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List of abbreviations

AFC	Antral follicle count
AI	Artificial insemination
AMH	Anti-Mullerian hormone
ANOVA	Analysis of variance
AP	Age at puberty
APA	Age adjusted pelvis area
AUC	Area under the curve
BCS	Body condition score
BW	Body weight
BWPA	Body weight adjusted pelvis area
BWT	Birth weight
CEI	Cow efficiency index
CI	Confidence interval
CIDR	Controlled internal drug release
CL	Corpus luteum
cm	centimetre
d	days
EBV	Estimated breeding value
EPD	Estimated progeny difference
FSH	Follicle stimulating hormone

GL	Gestational length
IQR	Inter quartile range
kg	kilogram
KR	Kleiber ratio
LBW	Lean body weight
LBWPA	Lean body weight adjusted pelvis area
LH	Luteinising hormone
mm	millimetre
MSD	Mating start date
OR	Odds ratio
PA	Pelvis area
PA:BW	Pelvis area to body weight ratio
PD	Pregnancy diagnosis
PGF	Prostaglandin $F2_{\alpha}$
R	Rand (South African currency)
ROC	Receiver operating characteristic
RTS	Reproductive tract score
SD	Standard deviation
Se	Sensitivity
Sp	Specificity
SPA	Standardised pelvis area

SUD	Standardised uterus diameter
TD	Transverse diameter of the pelvic canal
UD	Uterus diameter
US	Ultrasound
USA	United States of America
UT	Utah
VD	Vertical diameter of the pelvic canal
β	beta

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Introduction

This thesis presents the results of a study in a herd of beef cattle, including pre-breeding examinations of heifers and analysis of relevant records of the same animals, over a total period of 8 years. The broad objective of this study was to determine whether pre-breeding examinations of heifers can predict reproductive performance of the animals during their first, but also during subsequent breeding seasons in a seasonal breeding system. This knowledge could then be applied in the selection of animals that are likely to become efficient cows within a seasonal breeding system, prior to wasteful investments being made in keeping animals that are likely to fail to reproduce.

1.2. Fertility in female cattle

Fertility can be defined as the ability to reproduce. Although complete infertility is rare amongst cattle, relatively better or worse abilities to reproduce lead to significant effects on the effectivity of cattle production systems. Measures of fertility include the ability to reach puberty at a relatively young age, the ability to become pregnant during a restricted breeding period, the ability to calve without assistance and the ability to wean a calf (Wiltbank, 1994) (Figure 1.1). Although reproductive traits are ten times more economically important than production traits in beef cows, they have lower heritability (Wiltbank, 1994), causing many farmers to focus their selection goals on production traits, where faster genetic gain can be made (personal observation).

1.2.1. Antral follicle count

The bovine oestrous cycle is characterised by two, three or four follicular waves occurring during the oestrous cycle of 18 to 24 days (Youngquist, 2007). Recruitment of a new cohort of antral follicles therefore occurs every 6 to 10 days due to a rise in follicle stimulating hormone (FSH) (Youngquist, 2007). Although one, or sometimes more than one follicle is selected for dominance and subsequently inhibits the rest of the cohort of recruited follicles (Youngquist, 2007), the number of antral follicles within each wave (antral follicle count or AFC) is fairly constant per cow, and appears to be a function of follicular reserve (Cushman et al., 2009, Ireland et al., 2011). Follicular reserve in many species of mammals differs significantly between individuals, and decreases from birth until it reaches a level where normal ovarian function can no longer be maintained by the small number of follicles left (Ireland et al., 2011). Heifer calves are born with between 10,000 and 350,000 healthy follicles and oocytes (Erickson, 1966), which rapidly decreases to 2,000 to 40,000 by one year of age (Ireland et al., 2008). Stage of the oestrous cycle does not significantly affect AFC, and the AFC on the left and right ovaries are highly correlated, making it a useful estimate of follicular reserve at random stages of the oestrous cycle, even when only one ovary is examined (Cushman et al., 2009, Ireland et al., 2011). Cushman et al. (2009) further demonstrated a significant correlation between the AFC and the ultrasonographic length of the ovaries as well as the birth weight (BWT) of 406 crossbred beef heifers, but no association with reproductive tract score (RTS) or age of the heifers. Antral follicle count is also significantly associated with pregnancy outcome (Cushman et al., 2009), response to superovulation, oocyte quality and levels of anti-Mullerian Hormone (AMH) (Ireland et al., 2011). In cows, AFC is initially affected quadratically by age, increasing from birth to an age of 4 to 6 years in beef cattle, after which it slowly decreases again (Cushman et al., 2007).

1.2.2. Genotype x environment interaction

Beffa et al. (2009) demonstrated an unfavourable genetic correlation between fertility and growth rate, which means that current trends to improve growth traits of beef cattle have the potential to lead to the selection of reproductively inferior animals if reproduction performance is ignored or misinterpreted. Many different factors can affect fertility, the most important being environmental factors caused by management systems (Bourdon, 2000). Beffa et al. (2009) showed that cattle that are selected within a particular stable environment (management system) over a number of generations adapt to that environment and may perform differently in another environment. In a 40-year study of Afrikaner cattle in a nutritionally supplemented vs. a nutritionally restricted environment, it was shown that animals that were adapted to the supplemented environment (Genotype 1 in Figure 1.1) had a significantly lower calving rate when moved to a restricted environment, compared to animals that were adapted to the restricted environment (Genotype 2 in Figure 1.1), while the latter group did not perform differently when transferred to the supplemented environment (Beffa et al., 2009). In order to achieve relevant selection for a particular environment, it is therefore essential to maintain a management system with the minimum possible variation from year to year (Beffa et al., 2009). Apart from adequate selection for adaptability to an environment, genetic programming, especially during gestation, results in epigenetic effects that can determine the ability of an animal to perform optimally in an environment (Ireland et al., 2011).

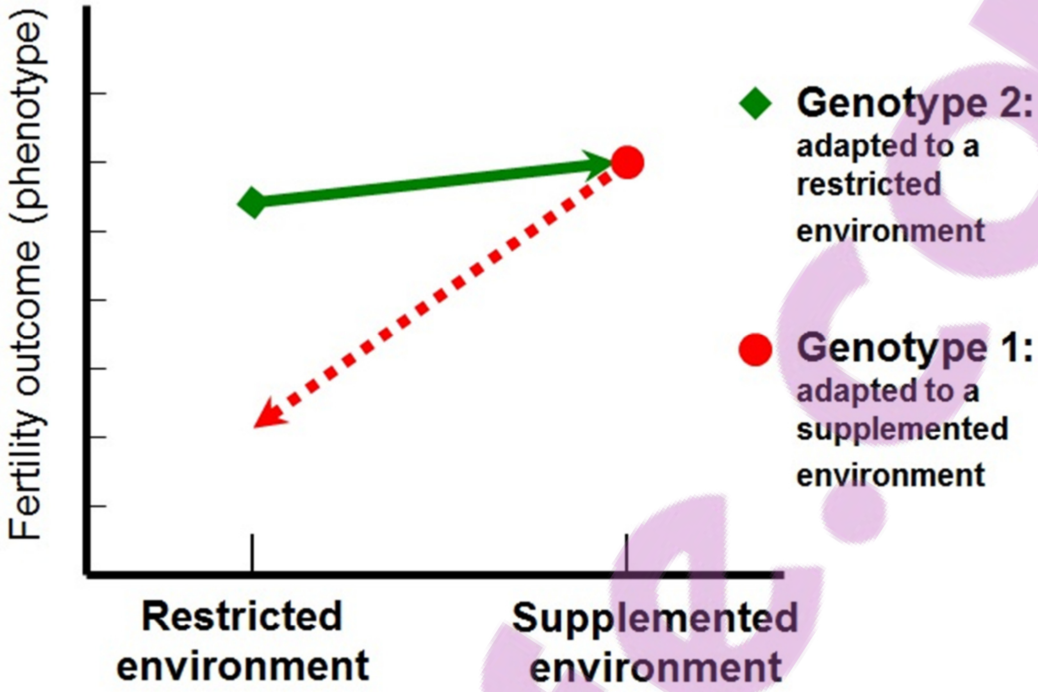

Figure 1.1: Genotype x environment interaction demonstrating the findings of Beffa et al. (2009) (adapted from Bourdon, 2000).

1.2.3. The onset of puberty in cattle

The onset of puberty in heifers is initiated by a decrease in oestradiol receptors in the hypothalamus and pituitary, ending the prepubertal negative feed-back and resulting in the first luteinising hormone (LH) surge and ovulation (Day et al., 1984, 1987). This shift occurs at a specific critical body weight (as a proportion of adult body weight) and age ranging from about 8 to 30 months (Pence et al., 2007). Various factors affect the age at puberty in individuals, and RTS provides an indirect measure of pubertal development (Andersen et al., 1991, Pence and BreDahl, 1998). Heifers that calve early tend to calve early in subsequent seasons and have increased lifetime production (Lesmeister et al., 1973, MacGregor and Casey, 1999, Pence et al., 2007, Stevenson et al., 2008). Maximum follicle diameter is correlated with uterus, cervix and vaginal diameter, and increases in the 10 weeks prior to first ovulation in heifers, due to increased LH pulse frequency (Desjardins and Hafs, 1969, Day et al., 1984, 1987, Bergfelt et al., 1994, Honaramooz et al., 2004, Cushman et al., 2009).

1.3. Restricted breeding seasons in beef cattle

In feral cattle herds, as in wild ruminants, breeding is synchronised with the natural availability of good quality feed (Spitzer, 1986). Restricted breeding and calving during the optimal season are key principles in good cow-calf management (Denham et al., 1991, Engelken et al., 1991), in order for critical times of nutritional needs during the reproductive cycle to be synchronised with the availability of fodder (Figure 1.2).

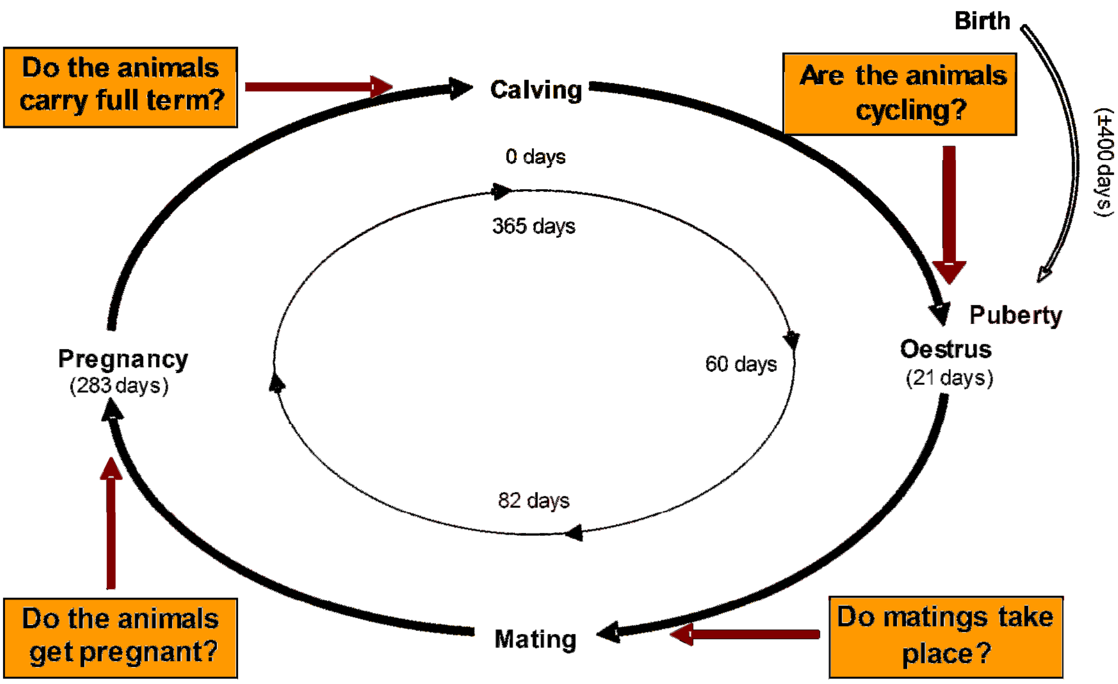

Figure 1.2: Major events in the reproduction cycle of heifers and cows that may affect reproduction efficiency in restricted breeding systems

Advantages of restricted breeding include enhanced production potential due to improved fodder flow, the opportunity to perform pre-breeding management procedures on the whole herd, a more uniform calf crop that improves marketability, and improvement in general calf management (Chenoweth, 2005a). However, very short breeding seasons (45 d) have been challenged due to the negative effect on calving rate, particularly in breeds with long gestational length (GL) such as *Bos indicus* breeds (Deutscher et al., 1989, Azzam et al., 1990, Denham et al., 1991). Proper management and selection of heifers (using body weight, conformation, growth performance, estimated breeding values, RTS and pelvimetry) before breeding are essential to the success of such systems, due to early, unassisted calving during the first calving season resulting in improved longevity and lifetime production in seasonal breeding systems (Grass et al., 1982, Larson, 2005, Cushman et al., 2013, Perry and Cushman, 2013). MacGregor and Casey (1999) demonstrated that calving date was a more repeatable measure of reproductive success than calving interval, specifically in herds with restricted breeding using more than 11,000 data points in the same herd studied in this thesis.

Due to the fact that weaning typically takes place on a single day in seasonal breeding systems (Larson, 2005), the production of a beef cow (kg of weaned calf produced) is highly dependent on her reproductive efficiency (being able to calve early during each annual calving season). However, other factors such as ability to survive, frame size, milk production, feed conversion rate and conformation also influence the production ability of a cow, therefore reproduction and production can be measured as separate outcomes in beef cattle.

