratio (Nkrumah et al, 2004). Selection for fertility in beef females is mostly done on their historical data, or on data of their parents, such as inter-calving period, days to calving etc. Due to its relatively low heritability (compared to production traits), selection for fertility is often neglected in breeding programmes, although it is known that fertility has the most significant economical effect on the cow-calf enterprise (Chenoweth and Sanderson, 2001). From the literature review done in this study, it seems that RTS could be a valid way of selection for fertility before the onset of the first breeding season, thus apart from advancing the opportunity to select, also excluding confounding due to bull effects.

The purpose of this part of the study was to compare RTS to mass, and simultaneously to age and BCS before breeding as predictors of production and reproduction outcomes. It is important to keep in mind that mass will logically be associated with age and BCS, which may have diluted the significance of the association of these 3 traits with outcomes in Tables 8.13, 8.16, 8.17, 8.19, 8.20 and 8.21.

The difference in *P*-value for RTS between Tables 8.21 and 8.22, confirms that the effect that RTS has on calf weaning mass, is via its effect on days to calving: once days to calving is added to the multiple regression model, RTS does not have an independent association with calf weaning mass.

The economically most significant outcomes for the cow-calf producer are weaning rate and weaning mass (see Figure 9.1). Because of the severe calf mortality problem during this study, the researcher decided to look at pregnancy rate rather than weaning rate, seeing that calf mortality had a temporal pattern that could bias the effect of the pre-breeding predictors. Table 9.1 is a summary of the *P*-values shown in Tables 8.13, 8.17, 8.18, 8.20 and 8.23 for the association between pre-breeding measurements (RTS, mass, BCS and age) and reproduction and production outcomes. RTS and BCS, which are arguably the more subjective measurements compared to mass and age, showed independent association with production outcomes in more instances than the objective measurements. The fact that RTS is a subjective measure does not negatively affect the strength of association of RTS with the outcomes. RTS showed a significant independent association with all the outcomes (Table 9.1) while BCS showed significant independent association with two of the outcomes. Age tended to have an independent association with weaning mass, but this association was not significant, while pre-breeding mass had a near-significant independent association with days to calving only. Table 9.1 demonstrates that RTS had a unique association (independent of mass, BCS and age) with production as well as reproduction outcomes.

Table 9.1: Summary of P-values for association between pre-breeding predictors of heifer performance, and reproduction and production outcomes using multiple regression

Reproduction and production outcomes	Multiple regression <i>P</i> -values for predictors of heifer performance			
	RTS	Mass	BCS	Age
Days to first AI	0.02	0.15	<0.01	0.57
Pregnancy rate to 50 day AI season	<0.01	0.15	0.58	0.74
Final pregnancy rate	<0.01	0.41	0.70	0.23
Days to calving	<0.01	0.06	0.05	0.27
Weaning mass	0.02	0.58	0.52	0.09

Comparing RTS with Kleiber ratio as predictors of heifer performance

Kleiber ratio is calculated as growth (ADG) per metabolic mass ($\text{mass}^{0.75}$), and because ADG is a factor of mass, age and birth mass, Kleiber ratio is therefore also a factor of mass, age and birth mass. The variation in birth mass is relatively small, and Kleiber ratio is mainly a function of mass and age. For this reason, Kleiber ratio could not be included in multiple regression models where mass and age before the breeding season were used as input variables, because it would be too highly correlated with them. To compare the suitability of RTS and Kleiber ratio in predicting production and reproduction outcomes, univariable regression was used. Simple logistic regression was used for pregnancy outcomes, and linear regression for numerical outcomes. The R^2 – value in linear regression is an indication of the amount of variation in the outcome that can be attributed to the input variable (or whole model for multiple regression) (Noordhuizen, 2001). In logistic regression, the same interpretation cannot be used, but the pseudo- R^2 -value can still be used to compare the fit of different models. Table 9.2 is a summary of the results of univariable regression models for PD to AI, days to calving, calf weaning mass and subsequent PD to AI, with RTS or Kleiber ratio as input variable. The results in Table 9.2 show that RTS explains more of the variation in fertility outcomes such as PD and days to calving, and may thus be a better predictor for these than Kleiber ratio, while being comparable to Kleiber ratio in predicting production outcome (calf weaning mass).