1.4. Reproductive tract scoring

The ability to select young heifers that will reproduce effectively in a seasonal breeding system has advantages over the alternative approach of waiting until reproductive failure occurs (Chenoweth, 2005a). Andersen et al. (1991) described a system to score the pubertal stage of heifers by transrectal palpation, which is further described in Chapter 2. Reproductive tract score predicts anoestrus and pregnancy failure in heifers, and is a valid selection tool to enhance reproductive performance of herds (Andersen et al., 1991, Pence et al., 2007, Archbold et al., 2012b, Gutierrez et al, 2014). However, oestrous cycle stage and proportion of heifers in anoestrus affect the accuracy of RTS (Archbold et al., 2012a) and the complexity of the RTS system affects its repeatability (Rosenkrans and Hardin, 2003). In addition, other tests with the potential to improve RTS, such as ultrasonography of the reproductive tract and measurement of AMH have become available (Ireland et al, 2009). Reproductive tract scoring as a selection tool in beef heifers has not been compared to other methods used to select heifers, and the association of RTS with reproductive performance of the subsequent breeding seasons has not been described before. Pre-breeding ultrasonographic measures of the reproductive tracts of heifers that have been used to predict reproductive outcome include the presence or absence of a corpus luteum (Archbold et al., 2011), the antral follicle count (Ireland et al., 2009) and the thickness of the endometrium in one of the uterine horns (Monteiro et al., 2013).

1.4.1. Accuracy and precision

Accuracy is commonly defined as an indication of the ability of a measurement to represent the true value, whereas precision is the ability of a measurement to produce consistent values when repeated (Thrusfield, 2007). Precision can further be divided between repeatability, being the degree of agreement between sets of observations made by the same operator (also called within operator repeatability), and reproducibility, which is the degree of agreement between sets of observations made by different operators on the same group of animals (also called between operator repeatability) (Thrusfield, 2007). Accuracy is typically measured as the sensitivity, specificity, and positive and negative predictive values of diagnostic or prognostic tests, whereas precision, in the case of dichotomous tests (such as diagnostic tests), is typically measured as agreement beyond chance, using the Kappa statistic (Dohoo et al., 2003a). For categorical data, adaptations of the Kappa statistic, such as multi-category analyses, can be used (Rosenkrans and Hardin, 2003).

The accuracy of RTS may potentially vary depending on age, body weight (BW), stage of pubertal development, breed and other unknown factors. However, reasons for misclassification of RTS in heifers are not well described. Spire and Holtz (1995) 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. Rosenkrans and Hardin (2002) found the RTS system to be moderately to substantially repeatable between and within veterinarians respectively, using multicategory Kappa statistics (Kappa values 0.46 and 0.64 respectively), and also to distinguish between prepubertal (RTS 1, 2 or 3) or post pubertal (RTS 4 or 5) heifers (binary outcome Kappa values 0.58 and 0.72 respectively). Archbold et al. (2011) concluded that the ultrasonographic presence of a CL was more accurate and repeatable than serum progesterone levels in determining luteal status in pubertal heifers when examined at a single point in time.

1.5. Dystocia in cattle

Dystocia is defined as the failure to complete partus and is common at first calving in heifers (Youngquist, 2007). Different scoring systems have been used to quantify the degree of intervention or assistance required in order to solve a dystocia case (Zaborski et al, 2009). The importance of dystocia in cattle extends beyond the loss due to perinatal mortality and veterinary costs. It also includes increased risk of respiratory and gastro-intestinal disease due to lower passive immunity transfer and higher cortisol levels (Lombard et al., 2007). These increased risks resulted in a 2.8 times higher risk of mortality by the time of weaning in calves born to dams that required assistance during the birth process (Barrier et al., 2013).

1.5.1. Factors affecting dystocia in cattle

Several factors are associated with an increased risk of dystocia in cattle, of which cow parity 1 vs. >1, relatively heavier calves, male vs. female calves and relatively smaller pelvic size as measured by pelvis area (PA) are the most important. Zaborski et al. (2009) and Meijering (1984) reviewed the factors affecting dystocia in cattle, which are summarised in Figure 1.3, where the interrelationships are also shown.

Factors can be classified as direct factors, being presentation of the calf and the uterus (such as uterine torsion), phenotypic factors related to the calf (calf BWT, twinning and perinatal mortality) or cow (GL, PA, BW and BCS at calving), non-genetic factors (parity, year and season of calving, cow age at calving, calf gender, nutrition and metabolic disorders such as hypocalcaemia, ketosis and delayed regression of the CL) and genetic factors (cow and bull breed, inbreeding and muscular hypertrophy) (Meijering, 1984; Zaborski et al., 2009).

Breed differences in the occurrence of dystocia are attributed to differences in the relative BWT of calves, pelvis structure and large variation in pelvis dimensions in some breeds (Citek et al., 2011, Nogalski and Mordas, 2012). Micke et al. (2010a) demonstrated that PA was associated with dystocia in beef heifers calving at 3 years old where the dystocia incidence was only 14%, and that this association was not altered by changing the level of nutrition during the first or second trimester of pregnancy. External pelvis measures are not correlated with internal pelvis dimensions and have not been associated with dystocia outcome (Lalrintluanga and Lallianchhunga, 2012).

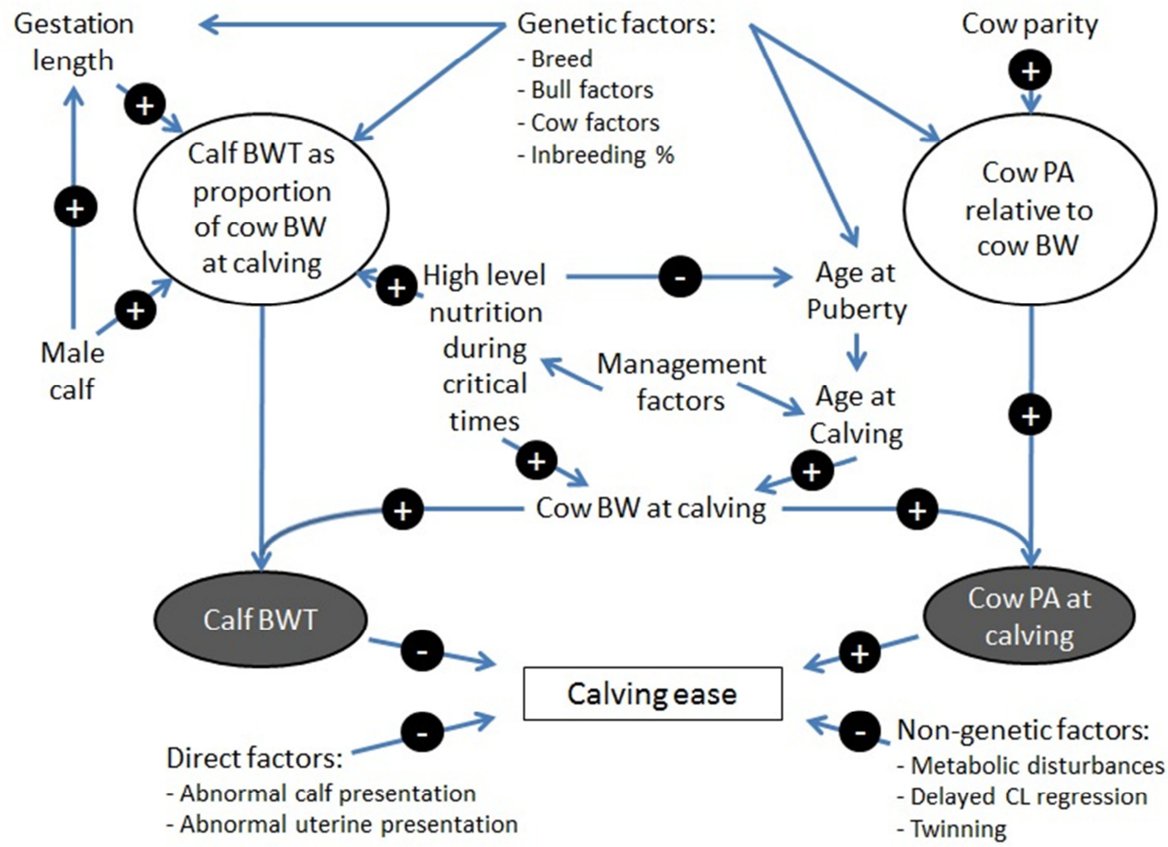

Figure 1.3: Diagram illustrating factors affecting dystocia in cattle and their interrelationships (BWT = birth weight, BW = body weight, PA = pelvis area).

Although pelvimetry did not form part of their study, Uzmay et al. (2010) determined that calf BWT and GL were the most significant independent risk factors contributing to the occurrence of dystocia in primiparous Holstein heifers, whereas no factors could be shown to have significant effects on the occurrence of dystocia in multiparous cows. In primiparous heifers, which have a significantly increased risk of dystocia, foeto-maternal disproportion (due to calf BWT being too high and/or PA being too low) therefore appears to be the most common reason for dystocia (Price and Wiltbank, 1978a). Cook et al. (1993) constructed a stochastic model of two strategies to reduce dystocia incidence and severity in heifers from published parameters. The two strategies were either using negative estimated progeny difference (EPD) for BWT in bulls or culling 20%, 40% or 60% of heifers based on PA. Pelvis area measures used in this study were not corrected for age or BW of the heifer. They concluded that selection of sires with negative BWT EPD was much more effective in reducing dystocia incidence and severity.

1.5.2. Factors affecting calf birth weight

Holland and Odde (1992) reviewed the factors affecting calf BWT, which can be summarised as follows: Sire and dam effects are similar in magnitude, and contribute most significantly to the BWT of the calf; heritability estimates are approximately 0.45. Breed effect is more significant than variation within breed, and heterosis is particularly outspoken when a *Bos taurus* breed is crossed with a *Bos indicus* breed, leading to larger calves being born, whereas inbreeding leads to smaller calves being born (inbred calves more than calves of inbred dams). Male calves are heavier at birth due to testosterone production from day 45 of gestation, whereas male calves also have a longer GL, which is associated with heavier calves at birth due to foetal growth of 100 to 250 g/d during the last days of gestation. However it is still unclear whether calf BWT is a function of GL, or vice versa.

Heavier cows give birth not only to heavier calves, but also to relatively heavier calves, as a proportion of cow BW at calving. The cause of this is unclear, but thought to be due to a limitation on the physiological maintenance of the foetus in smaller cows (Holland and Odde, 1992). Similarly, twin and triplet calves are lighter at birth, despite the fact that initial foetal growth rate does not differ. Calf BWT is also associated with dam age, with cows of 4-5 years old giving birth to calves that weigh 4-5 kg more than those of heifers. This is thought to be associated with the BW of the cow at calving, although other reports have shown a similar association between cow age and GL (Holland and Odde, 1992). A few classic experiments have demonstrated the effect of the ability of a cow to produce a heavier calf, where Brahman as well as Charolais embryos that were transplanted into Charolais cows resulted in calves with heavier BWT than when transplanted into Brahman cows (Holland and Odde, 1992). However, Cook et al. (1993) concluded that our ability to predict calf BWT in individual animals is poor.

Restricting energy intake of a cow or heifer during the third trimester of pregnancy, when 75% of foetal weight gain occurs, results in decreased calf BWT when compared to unrestricted intake. However it also results in decreased BW gain of the heifer and therefore the possibility of decreased pelvis development, and does not influence dystocia (Laster, 1974; Schoonmaker, 2013). Restricted feed intake during any stage of gestation further leads to restricted endocrine and organ development and altered genetic programming which may result in smaller follicular reserve and poor performance of the calf throughout life (DaSilva et al., 2002; Ireland et al., 2011; Cushman et al., 2012; Echternkamp et al., 2012; Schoonmaker, 2013). Micke et al. (2010b) demonstrated that restriction of nutrition during the first and second trimester of pregnancy also affects foetal growth trajectory and calf BWT. In their experiment nutritional restriction during the first trimester of pregnancy resulted in reduced foetal growth rate noticeable as early as d 39 of pregnancy, followed by compensatory growth during the second trimester when nutritional level was normalised, ultimately resulting in increased calf BWT. Nutritional restriction during the second trimester of pregnancy resulted in reduced foetal growth rate during that period and reduced calf BWT.

1.5.3. Pelvimetry

Although the internal pelvic canal is elliptical in shape and not rectangular, PA has conveniently been calculated as the product of the vertical (VD) and transverse (TD) pelvis diameter (Price and Wiltbank, 1978; Van Donkersgoed et al., 1993), most commonly measured using a Rice pelvimeter (Figure 1.4).

Different instruments can be used to measure pelvis dimensions, of which the Rice pelvimeter™ (Lane Manufacturing, Denver, Colorado) and Krautmann instrument are the best described (Wolverton et al., 1991, Van Donkersgoed et al., 1993).

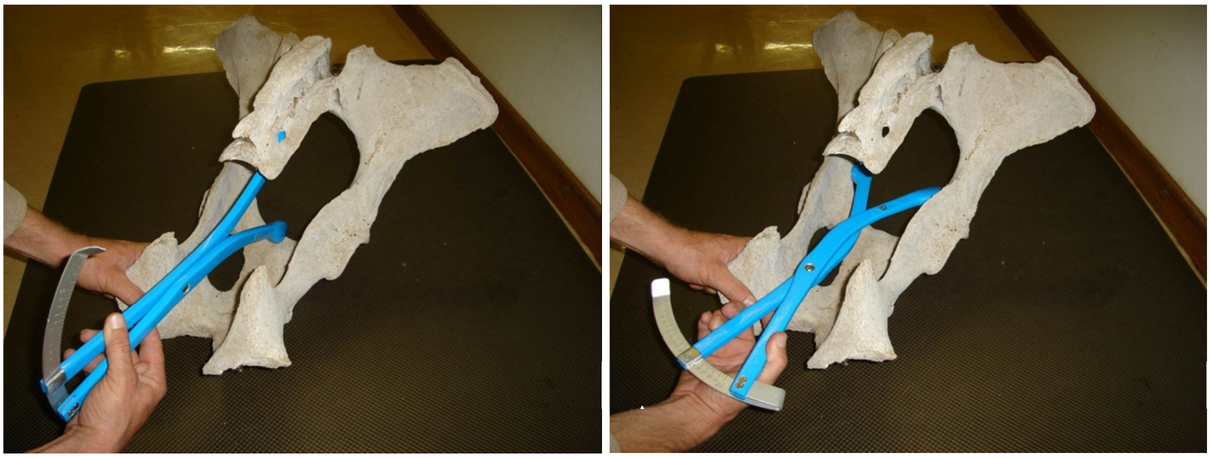

Figure 1.4: Measuring the vertical diameter (A) and transverse diameter (B) of the pelvic canal using a Rice pelvimeter.

The Rice pelvimeter is a calliper device consisting of a pair of aluminium arms and a stainless steel scale with 0.5 cm graduations. The repeatability of PA is reported to be moderate to high when a cut-off of the lowest 10% is used, and similar within and between instruments and veterinarians with Kappa values ranging from approximately 0.30 to 1.00 (Wolverton et al., 1991, Van Donkergoed et al., 1993). However, from the data of Van Donkersgoed et al. (1993) it appears that the Rice pelvimeter has superior repeatability within and between veterinarians. Kolkman et al. (2009) showed a strong correlation between pelvis measurements using the Rice pelvimeter in live Belgian Blue cattle and measurements obtained from the same animals after slaughter, although actual agreement was not assessed.

Fitzhugh et al. (1972) found that pelvic TD:VD ratio, as well as VD:BW decreased after weaning, and Price and Wiltbank (1978) suggested that this indicates earlier maturity of pelvis development, in particular TD, relative to BW development. An experiment in which two age cohorts of calves (131 castrated male, 78 intact female) were randomised to implantation with progesterone and oestradiol or untreated controls provided further support of this theory. Implantation resulted in larger TD but not VD of the pelvis at weaning (pre-pubertal), and larger TD as well as VD at the time of breeding (Lesmeister, 1976). Ramin et al. (1995) found that VD of the pelvis, PA and PA:height at the withers were all significantly associated with growth rate, and negatively associated with age at puberty in dairy heifers. Zaborski (2009) further mentions that poor nutrition during the development of heifers is an important risk factor for dystocia due to retarded growth with resultant poor development of the pelvis.

Applications of pelvis measures in bulls include using it at breeding soundness examination and as part of performance testing. Garcia-Paloma et al. (2011) suggested that bulls examined for breeding soundness with PA more than two standard deviations (SD) lower than the mean for the group (147 cm^2 in their study of 407 bulls of the Asturiana de los Valles breed) should be interpreted as having PA of questionable size. In a study performed by Singh et al. (2010) poor libido was associated with smaller PA in bulls. Siemens et al. (1991) recommended adjustment of PA by either 0.21 cm^2 per day of age or 0.15 cm^2 per kg BW in yearling bulls, for the purpose of performance testing.

1.5.4. Relative pelvis size

Van Donkersgoed et al. (1993) studied the usefulness of three different applications of pelvis measures in heifers (raw PA data, PA:BWT ratio and PA:pre-breeding BW ratio) and found sensitivity (Se) and specificity (Sp) for the occurrence of dystocia ranging from 18 to 90% and 35 to 92% respectively. Using a 16% culling rate with the PA:pre-breeding BW ratio they reported a Se and Sp of 28 and 86% respectively. Van Donkersgoed et al. (1993) concluded from this study that pelvimetry performed poorly as an on-farm test to reduce dystocia incidence due to its poor accuracy and precision, and they suggested that there was no evidence to support its further use. Basarab et al. (1993) reached the same conclusion, and found that using raw PA as culling tool resulted in culling of lighter heifers that produced calves with lower BWT, while using PA:BW ratio as culling tool resulted in culling of heavier heifers that gave birth to heavier calves.

Deutscher (1988) recommended adjustment of PA data to an age of 365 d by 0.27 cm^2/d of age in yearling heifers, based on findings of Price and Wiltbank (1987b) describing the correlation between age and PA in different breeds of cattle. Ramin et al. (1995) found a linear correlation between PA and age in dairy heifers between 12 and 18 months of age. However, Laster (1974) showed that variation in PA was mostly influenced by BW and breed. Van Donkersgoed (1997) confirmed that the use of age-adjusted PA using a standardised growth factor should be questioned due to many other factors affecting PA. Therefore, a satisfactory application of pelvimetry data for selection of heifers against dystocia does not currently exist.

1.6. Hypothesis and research questions

The general hypothesis tested in this study was that reproductive performance of beef heifers cannot be predicted before the onset of the first breeding season.

The following research questions are addressed in this thesis:

1. Is RTS a valid scoring tool to predict reproductive performance of restricted bred beef heifers in South Africa?
2. Is RTS a valid scoring tool to predict production performance of beef heifers in South Africa?
3. How does ranking of beef heifers by RTS compare to ranking by BW, age, BCS and Kleiber ratio with respect to their associations with reproduction and production performance?
4. Is pre-breeding RTS of beef heifers associated with long-term survival due to reproductive success in a restricted breeding system?

5. Is pre-breeding RTS of beef heifers associated with reproductive success after adjusting for its association with anoestrus during the first restricted breeding season?
6. Which factors may affect the accuracy of RTS in predicting reproductive success in beef heifers?
7. What are the associations between PA, BW, calf BWT, and calving and dystocia outcomes in beef heifers?
8. Is PA a valid culling tool to reduce the incidence of dystocia in beef heifers?
9. Which method of adjusting PA data has the strongest association with dystocia outcome?
10. Which method of adjusting PA data has the strongest association with BW and calf BWT?
11. Which ultrasonographic measures of the reproductive tract of beef heifers are independently associated with anoestrus and failure to become pregnant during a restricted breeding season?
12. Can ultrasonographic measures of the reproductive tract of beef heifers provide a prognostic model for reproductive outcomes comparable to RTS?
13. Does PA data provide any additional prognostic value for reproductive outcomes, apart from dystocia, in beef heifers?

1.7. Description of the study herd

The study population was the Bovelder cattle herd managed at Johannesburg Water's Northern Farm, which has since been terminated. The farming system and cattle breed type have been previously described (Paterson et al., 1980, Schoeman and Jordaan, 1998, MacGregor and Casey, 1999, Holm et al., 2008). In brief, this was a seasonally bred commercial beef farm (closed herd) that produced primarily breeding animals and also weaner calves for the feedlot industry, whereas cull animals were slaughtered for the formal beef industry. The farm was situated next to the water purification works of Johannesburg Water, near the Jukskei River north of Johannesburg, where second grade purified water was used to irrigate pastures and crops as cattle fodder.

Animals with parity 0, 1, 2, and ≥3 were managed in separate groups, and a single inseminator was assigned to each group. Heifers were typically aged between 12 and 16 months at the onset of first breeding, when they weighed between approximately 200 and 400 kg; however, it was usual that not all heifers had reached puberty by that time. Breeding started on the same day every year (15 October for heifers, and 1 November for cows). Breeding consisted of a 50-day AI period, followed 5 to 10 days later by a 42-day bull breeding period. Pregnancy diagnoses (PD) were performed by transrectal palpation between 23 March and 26 April of every year. The farm had an efficient data capture system that had been in place for more than 20 years, facilitating access to information. The fact that this farm was a municipality owned farm for many years had a positive effect on the quality of data generated, due to the fact that management protocols were fixed and were very seldom deviated from, leading to a herd that was well adapted to the system. However, the farm was commercially driven and culling policies were based on economic values applicable to most commercial beef farms in South Africa.

1.8. Study objectives and outline of the thesis

The research reported in this thesis consisted of a series of four observational studies using data collected from the study herd from 2002, when the first age cohort were born, until 2010, when the herd was sold. All four studies consisted of pre-breeding examinations that were performed during the weeks prior to breeding, followed by collection and analysis of the reproduction and production data of the herd.

The first study, reported in Chapter 2, addressed research questions 1-3. Reproductive tract scoring by transrectal palpation on a 5-point scale was performed 1 day before the start of breeding on a group of heifers (n = 272, born 2002) of which half were randomised within RTS category to a synchronisation programme using prostaglandin $F_{2\alpha}$. Heifers were followed through their second breeding season until they had weaned their first calves. The objectives of this study were to determine whether RTS is a valid tool to predict reproductive (pregnancy outcome and days to calving) and production performance (total kg of calf weaned) in beef heifers.

Chapter 3, which addressed research questions 4-6, describes a 7-year longitudinal study in which 292 beef cows in two age cohorts (born 2002 and 2003) were observed from 1-2 days before their first breeding season, when RTS was performed, until they had weaned up to five calves.

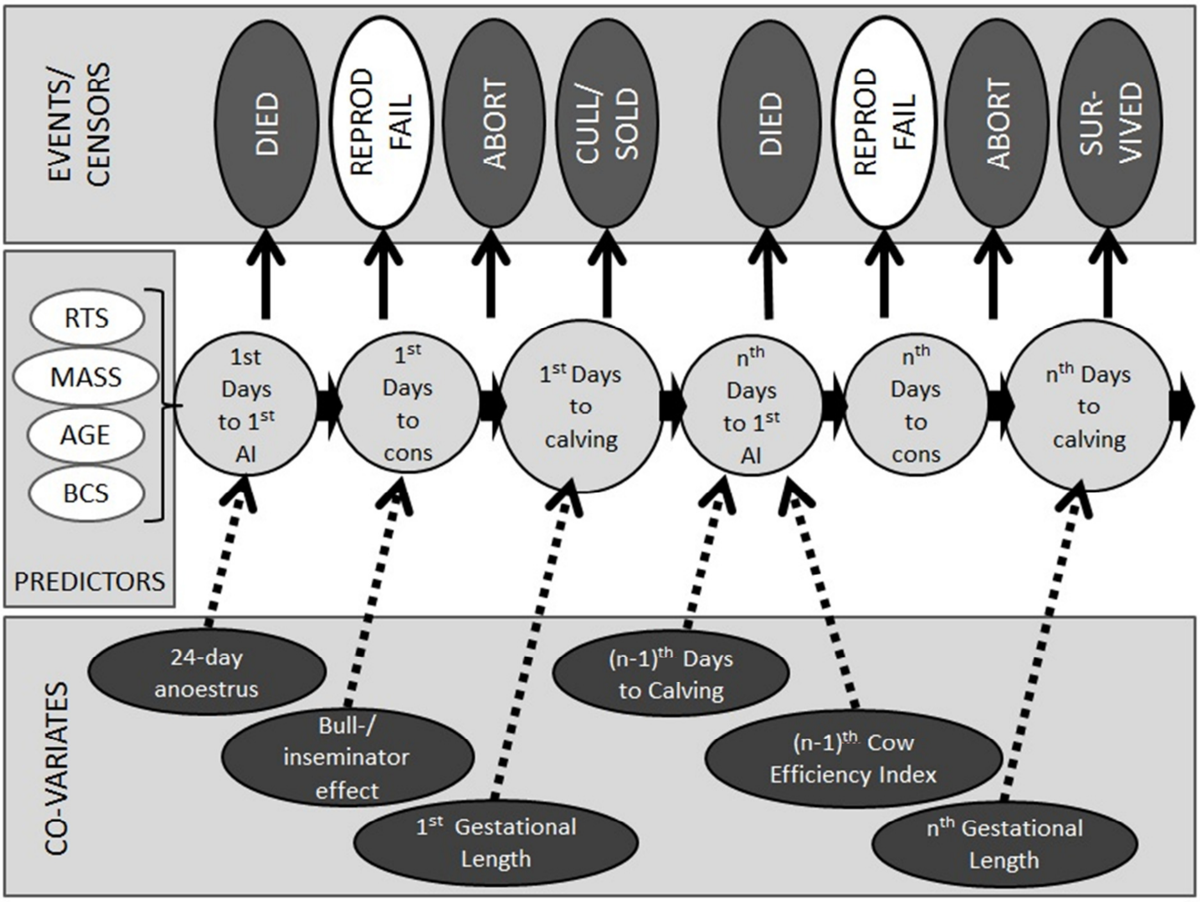

Figure 1.5: Causal pathway of events leading to reproductive failure during the n^th year in Chapter 3.

The objectives of this study were to determine whether RTS is a valid culling tool to select for heifers that are more likely to survive longer in a restricted breeding system, and specifically whether initial RTS category is associated with reproductive performance in subsequent breeding seasons in heifers that became pregnant during their first breeding season. Another objective of this study was to determine factors that affect the possible misclassification of RTS in beef heifers. Figure 1.5 is a diagrammatic representation of the causal pathway of events leading to reproductive failure during the n^{th} year that was used to construct the Cox proportional hazard analysis models for years to reproductive failure that were used to analyse the data in Chapter 3.

Chapter 4 addressed research questions 7-10. The effects of six culling strategies, based on pre-breeding PA data, on calving and dystocia rates and on pre-breeding and calf BWT were compared. Hypothetical culling of heifers was applied within age cohort after ranking them by PA and by five different methods of adjusting PA. The objective of this study was to compare the effects of the different culling strategies on calving and dystocia rates and on pre-breeding and calf BWT, in order to determine if PA is a valid predictor of dystocia outcome, and to establish whether adjusting PA data to the BW or the age of the heifer at breeding improves its prognostic value.

Research questions 11-13 were addressed in Chapter 5, where two age cohorts of heifers (n = 488, born 2007 and 2008) were followed from before first breeding until their first pregnancy test. Outcomes of this study were the observation of oestrus during the first 50 days of the breeding season, and pregnancy after the artificial insemination and bull breeding periods. Multiple logistic regression models were constructed using the hypothetical causal pathway diagram in Figure 1.6. The objectives of this study were to establish the most appropriate prognostic model for pregnancy failure and days to calving, using various ultrasonographic measures of the reproductive tract and pelvis measures of beef heifers, and then to determine if such a model is superior to RTS and if it adds prognostic value to RTS.

The four studies presented in Chapters 2 to 5 are then further discussed in the General Discussion in Chapter 6. Some further analysis of the combined data is presented, along with additional data collected during the study, to emphasise certain points. In addition, an outline of a cost-benefit analysis based on the data from the study is proposed, the details of which are presented in the Appendix. General conclusions with recommendations, and new research questions that resulted from this work, are presented in Chapter 7.