Table 9.2: A summary of the R^2 - and P – values from univariable regression models for various outcomes, with RTS or Kleiber ratio as the input variable

Variable	PD to AI		Days to calving		Calf weaning mass		Subsequent PD to AI	
	R^2	P	R^2	P	R^2	P	R^2	P
RTS	0.33	<0.01	0.08	<0.01	0.05	<0.01	0.84	<0.01
Kleiber ratio	< 0.01	0.05	0.02	0.10	0.03	0.05	< 0.01	<0.01

If RTS had been used as a selection criterion in this group of heifers before breeding, and RTS 2 was used as the cut-off point (from Table 8.14), selection of the best 94% of heifers would have increased the pregnancy rate to the 50 day AI season from 56% to 58% ($P = 0.79$). Using RTS 3 as the cut-off point (selecting the best 68% of heifers) would have resulted in an increase in pregnancy rate to the 50 day AI season from 56% to 64% ($P = 0.10$). Although impractical because of the proportion of heifers that would have needed to be culled, using RTS 4 as cut-off would have resulted in an increase in pregnancy rate from 56% to 73% ($P < 0.01$). It seems that in this group of heifers it would have been most sensible to use RTS 3 as cut-off for selection. Of course, this will not always be the case, and it depends on the timing of RTS and the level of puberty reached by the group of heifers. If the best 68% of heifers in this group were selected using Kleiber ratio, it would not have increased pregnancy rate to the 50 day AI season (56% vs. 57%, $P = 0.96$). The superiority of RTS as a selection tool for fertility outcome is well demonstrated by this.

RTS cannot be compared to BCS in the same way as above, because the cut-off points for RTS and BCS are different. Receiver-operating characteristic (ROC) analysis is a useful tool to compare the predictive value of RTS and other measures on pregnancy outcome, although the idea of RTS is not simply to predict pregnancy outcome, but rather as a selection tool for fertility. RTS has a higher area under the curve (AUC) value using ROC analysis (0.65) than BCS or Kleiber ratio, and the latter two do not add any predictability to RTS when used in a combined model (Figure 8.15). In fact, both BCS and Kleiber ratio have AUC values of 0.5, indicating that they are not useful for predicting pregnancy outcome.

Due to its ease of measurement, good heritability and association with feed conversion ratio and therefore energetic efficiency (Nkrumah, 2004), Kleiber ratio has been used on many beef farms for many years as one of the (or the only) selection tools for replacement heifers. This evidence suggests that RTS is a valid way of selecting for heifer fertility before the heifers are bred for the first time, rather than to use performance indices after the breeding or calving season as culling measures. Evidence from this study suggests that selecting for RTS will not select against production measures such as Kleiber ratio, although the analyses are not sufficient to prove that for certain. It must be noted that RTS,

although it has good association with production outcomes (such as calf weaning mass), is primarily an indicator of age at puberty and therefore fertility, and should not be used as the only selection measure for heifers.

Dystocia and calf birth mass

The temporal pattern in calf birth mass has three possible explanations. Firstly, the heifers used in this study were still growing at the time of their first calving season, and for this reason heifers calving later in the season would probably have been older and heavier, and would therefore have produced heavier calves (Holland and Odde, 1992) than their herd mates that calved early in the season. Secondly, a true seasonal effect could have occurred due to changes in environmental temperatures and nutrition (Holland and Odde, 1992) and lastly the fact that different bulls were used in the bull breeding season to those used for AI, could have led to a bull effect on calf birth mass (Holland and Odde, 1992).

The findings of the strong association between calf birth mass and dystocia in this study are in agreement with findings by Andersen et al (1993). Because of this strong association it makes sense to investigate associations with calf birth mass as a risk factor for dystocia. According to Table 8.20 heifer mass before the onset of the breeding season is significantly associated with calf birth mass, and could possibly be an indirect risk factor for dystocia.

The effect of synchronisation on the outcome of RTS

From the results in Tables 8.24 and 8.25 it is clear that the most significant difference in pregnancy rates and days to calving between the TEST and CONTROL groups occurred amongst heifers with RTS 3. In the CONTROL group, the pregnancy rate results as well as the median days to calving results of heifers with RTS 3 compared with those with RTS 1 and 2 (i.e. part of the pre-puberal group), while in the TEST group it compared with results of heifers with RTS 4 and 5 (i.e. part of the post-puberal group).

Median days to calving was 21 days for the TEST group, and 29 days for the CONTROL group ($P = 0.06$), however with all the heifers with RTS 3 removed median days to calving was 28 and 25 for the TEST and CONTROL groups respectively ($P = 0.84$). One can reason from this, that it seems likely that the PGF/6 protocol had a “protective” effect against poor performance in the group of heifers with RTS 3. This group of heifers are those that were at the brink of reaching puberty (Anderson, 1991). Logically, the more mature a heifer’s reproductive tract, the more favourably she should respond to PGF treatment, because the more likely it is that she has a CL susceptible to prostaglandin. The finding above was therefore quite surprising, and it will be interesting to see if it is repeatable. One could hypothesise that prostaglandin (under normal circumstances produced by the barren uterus) plays a role in the termination of the negative feedback mechanism of oestradiol on the hypothalamus-pituitary gonadotropin axis that occurs prior to puberty.