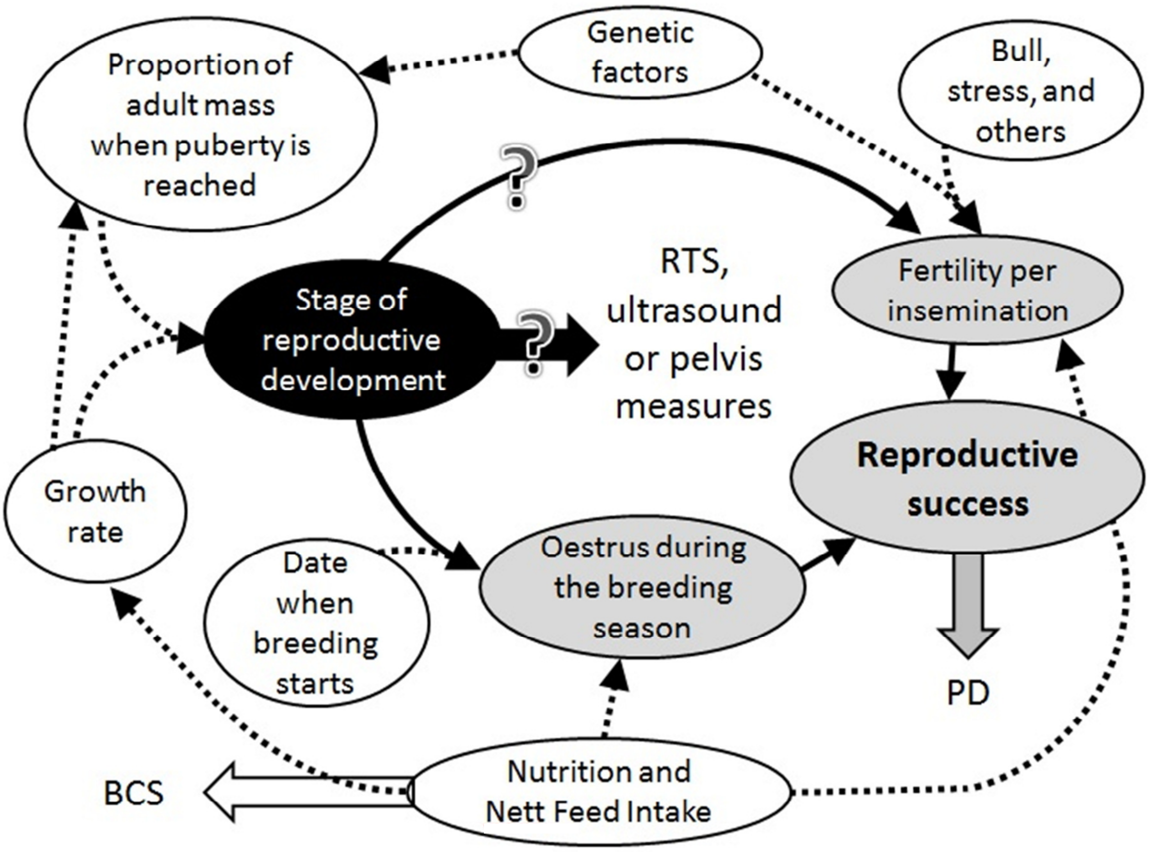

Figure 1.6: Hypothetical causal pathway used to construct multiple logistic regression models of pregnancy outcome in Chapter 5.

CHAPTER TWO

THE VALUE OF REPRODUCTIVE TRACT SCORING AS A PREDICTOR OF FERTILITY AND PRODUCTION OUTCOMES IN BEEF HEIFERS

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2.1. Abstract

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. This study was performed concurrently with another study testing the economic effects of an ooestrus synchronisation protocol using prostaglandin.

Reproductive tract scoring (RTS) by rectal palpation was performed on the group of heifers one day before the onset of their first breeding season. The effect of RTS on several fertility and production outcomes was tested, and the association of RTS with the outcomes was compared to that of other input variables such as body weight, age, body condition score and Kleiber ratio using multiple or univariable linear, logistic or Cox regression. Area under the curve for receiver operating characteristic analysis was used to compare the ability of different input variables to predict pregnancy outcome.

After adjustment for weight and age, RTS was positively associated with pregnancy rate to the 50 day AI season ($P < 0.01$), calf weaning weight ($r = 0.22$, $P < 0.01$) and pregnancy rate to the subsequent breeding season ($P < 0.01$), and negatively associated with days to calving ($r = 0.28$, $P < 0.01$). RTS was a better predictor of fertility than was Kleiber ratio, and similar in its prediction of calf weaning weight.

It was concluded from this study that RTS is a predictor of heifer fertility, compares well with other traits used as a predictor of production outcomes and is likely to be a good predictor of lifetime production of the cow.

Keywords: age at puberty, beef heifers, cattle, pregnancy rate, reproductive tract scoring, weaning weight, Kleiber ratio

2.2. Introduction

In the past, conformation, body weight, body condition score and calculated indices such as Kleiber ratio (KR) (Kleiber, 1947, Scholtz and Roux, 1988) have been used to select heifers for breeding. However, selection based on age at puberty (AP) is desirable due to its correlation with fertility outcomes, and ultimately with lifetime production of the cow through repeated early calving dates (Andersen et al., 1991). Age at puberty in heifers is conveniently defined as the age at which a heifer displays visual signs of oestrus for the first time (Pineda, 2003). Age at puberty is to some extent breed-determined and is a moderately heritable trait ($h^2 = 0.43$) with a favourable association with weaning weight and yearling weight of the offspring (Brinks, 1994).

Andersen et al. (1991) developed a standardised reproductive tract score (RTS) system to measure AP in heifers indirectly. This method involves palpation of the reproductive tract and ovarian structures per rectum and is scored from 1 to 5 (Table 1). Three possible applications of the RTS system have been recommended: firstly as a screening test to determine the pubertal status of heifers before the breeding season (Andersen et al., 1991), secondly as an indication of the nutritional requirements of heifers when sufficient time is allowed before the breeding season (Andersen et al., 1991), and thirdly as a selection tool for AP (Pence and Bredahl, 1998; Pence et al., 2007). Reproductive tract scoring as a method of selection has been found to be correlated with AP, response to synchronisation and pregnancy rate to synchronised oestrus, and has an estimated heritability (h^2) of 0.32 (Andersen et al., 1991). Reproductive tract scoring is a repeatable (between and within veterinarian) and accurate measure of pubertal status (Rosenkrans and Hardin, 2003).

The objective of this study was to compare the usefulness of RTS as a predictor of fertility and production outcomes with other selection measures such as KR, body condition score (BCS), weight or age at the onset of the first breeding season.

2.3. Materials and methods

This was a prospective study performed simultaneously with a study to determine the economic effects of oestrus synchronisation using prostaglandin on 272 Bovelder heifers at Johannesburg Water's Northern Farm (Holm, 2006; Holm et al, 2008).

The heifers' ages at the start of the breeding season ranged from 364 to 486 (median 431) days, while their body weight ranged from 261 to 407 (median 316) kg. Two days before the onset of the insemination season (day -1), all heifers were weighed, body condition scored (BCS) using a 5-point scale and reproductive tract scored (Andersen et al., 1991) (Table 2.1). Kleiber ratio was calculated as growth rate per metabolic weight (average daily gain/end weight$^{0.75}$), using the birth weight as the start weight, and the weight on day -1 as the end weight. To avoid potential bias caused by synchronisation, heifers were ranked firstly by RTS and secondly by body weight, and then block randomised in pairs to either the synchronised or the unsynchronised group (Holm et al, 2008). As a result of this, each RTS category contained exactly 50% synchronised and 50% unsynchronised heifers.

Table 2.1: Reproductive tract score (RTS) system (Andersen et al., 1991)

		Ovaries			
RTS category	**Uterine horns**	**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

Frozen semen of 11 different bulls was allocated to heifers according to normal farm practice. Farm management and other staff were blinded to categories (synchronisation and RTS), and heifers were managed as one group. The artificial insemination (AI) season started on 15 October 2003 (day 1) and lasted for 50 days. Oestrus detection was done by visual observation and marking during each night. Upon detection of standing oestrus heifers were inseminated once a day at 0900 h by one experienced inseminator. After a window period of 5 days, there was a period of 42 days natural breeding with bulls, using a multisire system with a heifer:bull ratio of 35:1.

Day of the AI season and semen batch were recorded for all inseminations during the breeding season. A veterinarian palpated the heifers per rectum to determine pregnancy status 90 days after the removal of bulls. Pregnancy status was confirmed by calving date. Abortions, dystocia, birth date, birth weight, gender of calf, calf mortality, cow mortality, and weaning weight were subsequently recorded. The subsequent breeding season started on 1 November 2004, consisting of a 50-day AI period followed 14 days later by a 42-day bull breeding period. No oestrus synchronisation was used, but similar records were collected during the subsequent breeding season. All calves were weaned on the same day (29 March 2005), and the trial was terminated on 1 April 2005.

Days to first insemination was defined as the day of the breeding season on which a heifer was inseminated for the first time. Days to calving was defined in a similar fashion, and the first day of the calving season was defined as the day when the first calf was born. When a heifer did not achieve the specified status by the end of the time period a maximum value was given to that heifer (eg. 50 in the case of days to first insemination), but these values were censored for the purpose of Cox regression.

Effects of age, weight and BCS on RTS were assesed using multiple linear regression. The various outcomes (days to first AI, pregnancy rates, days to calving and calf weaning weight) were then compared between categories of RTS. Proportions were compared using the Fisher exact test and means and medians were compared using ANOVA with the Tukey-Kramer multiple comparison test and Kruskal-Wallis one-way ANOVA respectively. The effects of RTS on the outcomes, adjusted for weight, BCS and age, were then estimated using Cox regression for days to AI and days to calving, logistic regression for pregnancy rates and multiple linear regression for weaning weight. The usefulness of each of the predictor variables (age, weight, BCS, KR and RTS) when used alone to predict the outcomes were compared using the R^2 statistic for linear regression models (weaning weight), the pseudo R^2 values for Cox regression models (days to AI and days to calving) and the area under the curve (AUC) of the receiver operating characteristic (ROC) curve for binary outcomes (pregnancy rates). Areas under the ROC curves were compared using the algorithm of DeLong et al (1988). Statistical analyses were done using NCSS 2004 (NCSS, Kaysville, UT, USA), Epicalc 2000 (http://www.brixtonhealth.com/epicalc.html) and Stata 10.1 (StataCorp, College Station, TX, USA).

2.4. Results

Table 2.2 gives a summary of the five RTS categories on day -1, and shows that heifers with RTS 1 and 2 were younger than those with RTS 3, 4 and 5 while heifers with RTS 3 were younger than those with RTS 5. It further shows that heifers with RTS 2 and 3 were lighter than those with RTS 4 and heifers with RTS 2 and 3 had lower BCS than those with RTS 4 and 5.

Table 2.2: Summary of RTS categories on day -1 of the breeding season (heifers born 2002)

RTS	Number	Age in days (mean; 95% CI)	Weight in kg (mean, 95% CI)	BCS (1-5 scale) (Mean, 95% CI)
1	16	420^{a} 408-432	309^{ab} 291-327	3.8^{ab} 3.6-4.0
2	70	423^{a} 417-428	309^{a} 303-316	3.7^{a} 3.6-3.8
3	81	432^{b} 428-436	313^{a} 307-319	3.7^{a} 3.6-3.8
4	74	434^{bc} 430-438	320^{b} 315-326	3.8^{b} 3.7-3.9
5	30	439^{c} 432-446	318^{ab} 308-329	3.9^{b} 3.7-4.0

^{abc} Values within columns with no superscripts in common differ significantly

Using simple linear regression, age, weight and BCS before the onset of the breeding season were each associated with RTS (P = 0.03, P < 0.01 and P < 0.01 respectively). However in a multiple regression model of RTS using age, weight and BCS as predictors, only pre-breeding age was independently associated with RTS (P < 0.01) (Table 2.3).

Table 2.3: Effects of pre-breeding age, weight and BCS on RTS (multiple regression)

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

In a multiple regression model, age, weight and BCS were all significantly associated with pre-breeding KR (P < 0.01) while RTS was not independently associated with KR (P = 0.76) (data not shown).

The univariable effects of pre-breeding RTS on pregnancy rates, days to first AI, days to calving and weaning weight of the calves are summarised in Table 2.4.

Using logistic regression, RTS and weight before the onset of breeding showed positive univariable associations with pregnancy rate after the first breeding season (P < 0.01

and *P* = 0.04 respectively) whereas age, BCS and KR did not (*P* = 0.07, *P* = 0.17 and *P* = 0.28 respectively). Associations between predictor variables measured before the first breeding season and pregnancy rate after the second breeding season were significant for RTS (*P* = 0.02), weight (*P* = 0.01) and BCS (*P* = 0.03), but were not significant for age (*P* = 0.10) and KR (*P* = 0.73).

Table 2.4: Summary of reproduction and production outcomes by RTS category in beef heifers

RTS	Pregnancy rate (%) to AI period	Final pregnancy rate (%)	Median days to calving	Mean calf weaning weight (kg)	Proportion of heifers present at start of subsequent season (%)	Pregnancy rate (%) to subsequent AI period
1	31^{a}	56^{a}	53.5^{ab}	194^{ab}	50^{ac}	63^{ab}
2	40^{a}	76^{a}	52^{a}	186^{a}	51^{a}	61^{a}
3	53^{a}	81^{ab}	28^{bc}	213^{b}	57^{a}	72^{b}
4	70^{b}	92^{b}	15^{c}	207^{b}	80^{b}	85^{b}
5	80^{b}	93^{b}	18^{c}	213^{b}	70^{bc}	90^{b}

^{abc} Values within columns with no superscripts in common differ significantly

Univariable Cox regression analyses of days to first AI and of days to calving showed negative associations with pre-breeding RTS and BCS (*P* < 0.01), but no significant association with pre-breeding weight, age or KR. Univariable linear regression of calf weaning weight showed a significant association with pre-breeding RTS (*P* < 0.01), age (*P* = 0.04) and KR (*P* = 0.05), but not with BCS (*P* = 0.37) or weight (*P* = 0.65). Calves of heifers with RTS 1 or 2 (n = 33) had a mean weaning weight of 186.7kg (95% CI 176.0 – 197.4 kg), differing significantly from calves of heifers with RTS 3, 4 or 5 (n = 102) with a mean weaning weight of 210.1 kg (95% CI 203.8 – 216.4 kg) (*P* < 0.01).

Table 2.5 shows a summary of the multivariable (logistic, Cox and linear) regression models for the various outcomes using pre-breeding RTS, age and weight as predictor variables. It shows a consistently significant association between RTS and the outcomes (*P* < 0.01), but not for age or weight. When days to calving was added as predictor variable to the logistic regression model for pregnancy to the subsequent AI season, days to calving was significantly associated with the outcome (*P* < 0.01) but RTS was not (*P* = 0.09). When an interaction term between synchronisation group and RTS was included as predictor variable in these models, this interaction term was not associated with any of the outcomes (*P* > 0.2).

Table 2.5: Multivariable associations of pre-breeding RTS, weight and age with some important production and reproduction outcomes in beef heifers

Predictor variable	Pregnancy after the first AI season[1] (odds ratio; 95% CI; *P* – value)	Pregnancy after the subsequent AI season[1] (odds ratio; 95% CI; *P* – value)	Days to first AI[2] (hazard ratio; 95% CI; *P* - value)	Days to calving[2] (hazard ratio; 95% CI; *P* – value)	Calf weaning weight[3] (Coefficient; 95% CI; *P* - value
RTS (1 – 5)	1.78 1.38 – 2.29 <0.01	1.64 1.15-2.33 <0.01	1.18 1.04 – 1.32 <0.01	1.25 1.09 – 1.44 <0.01	6.49 1.14 – 11.84 <0.01
Body weight (kg)	1.01 1.00 – 1.02 0.10	1.00 0.99-1.02 0.85	1.00 0.99 – 1.00 0.84	1.00 0.99 – 1.00 0.18	-0.04 -0.28 – 0.20 0.73
Age (days)	1.00 0.98 – 1.01 0.76	1.01 0.99-1.02 0.34	1.00 1.00 – 1.01 0.31	1.00 1.00 – 1.01 0.32	0.24 -0.04 – 0.53 0.09

[1] Data from multiple logistic regression models
[2] Data from Cox regression models
[3] Data from multiple linear regression model

In Table 2.6 the usefulness of each pre-breeding variable when used on its own for predicting various economically important outcomes are compared. For each outcome, RTS was a better predictor (explained more of the variation in the outcome) than any of the four other pre-breeding variables.

Table 2.6: Univariable predictive ability of five pre-breeding variables for some important production and reproduction outcomes in beef heifers

Predictor variable	Pregnancy after the first AI season[1]	Pregnancy after the subsequent AI season[1]	Days to first AI[2]	Days to calving[2]	Calf weaning weight[3]
RTS	0.67	0.66	0.03	0.05	0.05
BCS	0.56	0.53	0.03	0.02	<0.01
Weight	0.58	0.53	<0.01	<0.01	<0.01
Age	0.54	0.57	<0.01	<0.01	0.03
Kleiber ratio	0.51	0.42	<0.01	0.01	0.03

[1] Area under the curve for receiver operating characteristic analysis
[2] Pseudo-R^2 value for univariable Cox regression
[3] R^2 value for univariable linear regression

The AUC of the ROC curve for RTS (0.67) was significantly greater than that for age, BCS, KR ($P < 0.01$) and weight ($P = 0.045$). For prediction of pregnancy to the subsequent AI season the AUC of the ROC curve for RTS (0.66) was significantly greater than that for

BCS (*P* = 0.04), KR (*P* < 0.01), and weight (*P* = 0.02) but did not differ from the AUC for age (0.57) (*P* = 0.14).

2.5. Discussion

Some studies have clarified the basic principles of the onset of puberty (Day et al., 1984; Day et al.,1987; Foster, 1994). Puberty in cattle occurs when a certain level of somatic development (critical body weight) is reached, causing the pre-puberal negative feedback of oestradiol on the pituitary and/or hypothalamus to be terminated, and leading to the first ovulation. Environmental factors affecting the onset of puberty in heifers include nutrition, seasonal effects, climate and biostimulation (Pineda, 2003). Figure 2.1 summarises the factors affecting AP, and also the pathways through which AP influences production outcomes.

In this study RTS was associated with age, weight and BCS before the first breeding season, but it appears that in this group of heifers RTS was associated more strongly with age than with weight of the heifer (Tables 2.2 and 2.3). This is in contrast with the older theory that a heifer needs to reach a specific level of somatic development (weight) for the onset of puberty to be induced (Day et al., 1984; Day et al., 1987; Foster, 1994), and may indicate that there is an age-related induction of puberty which is not related to the weight of the heifer, in agreement with Yelich et al (1995) and Pence et al (2007). On the other hand, it may also indicate some variation in the critical weights of individual heifers that needed to be achieved to induce puberty, meaning that there was some scope for selection for AP in this population. Reproductive tract score was not associated with pre-breeding KR in this study as was shown using multiple regression, while age, weight and BCS all contributed to the variation in KR.

Reproductive tract score was associated with all important fertility and production outcomes in this study (Table 2.4), which is in agreement with previous studies (Andersen et al., 1991; Pence and BreDahl, 1998; Pence et al, 2007). In general, heifers with RTS 1 and 2 had significantly longer days to first AI and days to calving, and significantly lower pregnancy rates and calf weaning weights than those with RTS 4 and 5. After adjustment for weight and age, RTS showed a significant association with all the outcomes shown in Table 2.5, and these associations were not confounded by synchronisation group in this study. These results, along with those shown in Table 2.6, indicate that variation in RTS accounted for more of the variation in the fertility and production outcomes than did variation in weight, age or KR. This indicates that RTS represents a measure of the true genetic variation of AP within the population, which is in agreement with Pence et al. (2007).

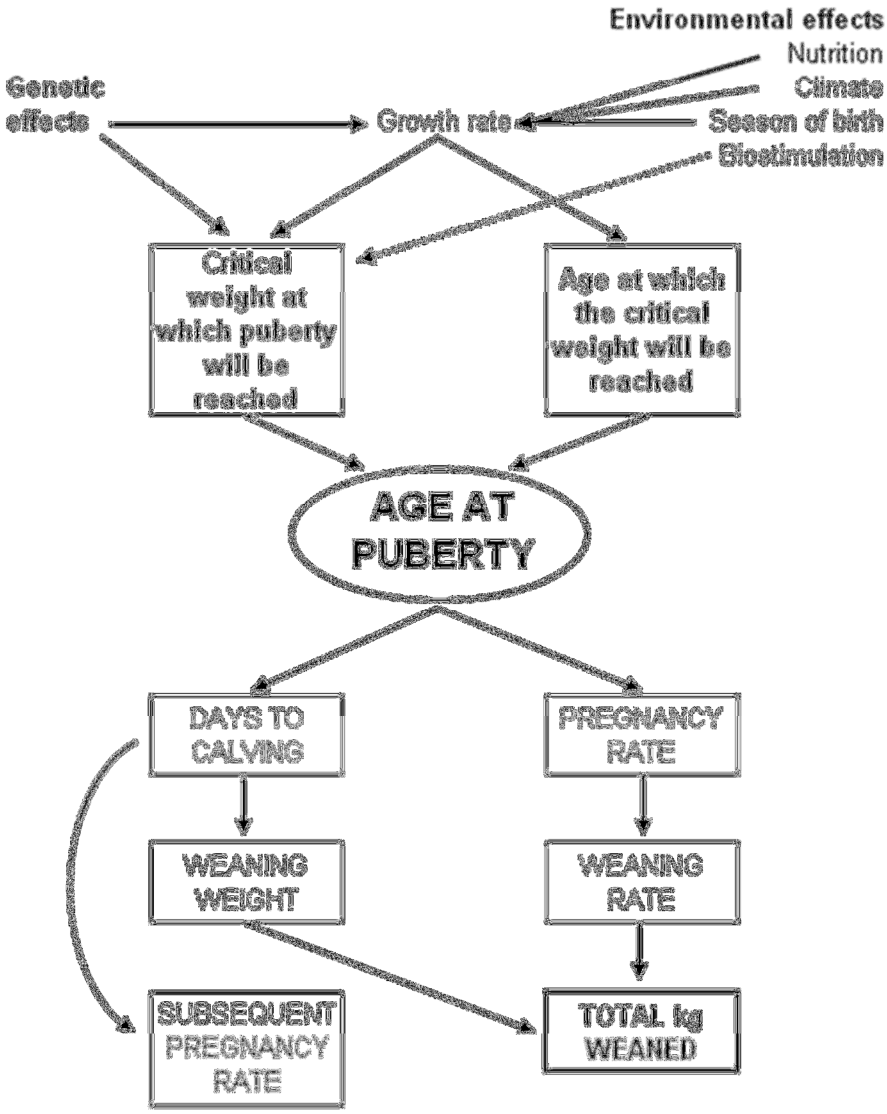

Figure 2.1: Diagram illustrating factors affecting age at puberty (AP), and the pathways through which AP may influence production outcome.

In this study RTS and BCS, which are the more subjective measurements (compared to weight and age), explained more of the variation in the fertility and production outcomes than did the objective measurements (Table 2.6). This supports the findings of Rosenkrans and Hardin (2003), that RTS has good accuracy despite less favourable repeatability. The subjectivity of RTS is not only caused by the less favourable repeatability, but also by the complexity of the scoring system. It is the experience of the authors that many heifers do not fit a particular RTS score exactly, and it is for the operator to decide which of the measures to weigh heaviest (Holm, 2006). More research is needed to clarify which of the different measures of the RTS system gives the best prediction of reproduction outcome, this may improve the accuracy of RTS. Ultrasound may also improve the repeatability of RTS.

2.5.1. Comparing RTS with other predictors of heifer performance

Reproductive tract score showed a consistently stronger association with fertility and production outcomes than did KR (Table 2.6). This is evidence that RTS can be used as a primary selection tool for heifers before the onset of breeding without any detrimental effect on production. The association of RTS with weaning weight of the offspring was mostly indirectly through its effect on days to calving. This was shown by the multiple regression model for weaning weight of the offspring: RTS was significantly associated with weaning weight of the offspring, but this association was not significant when days to calving was added to the model as predictor.

If RTS had been used as a selection criterion in this group of heifers before breeding, using RTS 2 as the cut-off point (from Table 2.4), thereby selecting the best 94% of heifers, the pregnancy rate to the 50 day AI season would not have increased (56% vs 58%, P = 0.79). Using RTS 3 as the cut-off point, thus 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 (62%) 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, as it depends on the timing of RTS and the proportion of heifers in the group that have reached puberty by that time. If the best 68% of heifers in this group were selected using KR, 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 is well demonstrated by this, despite the fact that day -1 was probably not the best time to use RTS as selection tool in this group of heifers. Although speculative, one could suspect that scoring heifers one or two months earlier may have resulted in stronger associations with the outcome. More research is needed to determine the best time to do RTS on yearling heifers as a selection tool for fertility.

Receiver-operating characteristic (ROC) analysis is a useful tool to compare the predictive ability 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. The AUC of the ROC curve provides a summary of the overall ability of a diagnostic test or predictor variable to correctly classify or predict a binary outcome. In this study the AUC can be interpreted as the probability that a randomly chosen pregnant (to the 50-day AI period) heifer had a greater pre-breeding RTS than a rondomly chosen non-pregnant heifer. It is clear that, although RTS was nowhere near perfect (AUC = 1), it was significantly better than that of any of the other measures (Table 6). On the other hand the AUC for BCS, age and KR were not significantly different from 0.5, indicating no predictive ability.

2.5.2. Long term benefits of using RTS as selection tool

Selecting for RTS leads to a reduction in days to calving (Table 2.4), which allows heifers more time to recover from the stress of calving and to become prepared for the next breeding season. First calf cows are known to be the group under most pressure to re-conceive 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 detriment of fertility (Chenoweth and Sandersen, 2001). Reproductive tract score was shown in this study to influence not only the immediate calving season, but also the pregnancy rate to the subsequent breeding season (Table 2.4). It was shown in this study that the association of pre-breeding RTS with the pregnancy rate to the second breeding season was not direct, but was confounded by the association between RTS and days to calving during this first 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, 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 Sandersen, 2001) and was also shown using these data (Holm, 2006).

It can be seen here that one should take into account not only the direct benefit of using RTS as selection tool for heifers, but also the effect that selection using RTS will have during the subsequent breeding seasons, and therefore on lifetime production of the cows.

Due to its ease of measurement, good heritability and association with feed conversion ratio (Nkrumah, 2004), KR has been used as an important selection tool for replacement heifers. Evidence from this study suggests that selecting for RTS will not select against production measures such as KR, due to their poor association with each other. However, RTS is primarily an indicator of age at puberty, and could be used in addition to production parameters such as KR in a selection policy.

2.6. Conclusion

Reproductive tract score before the onset of the breeding season is a predictor of heifer reproductive performance, even after adjustment for age, weight and BCS. It is a better predictor of fertility than other traits commonly used (weight, BCS, KR), compares well with these traits in predicting production outcomes, and is likely to be a predictor of lifetime production of the cow.

CHAPTER THREE

FACTORS AFFECTING THE USEFULNESS OF REPRODUCTIVE TRACT SCORING AS A CULLING TOOL ANALYSED BY LONG TERM REPRODUCTIVE PERFORMANCE IN BEEF HEIFERS

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3.1. Abstract

In a 7-year longitudinal study 292 beef cows in a restricted breeding system were observed from 1-2 d before their first breeding season, when reproductive tract scoring (RTS) was performed, until weaning their 5th calves. The objectives were to determine whether pre-breeding RTS in heifers is a valid tool to predict long-term reproductive performance, and secondly to investigate factors that may influence its predictive ability. Outcomes measured were failure to show oestrus during the first 24 d of the first 50-day AI season, failure to become pregnant during each yearly AI season (reproductive failure), days to calving from the start of each calving season, and years to reproductive failure. The effect of RTS on each outcome was adjusted for year of birth, pre-breeding age, BW and BCS, and for 24-day anoestrus, bull, gestation length, previous days to calving and previous cow efficiency index where applicable. During their first breeding season, heifers with RTS 1 were more likely to be in anoestrus for the first 24 d (OR 6.1, 95% CI 2.2, 16.7), and were also more likely to fail to become pregnant even after adjusting for 24-day anoestrus (OR 3.2, 95% CI 1.2, 8.6), compared to those with RTS 4 or 5. Animals with RTS 1 or 2 were at increased risk of early reproductive failure compared to those with RTS 4 or 5 (HR = 1.4, 95% CI 1.0, 1.9) despite the fact that RTS was not associated with calving rate or days to calving after the second calving season. Although RTS at a threshold of 1 had consistent specificity of 94-95% for both 24-day anoestrus and pregnancy failure, its predictive value was lower in the age cohort with a higher prevalence of anoestrus. Most animals with RTS 1 or 2 that were subsequently detected in oestrus were in early to mid di-oestrus at the time of scoring; repeating RTS on low scoring animals after 7 d may therefore improve specificity. We conclude that RTS is a valid culling tool to improve long-term reproductive success in a seasonal breeding system, by excluding heifers that are likely to fail to become pregnant or likely to calve late in their first calving season. We further conclude that the predictive value of RTS decreases with increasing prevalence of anoestrus and at certain stages of the oestrous cycle, and that RTS may predict pregnancy failure due to causes other than anoestrus.