Some caution has to be taken when making this assumption, as it is possible that some heifers with RTS 3 had in fact reached puberty, but were at a stage of their cycle when structures were not palpable on the ovaries. This typically occurs for the first 2 days after ovulation, which would mean that those heifers would have had CL susceptible to PGF by day 6 (keeping in mind that RTS was performed on day -1). It is however unlikely that such a large proportion of heifers with RTS 3 would have been at that stage of the oestrus cycle on the same day, and the fact that all other effects of RTS have shown reasonably constant monotonic changes from one RTS category to the next (Tables 8.14, 8.15, 8.18 and 8.23).

The effect of induction of puberty in heifers by exogenous progestagens has been reported and studied before (Hall et al, 1997), but to our knowledge this effect has not been described for prostaglandins. Further research is required in this field.

Long-term benefits of using RTS as selection tool

Selecting for RTS leads to a reduction in days to calving (Table 8.18), which allows the heifers more time to recover from the stress of calving and to be prepared for the next breeding season. First calf cows are known to be the age group of under most pressure to reconceive in the subsequent breeding season, due to the fact that they are still growing and also nursing a calf, which puts tremendous pressure on their energy and protein metabolism, to the disadvantage of fertility (Chenoweth and Sanderson, 2001). RTS has been shown in this study to not only have effect on the immediate calving season, but also on the subsequent calving season. The proportion of heifers with RTS 4 and 5 that remained in the herd until their second breeding season was 80/104 (77%), while that proportion for heifers with RTS 1 to 3 was 90/167 (54%), demonstrating a significantly increased survival of heifers with higher RTS ($P < 0.01$).

Apart from this, amongst the heifers that were retained until their second breeding season, there was a strong association between RTS (before first breeding season) and pregnancy outcome of the second breeding season (Table 8.23), most likely due to the effect of RTS on days to calving. The effect of days to calving on pregnancy rate of the subsequent breeding season is well known (Chenoweth and Sanderson, 2001), and was also demonstrated in this study (Figure 8.9). Using the data presented in Table 8.23, it can be calculated that using RTS 3 as cut-off for selection would have tended to increase the pregnancy rate of the subsequent breeding season from 76% to 81% ($P = 0.37$). Further, if Kleiber ratio had been used to select the best 68% of heifers, it would have tended to decrease the pregnancy rate to the subsequent breeding season from 76% to 68% ($P = 0.19$).

It can be seen here that one has to take account not only of the direct benefit of using RTS as selection tool for heifers, but also the effect that selection using RTS will have on life production of the cows.

10. CONCLUSIONS

The effects of the PGF/6 synchronisation protocol

It is concluded that a PGF/6 protocol can lead to an increase in the total mass of calves weaned from a limited calving season, most likely by decreasing the days to calving, but also by increasing the number of calves born, and increasing the ratio of male to female calves born. Seasonal patterns in growth and mortality rates (caused by disease or climate) can also contribute to an increase in the total mass of calves weaned if the concentrated calving season is synchronised with the “safe” season, but can similarly have a negative effect if synchronised with the “unsafe” season. This represents an increased risk of a high incidence of production loss or mortality. Other possible benefits from the PGF/6 programme that require verification and further study include reduced abortion rate and induction of puberty.

Cost effectiveness of synchronisation

It is concluded that a practical way to predict the cost effectiveness of an oestrus synchronisation protocol is to determine the ratio between the future value of the total cost of the programme and the future price of weaner calves per kg live mass. This ratio represents the minimum increase in mean weaning mass that has to be achieved for the programme to be cost effective if no increase in weaning rate is achieved. Further research is required to determine the likely increase in mean weaning mass achievable by different synchronisation protocols. One also needs to consider the increased risk of severe production loss due to a concentrated calving season. Further research is required to quantify this risk.

Reproductive tract scoring as a predictor of heifer performance

It is concluded that RTS is a unique predictor of heifer performance, despite being correlated with age, mass and BCS before the onset of the breeding season. It is a better predictor of fertility, compares well with other traits in predicting production outcomes, and is likely to be a predictor of life production of the cow. Further research is required to determine which measurements taken during the scoring process are most strongly associated with heifer performance, and also to determine their heritability and associations with other traits.

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