Keywords: beef cattle; culling; fertility; heifer selection; predictive ability; reproductive tract score

3.2. Introduction

Reproductive traits are 10 times more economically important than production traits in beef cows (Wiltbank, 1994). Restricted breeding and calving during the optimal season are key principles in good cow-calf management (Denham et al., 1991, Engelken et al., 1991). Proper management and selection of heifers (using BW, conformation, EBV, reproductive tract score and pelvimetry) before breeding are essential to the success of such systems (Grass et al., 1982, Larson, 2005).

The onset of puberty in heifers is initiated by a decrease in oestradiol receptors in the hypothalamus and pituitary, ending the prepubertal negative feed-back and resulting in the first LH surge and ovulation (Day et al., 1984, Day et al., 1987). This shift occurs at a specific critical BW (as a proportion of adult BW) and critical age which varies amongst animals (Pence et al., 2007). Various factors affect the age at puberty in individuals, and reproductive tract scoring (RTS) provides an indirect measure of pubertal development (Andersen et al., 1991, Pence and BreDahl, 1998, Holm et al., 2009). Weaknesses of RTS include imperfect repeatability, subjectiveness and inconsistent associations with reproductive outcome (Rosenkrans and Hardin, 2003, Holm et al., 2009).

Short term reproductive performance may be predicted by RTS (Andersen et al., 1991, Pence et al., 2007, Holm et al., 2009) and we hypothesised that RTS may predict long-term survival in restricted bred heifers due to its association with pregnancy outcome and days to calving after first breeding, combined with reports that heifers calving early tend to calve early in subsequent seasons and have increased lifetime production (Lesmeister et al., 1973, Pence et al., 2007, Stevenson et al., 2008, Cushman et al., 2013). To the knowledge of the authors, a long-term study of the performance of heifers by RTS category has not been reported.

The objectives of this study were to determine the usefulness of RTS as predictor of long-term reproductive performance, and to investigate factors that may influence its predictive value.

3.3. Materials and methods

This was an observational study of 292 uniquely identified Bovelder beef cows born in either 2002 or 2003 (2002 and 2003 cohorts) that were followed from just prior to their first breeding season until they had weaned up to five calves. The farming system and breed type have been described previously (Paterson et al., 1980, Schoeman and Jordaan, 1998, Holm et al., 2008, 2009).

Reproductive tract scoring by transrectal palpation using a 5-point scale was performed on all heifers either 1 or 2 d before the onset of their first breeding season (Andersen et al., 1991). Scores 4 and 5 were combined in the analyses, after assuming that both categories were pubertal at the time of scoring (Stevenson et al., 2008), and were used as the reference category in Cox proportional hazards and logistic regression models. It was further assumed that heifers with RTS 1 or 2 were prepubertal, whereas those with RTS 3 were peripubertal (Stevenson et al., 2008). Body condition score (BCS) was determined at the same time using a 9-point scale (Marston, 2005). For the purpose of regression models and survival analysis, BCS was categorised into 2 categories: BCS $\leq$ 6 and BCS $\geq$ 7. Farm management and staff were blinded to RTS and BCS data throughout the study.

Animals with parity 0, 1, 2, and $\geq$ 3 were managed in separate groups, and a single inseminator was assigned to each group. The breeding season for heifers started on 15 October every year and consisted of 50 d of continuous visual oestrus observation, with once daily AI at 09h00. The breeding season for cows started on 1 November and consisted of 60 d of oestrus observation and AI in a similar way. Inter-oestrus periods of nulliparous heifers ranged from 16 to 24 d (mean 20 d). Days to first oestrus was defined as either the days to first insemination if it resulted in a pregnancy, or the days to the first insemination that was followed by a normal (16 to 24 d) inter-oestrus interval, or if neither of the above occurred it was the days to the last insemination. An animal was defined in oestrus or met-oestrus at the time of RTS if days from RTS to first oestrus ranged from 18 to 24 d. Similarly she was defined in early di-oestrus on the day of RTS if days to first oestrus ranged from 14 to 17 d, mid-cycle for days to oestrus from 9 to 13 d, late di-oestrus for days to oestrus from 5 to 8 d and pro-oestrus if days to first oestrus ranged from 1 to 4 d. If a heifer's days to first oestrus was more than 24 d, it was assumed that she was not yet cycling on the day of scoring, and this was defined as 24-day anoestrus.

Bulls were placed with cows for a period of 42 d in a multisire system at a maximum ratio of 1:40 cows, starting 5 to 10 d after the end of the AI period. All the bulls used for natural breeding and AI originated from the same herd and were allocated to cows based on growth performance and conformation, while controlling for inbreeding. Semen for AI was collected, processed and stored in a purpose-built facility on the farm. Seventeen AI bulls were allocated to 10 to 30 heifers each, and the ratio decreased to 1 to 10 cows per AI bull by the fifth parity.

Pregnancy diagnoses (PD) were performed by transrectal palpation (Sheldon and Noakes, 2002) between 23 March and 26 April of every year. Artificial insemination records of cows were available to the veterinarian during pregnancy diagnosis to assist in the differentiation between AI and clean-up bull pregnancies. Animals that were not pregnant to the AI season, as well as those that aborted, or that were confirmed pregnant to AI but failed to calve during the calving season, were sold as soon as their status was known.

Data collected during every AI and calving season included the following: bull allocated, first to fourth AI day (numbered from the first day of the AI season), pregnancy diagnosis, abortion and culling dates, calving date, dystocia score, twinning data, calf gender, calf BW at birth and BW of the cow and calf at weaning. Cow efficiency index (CEI) determined at each weaning event was defined as the weaning weight of the calf corrected to an age of 205 d divided by the metabolic weight of the cow at weaning ($BW^{0.75}$) (Kleiber, 1947).

Days to pregnancy was defined as the days from the start of the AI season to the last insemination for animals that were confirmed pregnant after the end of the breeding season. Gestation length (GL) was defined as the number of days from the last recorded AI until calving. Animals with GL < 266 d were either changed from “calved” to “aborted” if the birth weight of the calf was below 25kg and the calf did not survive, otherwise an earlier conception date was assigned if this was available from the AI records, or else the GL data was removed if neither was possible. For animals with GL > 299 d the pregnancy diagnosis data was changed from pregnant to not pregnant, as we assumed that the cow did not conceive during the AI season, or if the only recorded AI was early in the season and the calving date was too early for a bull pregnancy, the GL data was removed, in which case it was assumed that additional AI’s performed were not recorded.

Days to calving was defined as the number of days from the start of the calving season until each cow calved, and the first day of the calving season was defined as the day on which the first cow calved within each age cohort.

The study was terminated after the fifth intercalving interval had occurred for all remaining cows, which occurred in April 2009 and April 2010 for the 2002 and 2003 cohorts, respectively.

3.3.1. Analytical procedures

Data were analysed using NCSS 2007 (NCSS, Kaysville, UT, USA) and STATA 11.1 (StataCorp, Texas, USA). Independent proportions, means and medians were compared using the Fisher exact test, ANOVA and Kruskal-Wallis ANOVA, respectively. Standard deviations were provided with the means. Pregnancy proportions, RTS, BW and age of heifers were compared between different AI bulls used during the first breeding season.

Reproductive tract score, being the variable of interest, was initially used in univariable models of days to calving and pregnancy outcome for the first to the fifth breeding season, for each heifer cohort as well as for the combined data. The individual effects of other possible covariates were also estimated (pre-breeding age, BW, BCS and GL, and also the preceding season's days to calving and CEI in the case of the second to fifth calving seasons), whereafter the effect of RTS on the outcome was adjusted for covariates that were significant ($P < 0.05$) predictors on their own, using multivariable models. Artificial insemination bull was added as a random effect to the logistic regression models of pregnancy failure during the first AI season. The fit of the logistic regression models of pregnancy failure during the first AI season was evaluated using the Hosmer-Lemeshow goodness-of-fit test.

For the Cox regression model of years to reproductive failure, reproductive failure (the event of interest) was defined as a negative pregnancy diagnosis after the limited AI breeding season. Observations for reproductive failure were done once every year on the day of pregnancy diagnosis, and all data from animals that had left the herd since the previous observation (or the start of the study in the case of the first pregnancy diagnosis) were interval censored to the following day of pregnancy diagnosis. Censored data included those from cows that had aborted their previous pregnancy, cows that died or cows that were culled for any other reason. Artificial insemination bull used during the first AI season was added as a shared frailty to the Cox regression model of years to reproductive failure, whereas the proportional hazards assumption of the model was evaluated using Schoenfeld residuals, and by evaluating the log cumulative hazards plot of the curves of the RTS categories. Data from cows that were still in the herd (and confirmed pregnant) at the study termination were right censored to the last observation (Dohoo et al, 2003c).

Sensitivity (Se), specificity (Sp) and positive predictive value (PV+) were calculated for the ability of RTS 1, or 1 and 2 combined, to predict either anoestrus or pregnancy failure.

3.4. Results

Heifers with low RTS had higher rates of 24-day anoestrus and pregnancy failure when compared to those with higher RTS (Table 3.1). Lower RTS was independently associated with an increased odds of 24-day anoestrus in both years (Table 3.2). None of the other variables was independently associated with 24-day anoestrus even when RTS was removed from the models (*P* > 0.05), whereas most of the odds ratios presented in Table 3.2 changed by more than 30% when RTS was removed from the models.

Table 3.1: Outcomes of different categories of pre-breeding RTS after the 50-day heifer breeding season, by year of birth.

RTS	24-day anoestrus		Days to pregnancy[1]		Pregnancy failure	
category	2002 cohort	2003 cohort	2002 cohort	2003 cohort	2002 cohort	2003 cohort
1	4/13 (31%)^{a,b}	8/12 (67%)^{a}	24^{a,b} (14.5 - 26.5)	13.5^{a} (9.5 - 38)	8/13 (62%)^{a,b}	8/12 (67%)^{a}
2	11/43 (26%)^{a}	9/34 (26%)^{b}	22^{a} (16 - 39)	16.5^{a} (7 - 29)	25/43 (58%)^{a}	10/34 (29%)^{b}
3	12/53 (23%)^{a}	4/30 (13%)^{b}	13^{b,c} (3 - 26.5)	14^{a} (8.5 - 31.5)	28/53 (53%)^{a}	5/30 (17%)^{b}
4 – 5	7/75 (9%)^{b}	6/32 (19%)^{b}	9^{c} (3 - 15)	15^{a} (5 - 31)	24/75 (32%)^{b}	9/32 (28%)^{b}
Total	33/184 (18%)^{A}	28/108 (26%)^{A}	12^{B} (4 - 25)	15^{B} (7 - 31)	85/184 (46%)^{C}	32/108 (30%)^{D}

[1]Median (interquartile range)
[a, b]Proportions or medians within columns with different superscripts differ significantly (*P* < 0.05)
[A,B]Proportions or medians within rows with different superscripts differ significantly (*P* < 0.05)

Without adjusting for 24-day anoestrus, all heifers in the study with RTS 1 and RTS 2 (compared to RTS 4 and 5 combined) were more likely to fail to become pregnant during their first AI season (OR = 4.7, *P* = 0.002 and OR = 2.1, *P* = 0.025, respectively). After adjusting for 24-day anoestrus, pre-breeding BW and age, the associations became weaker, but were still significant, particularly in heifers born in 2002 (Table 3.3). Even when adjusting for anoestrus during the entire 50-day AI season, heifers with RTS 1 and 2 combined were more likely to fail to become pregnant than those with RTS 4 and 5 combined (OR = 1.9, *P* = 0.043). The random effect of AI bull in the logistic regression model of pregnancy failure during the first AI season was not significant (*P* = 1.000), and the fit of the logistic regression model of pregnancy failure after the first AI season was adequate (Hosmer-Lemeshow *P* = 0.129).

Table 3.2: Multivariable logistic regression models of factors associated with 24-day anoestrus, for the two birth cohorts separately and combined.

	2002 cohort			2003 cohort			Combined[1]		
Predictor	**OR[2]**	**95% CI**		**OR[2]**	**95% CI**		**OR[2]**	**95% CI**	
RTS 1	4.7	1.1	20.0	9.4	1.9	45.2	6.1	2.2	16.7
RTS 2	3.5	1.2	10.3	1.6	0.5	5.3	2.4	1.1	5.3
RTS 3	2.4	0.9	6.8	0.7	0.2	2.7	1.5	0.7	3.5
RTS 4 + 5	1.0	-	-	1.0	-	-	1.0	-	-
BW (per 10kg)	1.0	0.9	1.2	1.0	0.8	1.2	1.0	0.9	1.1
Age (w)	1.0	0.9	1.2	0.9	0.7	1.2	1.0	0.9	1.1
BCS 6 vs 7	1.7	0.7	3.8	0.7	0.3	2.0	1.3	0.7	2.3
Year of birth 2002 vs 2003	-	-	-	-	-	-	0.8	0.4	1.4

[1]2002 and 2003 birth cohorts combined
[2]Odds ratio

Heifers with RTS 1 had a lower calving rate during the first calving season than those with RTS 2, 3 and 4 and 5 combined, and heifers with RTS 4 and 5 combined calved earlier than heifers with RTS 2 and 3 (Table 3.4). In the second calving season, the remaining heifers with initial RTS 4 and 5 combined had a higher calving rate than those with RTS 2 or 3, and heifers with initial RTS 2 calved later in the second calving season than those with RTS 3 (Table 3.4). From the third calving season onwards, calving rates and days to calving in animals that remained in the herd did not follow any particular pattern (Table 3.4).

In the Cox regression model of days to first calving, heifers with RTS 3 were less likely to calve early in their first calving season than those with RTS 4 and 5, adjusted for the occurrence of oestrus during the first 24 d of the breeding season. Neither pre-breeding BW, age, BCS, RTS, previous days to calving nor CEI was consistently associated with pregnancy failure or days to calving in the second to fifth calving seasons. Only GL was a consistent predictor of days to calving in all calving seasons.

Table 3.3: Multivariable logistic regression models of factors associated with pregnancy failure after the first 50-day AI season, for the two birth cohorts separately and combined.

	2002 cohort			2003 cohort			Combined[1]		
Predictor	**OR[2]**	**95% CI**		**OR[2]**	**95% CI**		**OR[2]**	**95% CI**	
RTS 1	3.1	0.9	11.1	2.1	0.4	11.4	3.2	1.2	8.6
RTS 2	2.7	1.2	6.2	0.8	0.3	2.8	1.8	0.9	3.5
RTS 3	2.2	1.0	4.6	0.5	0.1	2.1	1.4	0.8	2.7
RTS 4 + 5	1.0	-	-	1.0	-	-	1.0	-	-
BW (per 10kg)	0.9	0.8	1.0	1.0	0.8	1.2	0.9	0.8	1.0
Age (w)	1.0	0.9	1.2	1.0	0.8	1.3	1.0	0.9	1.1
24-day anoestrus	2.0	0.9	4.4	10.1	3.5	29.4	4.0	2.1	7.7
Year of birth 2002 vs 2003	-	-	-	-	-	-	2.6	1.4	4.5

[1]2002 and 2003 birth cohorts combined
[2]Odds ratio

Table 3.4: Calving rate and median days to calving, by calving season, for different pre-breeding RTS categories.

Pre-breeding RTS	**First[1]**		**Second[1]**		**Third[1]**		**Fourth[1]**		**Fifth[1]**	
	calv rate[2]	**d to calv[3]**	**calv rate**	**d to calv**	**calv rate**	**d to calv**	**calv rate**	**d to calv**	**calv rate**	**d to calv**
1						24.5[a,b] (18-35)	3/6 (50%)[a]	17[a] (12-23)	2/3 67%[a]	20[a] (2-38)
1 – 2	48/102 (47%)[a]	299[a] (288-313)	31/48 (65%)[a]	306[a] (298-314)	24/31 (77%)[a]	27.5[a,b] (19-44)	13/18 72%[a]	30[a,b] (19-50)	8/13 62%[a]	28.5[a] (16-50)
3	45/83 (54%)[a,b]	298[a,b] (289-312)	31/45 (69%)[a]	293[b] (289-311)	21/31 (68%)[a]	22[a] (9-36)	16/21 76%[a]	29[b] (20-44)	10/16 63%[a]	20[a] (11-41)
4 – 5	69/107 (64%)[b]	292[b] (286-304)	55/69 (80%)[a]	301[a,b] (294-315)	36/55 (65%)[a]	32.5[b] (21-43)	24/36 67%[a]	27.5[a,b] (19-39)	16/24 67%[a]	18[a] (14-38)

[1]First to fifth calving season
[2]Calving rate as a proportion of the total number of heifers or cows bred
[3]Median days to calving (interquartile range)
[a, b]Proportions or medians within columns with different superscripts differ significantly (*P* < 0.05)

In univariable Cox regression models of years to reproductive failure, 24-day anoestrus during the first breeding season was the only consistent predictor in both birth cohorts (*P* = 0.036 and *P* < 0.001 for 2002 and 2003, respectively). When only pre-breeding measures were considered in a multivariable Cox regression model, RTS categories 1 and 2 combined (relative to RTS 4 and 5), and pre-breeding BW predicted years to reproductive failure independently after adjusting for year of birth (Table 3.5). The model did not violate the proportional hazards assumption (*P* = 0.326), and log cumulative hazards plots of the RTS categories remained reasonably parallel up to year 4. The bull assigned during the first AI season did not have a significant effect as shared frailty (*P* = 0.444).

Table 3.5: Multivariable Cox regression model of factors associated with the number of years to reproductive failure.

	Years to reproductive failure			
Predictor	**HR[1]**	**95% CI**		***P***
RTS 1 + 2	1.4	1.0	1.9	0.045
RTS 3	1.3	0.9	1.8	0.184
RTS 4 + 5	1.0	-	-	-
BW (per 10 kg)	0.9	0.9	1.0	0.043
Year of birth 2002	1.4	1.1	1.9	0.020

[1]Hazard ratio

The 2002 cohort of heifers was older (mean age 428.6±19.9 d vs. 420.8±14.7 d, *P* < 0.001), but lighter (mean BW 315.8±26.4 kg vs. 324.0±25.9 kg, *P* = 0.010) than those born in 2003 at the start of their first breeding season. In the 2002 cohort there were low but significant correlations between pre-breeding age and BW (Pearson's *r* = 0.32, *P* < 0.05), age and RTS (*r* = 0.29, *P* < 0.05), BCS and BW (*r* = 0.30, *P* < 0.05) and BCS and age (*r* = 0.21, *P* < 0.05). In the 2003 cohort none of the pre-breeding variables were significantly correlated. In the 2002 cohort fewer heifers were prepubertal (RTS 1 or 2) than in the 2003 cohort (56/184 vs. 46/108, *P* = 0.042) and more heifers tended to be pubertal (75/184 vs. 32/108, *P* = 0.060).

In the 2002 cohort, fewer heifers had inter-oestrus periods shorter than 16 d during the first breeding season than in the 2003 cohort (32/106 vs. 36/73, *P* = 0.012), whereas the proportion of inter-oestrus periods longer than 24 d was similar (19/106 vs. 7/73, *P* = 0.136). Median days to first oestrus, to first pregnancy and to first calving decreased with increasing RTS category in the 2002 cohort but this was not the case for the 2003 cohort (Table 3.1).

Reproductive tract score 1 had a Sp of 94-95% for 24-day anoestrus or pregnancy failure during the first breeding season. Increasing the cut-off level to RTS 2 decreased the Sp by at least 25%. The PV+ of RTS for 24-day anoestrus and pregnancy failure varied, depending on the true incidence of the outcome (Table 3.6).

Table 3.6: Ability of low RTS to predict anoestrus in the first 24 days of the breeding season or to predict failure to become pregnant during the first 50-day AI season, by birth cohort.

Outcome	Year of birth	True incidence	RTS	Culling %[1]	Se[2]	Sp[3]	PV+[4]
24-day anoestrus	2002	18%	1	7%	0.12	0.94	0.31
			1-2	30%	0.42	0.72	0.25
	2003	26%	1	11%	0.29	0.95	0.67
			1-2	43%	0.64	0.65	0.39
50-day pregnancy failure	2002	46%	1	7%	0.09	0.95	0.62
			1-2	30%	0.39	0.77	0.59
	2003	30%	1	11%	0.25	0.95	0.67
			1-2	43%	0.56	0.63	0.39

[1]Percentage animals that would be culled based on low RTS
[2]Sensitivity
[2]Specificity
[3]Positive predictive value

Fifty-seven of 77 heifers (74%) with RTS 2 showed true oestrus before d 24 of the first AI season, being similar to the rate for all heifers in the study (231/292; 79%). Of the 17 heifers that were never inseminated during the first AI season, none had scores 4 or 5.

Table 3.7: Number of heifers in each RTS category by number of days from scoring to the first occurrence of true oestrus (2003 cohort), with calculated stage of the oestrous cycle at scoring.

	Days to first true oestrus							
	1-4	**5-8**	**9-13**	**14-17**	**18-24**	**> 24**	**No oestrus**	**Total**
	Calculated stage of cycle at scoring[1]							
RTS	**Pro-oestrus**	**Late di-oestrus**	**Mid-cycle**	**Early di-oestrus**	**Oestrus or met-oestrus**	**Peri-pubertal**	**Pre-pubertal**	
1	0	0	2	2	0	2	6	12
2	4	6	1	10	3	7	3	34
3	5	3	9	6	3	4	0	30
4	4	2	2	6	6	6	0	26
5	2	2	1	0	1	0	0	6
Total	**15**	**13**	**15**	**24**	**13**	**19**	**9**	**108**

In the 2003 cohort the highest proportion of heifers with RTS 1 never showed oestrus during the 50-day AI season, whereas the highest proportions of heifers with RTS 2 and 3 were calculated to be in early di-oestrus and mid-cycle respectively at the time of scoring (Table 3.7). In the 2002 cohort this pattern was less evident, but there was a significant natural synchronisation that occurred: 48 of the 151 cycling heifers (32%) had their first oestrus during the first 4 d of the breeding season, whilst the expected proportion in an unsynchronised group is 4/20 (20%, or in this case 30/151, $P = 0.025$).

3.5. Discussion

3.5.1. RTS as predictor of long-term reproductive performance

In heifers born in both years RTS was associated with 24-day anoestrus independent of pre-breeding BW, age or BCS. This supports the theory that the BW and age when puberty is reached varies between animals (Pineda et al, 2003), even in a uniform group such as the animals in our study. In this study RTS predicted the outcome of the first breeding season, which has a very significant effect on the long-term survival of cows in a seasonal breeding system with a strict culling policy based on reproductive failure. Reproductive tract score predicted the number of years to reproductive failure independent of other pre-breeding measures (Table 3.5). However, RTS was not a predictor of pregnancy rate or days to calving in the remaining animals from the third to fifth calving season, and the effect of RTS on long-term reproductive failure appears to be determined by the outcome of the first two breeding seasons only (Table 3.4). It may be that the strict application of culling after reproductive failure in this system reduced variability in the animals surviving to subsequent seasons to the point that we were unable to draw any further conclusions. Of the original 39 animals with RTS 1 in this study, only 3 survived to the start of their fifth breeding season, supporting this statement. Further studies are needed to test the hypothesis that heifers with lower RTS have inferior reproductive performance in non-seasonal systems.

3.5.2. Factors affecting the predictive ability of RTS

The lack of clear correlations between pre-breeding measures in the 2003 cohort, as well as the fact that the 2003 cohort was heavier despite being younger than the 2002 cohort prior to breeding suggests different culling practices and growth rates in the heifers of the two age cohorts.

The higher proportion of short (< 16 d) inter-oestrus periods recorded in the first breeding season of the 2003 cohort compared to the 2002 cohort suggests that either there were more heifers with truly short inter-oestrus intervals in the 2003 cohort, or oestrus observation accuracy differed between years. Although not significantly different, the proportion of longer (> 24 d) inter-oestrus periods recorded in the 2003 cohort was nominally lower. We therefore assume that oestrus observation was most likely more sensitive and less specific in the 2003 cohort than in the 2002 cohort.

Lower RTS scores were generally associated with increased rates of 24-day anoestrus in both years of birth in this study. In the 2003 cohort, however, the higher rate of 24-day anoestrus was poorly predicted by RTS scores 2 to 5, when compared to the same results for the 2002 cohort. In particular, heifers with RTS 4 and 5 in the 2003 cohort had an unexpectedly high rate of anoestrus, despite the fact that oestrus observation sensitivity (Se) was likely higher. We therefore conclude that the incidence of true anoestrus was higher in the 2003 than in the 2002 cohort. This may have been caused by the younger age of the 2003 cohort or by differences in environmental or management factors during the first breeding seasons of the two cohorts (Larson, 2005).

The uniformity of the 2003 cohort due to apparently appropriate management and pre-selection may have influenced our ability to show significant differences between animals in this group. Although the same veterinarian applied RTS in the two cohorts, it is possible that due to the subjective nature of the RTS system the veterinarian expected to find a certain level of variability within the group, and may inadvertently have adjusted the categories to suit the group of animals. In addition to this, different growth rates between the two cohorts may have led to different rates of sexual development after RTS was applied, particularly in animals with RTS scores 2 and 3. The higher pre-breeding growth rate of the 2003 cohort (evidenced by their higher BW and lower age) suggests that if this trend continued into the first AI season, animals with RTS scores 2 and 3 may have developed significantly between the application of RTS and the end of the AI season.

Despite the lower rate of anoestrus recorded during the first 24 d of the breeding season in the 2002 cohort, the rate of pregnancy failure after the first 50-day AI season was higher in this group, and we therefore assume that other unmeasured factors, not related to cyclicity of the heifers, influenced fertility per insemination in this group.

The incidence of 24-day anoestrus was relatively low in the 2002 cohort, and it appears that anoestrus was mostly as a result of animals not having reached puberty at the onset of breeding, as indicated by the good ability of RTS 1, 2 and 3 to predict anoestrus relative to scores 4 and 5. Although other factors leading to reproductive failure seem to have existed in the first breeding season of the 2002 cohort, it appears from our analyses that RTS had the ability to predict pregnancy failure not only due to the failure to cycle during the first 24 d of the breeding season, but also due to other, unknown factors. It may be reasoned that these other factors may simply be the occurrence of anoestrus for the entire 50-day breeding season, however in multivariable analysis it was found that RTS predicted pregnancy failure also after adjusting for anoestrus during the entire 50-day AI season. This is supported by the fact that heifers with lower RTS tended to calve later in their first calving season, even after adjusting for 24-day anoestrus. The other factors leading to pregnancy failure in this breeding season could not be determined, making it difficult to speculate about the association between RTS and fertility which lies beyond cyclicity. Potential confounding due to differing oestrus observation accuracy between the two years cannot be ruled out.

In the 2003 cohort RTS did not predict pregnancy failure as accurately as in the 2002 cohort. Also, in the 2003 cohort RTS did not predict pregnancy failure after correction for anoestrus. It is therefore assumed that other factors leading to animals failing to show oestrus during the first 24 d of the breeding season, and that were not measurable by RTS, existed in this cohort. These may have included management factors such as nutrition, as well as other environmental factors such as weather (Larson, 2005). However, in this cohort there was a very strong association between 24-day anoestrus and pregnancy failure, and it appears that there were very few other factors leading to pregnancy failure except for heifers that were not observed in oestrus. It is thus concluded that for these two reasons RTS could not predict pregnancy failure as accurately as in the heifers born in 2002.

The Sp of RTS for 24-day anoestrus was coincidentally very similar to the Sp at the same cut-off for 50-day pregnancy failure within year of birth, and also similar between years of birth. However, the PV+ of RTS for pregnancy failure in the 2002 cohort was higher than the PV+ for 24-day anoestrus at the same RTS threshold. This occurred due to the higher incidence of pregnancy failure compared to 24-day anoestrus (Dohoo et al., 2003a), combined with the apparent ability of RTS to predict pregnancy failure independent of its association with cyclicity in this age cohort.

For the 2003 cohort the Se, Sp and PV+ of the lower two RTS thresholds were very similar for 24-day anoestrus and pregnancy failure, and this happened where the true prevalence of anoestrus during the first 24 d was similar to the true prevalence of pregnancy failure after the first 50-day breeding season. It seems thus that the ability of RTS to predict pregnancy outcome is firstly influenced by the true prevalence of anoestrus, and secondly by other factors causing pregnancy failure.

If we consider RTS as a test for anoestrus or late onset oestrus (with the threshold between RTS 2 and 3), then most animals with late onset oestrus were correctly classified as RTS 1 or 2. Cycling animals were present, however, and occurred in particular in the RTS 2 category. In the 2002 cohort these "false positives" did not follow any particular pattern, although in the 2003 cohort there seems to be an over representation of RTS 2 category heifers that were in fact in early di-oestrus at the time of scoring. This is a time of the oestrous cycle when the new CL after ovulation may still be small and embedded within the ovary, and therefore not easily palpable (Rosenkrans and Hardin, 2003, Fernández Sánches, 2008), particularly in the case of peripubertal heifers. Simultaneously, the new follicular wave after ovulation is developing, with the probability of detecting palpable follicles being low (Fernández Sánches, 2008). The apparent natural synchronisation that occurred early in the first AI season of the 2002 cohort led to many heifers in pro-oestrus at the time of scoring which appeared to have decreased the chance of incorrectly assigning low RTS scores to cycling heifers.

Due to the fact that apparent misclassifications occurred, particularly in low scoring animals at certain stages of the oestrous cycle, we suggest that repeating RTS 7 d later in low scoring animals may improve the Sp of the test.

3.6. Conclusions

Pre-breeding RTS is a valid culling tool to exclude beef heifers in anoestrus in a seasonal breeding system. Although culling by RTS enhances long-term reproductive success of the herd, this is achieved by the effect on the pregnancy outcome of the first two breeding seasons only. The predictive ability of RTS decreases with increasing prevalence of anoestrus and with an increasing proportion of heifers in met-oestrus or early di-oestrus at the time of scoring, whereas RTS may also be associated with other factors affecting success of insemination, unrelated to cyclicity.

CHAPTER FOUR

A NEW APPLICATION OF PELVIS AREA DATA AS CULLING TOOL TO AID IN THE MANAGEMENT OF DYSTOCIA IN HEIFERS

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4.1. Abstract

Although foeto-maternal disproportion is the major cause of dystocia in heifers, pelvis area (PA) is not recommended as a culling tool due to its relatively low importance and genetic correlation with calf birth weight (BWT), the most important factor associated with dystocia. The objective of this observational study of 484 limited bred yearling beef heifers was to compare the effects of different methods of adjustment of PA data for culling to select against dystocia. Multivariable analyses were used to determine predictors of PA, calf BWT and dystocia. Hypothetical culling rates of 10 and 20% were then applied after ranking heifers by each of the following: unadjusted PA; PA adjusted to 365 d of age by a factor of 0.27 cm^2/d (APA); PA:BW ratio (PA:BW); PA adjusted to the median BW of the group using the regression coefficient of PA on BW within age group (BWPA); and PA similarly adjusted to the median lean BW (LBWPA). Dam parity, sire, pre-breeding age, pre-breeding BW and pre-breeding BCS were associated with PA whereas dam parity, sire, own BWT, PA, AI bull and calf gender were associated with calf BWT ($P < 0.05$). Dam parity, calf BWT and either BWPA or LBWPA were the only independent predictors of dystocia ($P < 0.05$). Adjusting PA to BW or LBW improved the sensitivity and specificity to predict dystocia. After hypothetical culling by PA, retained heifers were heavier, had a higher calving rate and calves tended to be heavier at birth compared to culled heifers, but dystocia rates were not different. Culling by APA resulted in similar effects, except that dystocia rate tended to be lower in retained heifers. Culling by PA:BW resulted in lower dystocia rate in retained than in culled heifers, but retained heifers had lower pre-breeding BW than culls. Culling by BWPA and LBWPA resulted in lower proportions with dystocia and a tendency towards higher calving rates in the retained heifers, without affecting the pre-breeding BW or calf BWT. It is concluded that pelvimetry is a useful culling tool to aid in the management of dystocia in yearling heifers, and that adjustment of PA to median BW or LBW within age group improves its accuracy and avoids the undesirable side-effects.

Keywords: beef cattle, culling, dystocia, heifer selection, pelvimetry, pelvis area

4.2. Introduction

Foeto-maternal disproportion is the major cause of dystocia in heifers (Price and Wiltbank, 1978a), and attempts to prevent it have focused mainly on reducing the birth weight (BWT) of the calf (Andersen et al., 1993) and ensuring adequate breeding BW (King et al., 1993). Calf BWT and pelvis area (PA) contribute 33% and 12% respectively towards dystocia in heifers (Wolverton et al., 1991). Breed effect on the incidence of dystocia is attributed to differences in the relative calf BWT, pelvis structure and large variation in pelvis dimensions in some breeds (Price and Wiltbank, 1978a, Citek et al., 2011, Nogalski and Mordas, 2012). Heritability of calf BWT and PA are reported to be 0.44 and 0.46 respectively, but heritable traits predict calving ease poorly in individuals (Andersen et al., 1993, Van Donkersgoed 1997).

Cook et al. (1993) concluded that negative EPD for BWT in bulls was more effective in reducing dystocia rate and severity than culling heifers based on PA. Using bulls with low BWT or low BWT EPD to prevent dystocia has been challenged due to the correlation with lower growth rate and adult BW (Wolverton et al, 1991), and thus smaller PA. However, the benefit of increasing PA may in the same way be offset by increased calf BWT due to their genetic correlation (Andersen et al, 1993).

Adjusting PA to BW and to own BWT by ratios has been as disappointing as using raw PA for culling in heifers (Van Donkersgoed et al., 1993). Deutscher (1988) recommended culling of yearling heifers after adjustment of PA data to an age of 365 d by 0.27 cm^2/d of age, and a similar method is currently used in beef cattle management software in South Africa (BeefPro, BenguelaSoft CC, South Africa).

The objective of this study was to compare the accuracy and effects of different methods of adjustment of PA data for culling to select against dystocia.

4.3. Materials and methods

This was an observational study of 484 Bovelder beef heifers born in either 2006 or 2007 (2006 and 2007 birth cohorts) that were followed from just prior to their first breeding season until they had calved for the first time. The farming system, breed and location have been described previously (Paterson et al, 1980, Schoeman and Jordaan, 1998, Holm et al, 2008, 2009). Each animal in the study was uniquely identified.

Heifers were weighed within 1 month preceding the mating start date (MSD) (pre-breeding BW), and internal vertical diameter (VD) and transverse diameter (TD) of the pelvis were measured within the 7 d preceding the MSD by transrectal placement of a caliper type pelvimeter (Rice pelvimeter, Lane Manufacturing, Denver, Colorado) between the cranial end of the symphysis pelvina and the dorsal wall of the pelvis, and at the widest distance between the medial aspects of the corpora ossium iliorum, respectively (Wolverton et al, 1991, Cook et al., 1993, Van Donkersgoed, 1997). Body condition score (BCS) was determined at the same time using a 9-point scale (Marston, 2005). For the purpose of regression models, BCS was categorised into 2 approximately equal sized categories (<6 and ≥6) since relatively few animals had BCS <5 or >6. Farm management and staff were blinded to PA and BCS data throughout the trial.

The MSD was October 15 of each year and breeding consisted of a 50 d AI period followed 5 to 7 d later by a 42 d clean-up bull breeding period. Pregnancy diagnoses (PD) were performed by transrectal palpation (Sheldon and Noakes, 2002) on April 1 2008 (2006 cohort) and on March 3 2009 (2007 cohort). Animals that were estimated to have conceived during the bull breeding season and not during the AI season were sold as pregnant heifers.

Sire was defined as the sire of the heifer, and AI bull was the bull assigned to each heifer during the breeding season. All 7 and all 6 of the AI bulls allocated to the 2006 and 2007 birth cohorts respectively, and 40 of the 45 bulls that sired the heifers in this trial originated from the same herd. Bulls with own BWT up to a maximum of 33 kg were allocated to nulliparous heifers, and up to a maximum of 36 kg to primiparous cows, while controlling for inbreeding. In other data from this herd all bull calves born to 2 age cohorts of cows over 5 calving seasons (n = 393) had a median BWT of 36 kg (interquartile range 32 – 44 kg). This strategy to reduce the incidence and severity of dystocia in heifers and primiparous cows had been in use for approximately 25 years in the herd prior to this study, and heifers had never been selected based on PA prior to, or during this trial (R. J. Wood, Johannesburg Water, personal communication, 2007).

Farm data collected for each heifer included the following: dam parity, sire, birth date, BWT, occurrence of dystocia during her own birth, AI bull used, pregnancy diagnosis, calving date, calf BWT, calf gender and dystocia score (0 = no assistance, 1 = assistance required, 2 = surgical intervention required).

Pelvis area was calculated as the product of the VD and TD. Lean BW (LBW) was calculated for each heifer by adjusting the BW to a lean BWS (score 4, using the formula:

$$LBW = BW - [BCS - 4] \times b_1$$

where b_1 is the regression coefficient of BW on BCS within own birth cohort. In the same way, PA adjusted to BW (BWPA) and PA adjusted to LBW (LBWPA) were calculated for each heifer by adjusting to the median BW and median LBW within birth cohort, respectively, using the formulae:

$$BWPA = PA - [BW - Median(BW)] \times b_2$$

$$LBWPA = PA - [LBW - Median(LBW)] \times b_3$$

where b_2 is the regression coefficient of PA on BW, and b_3 is the regression coefficient of PA on LBW, within own birth cohort.

Finally, hypothetical culling was applied at 10 and 20% culling rates after ranking all the heifers enrolled at the start on each of the following five criteria: 1. Unadjusted PA (PA); 2. PA adjusted to 365 d of age by subtracting 0.27 cm^2 per day of age difference between each heifer's age and 365 d (APA) (Siemens et al., 1991, Price and Wiltbank 1978b); 3. PA:pre-breeding BW ratio (PA:BW) (Van Donkersgoed et al., 1993); 4. BWPA; and 5. LBWPA. Effects and side-effects of hypothetical culling after the different ranking procedures were determined by comparing pre-breeding BW, calving and dystocia rates, unassisted calving rate and calf BWT between those heifers that were culled and those that were retained, for each level of culling. Sensitivity (*Se*) and specificity (*Sp*) for the correct prediction of dystocia were also determined for each ranking procedure and for each culling level as follows:

$$Se = \text{number of culled heifers with dystocia} \div \text{total number of dystocia cases}$$

$$Sp = \text{number of retained heifers with unassisted births} \div \text{total number of unassisted births}$$

4.3.1. Analytical procedures

Data of the 2 birth cohorts were pooled, and subsequently analysed using NCSS 2007 (NCSS, Kaysville, UT, USA) and STATA 11.1 (StataCorp, College Station, TX, USA). Proportions were compared using the Fisher's exact test in the case of independent proportions, and means were compared using ANOVA with the Tukey-Kramer multiple comparison test.

Multiple regression models of PA, VD, TD and calf BWT, and logistic regression models of dystocia were constructed by first adding all available predictors as covariates. Initial covariates in the models of PA, VD and TD were parity of the dam (1, 2, ≥3), BWT of the heifer, pre-breeding BW, and BCS and age at pre-breeding examination. Initial covariates in the model of calf BWT were the same as for the model of PA except that age at calving replaced pre-breeding age, and calf gender and PA were also included as covariates. Initial covariates in the logistic regression model of dystocia were the same as for the model of calf BWT except that the heifer's own dystocia score and calf BWT were also included. Year of birth was forced into all models. This was followed by a step-wise reduction of covariates based on the highest Wald *P*-values until only covariates that were independently associated with the outcome ($P < 0.05$) remained. Following this, variables were added back into the model one by one and retained if significant. Variables were considered to be confounders if adding them to the models changed the coefficients of other covariates by more than 15%, in which case they were retained in the models. PA (the main variable of interest) was forced into the model and sire and AI bull were included as random effects and retained if significant ($P < 0.05$).

Sensitivity and specificity of different culling procedures for dystocia were compared using conditional logistic regression. Areas under the receiver operating characteristic (ROC) curves (ROC-AUC) for prediction of dystocia by the five different culling procedures were compared using the algorithm of DeLong et al. (1988).

4.4. Results

The heifers born in 2006 were older and heavier at the time of examination, had greater calf BWT, VD and larger PA than those born in 2007 (Table 4.1). The calving rate was also higher in the group born in 2006, but the dystocia rate was the same in the two years of birth. Only 4 (2.9%), and 2 (1.5%) dystocia cases requiring surgical intervention (score 2) were reported in heifers born in 2006 and 2007 respectively, therefore dystocia scores 1 and 2 were combined in further analyses.

Table 4.1: Pre-breeding and calving data by birth cohort.

	Year of birth							
	2006 (n = 225)				2007 (n = 259)			
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Pre-breeding BW (kg)	316[a]	28	226	405	292[b]	36	195	392
Pre-breeding age (d)	407[a]	21	336	459	401[b]	31	311	449
Pre-breeding BCS (1-9)	5.6[a]	0.6	5	8	5.8[b]	0.7	5	8
Pre-breeding TD[1] (cm)	11.1[a]	0.8	9	13	11.0[a]	0.9	8.5	13
Pre-breeding VD[2] (cm)	13.3[a]	0.8	11.5	16	12.0[b]	0.9	11	16
Pre-breeding PA[3] (cm^2)	148[a]	16	112.5	194	143[b]	18	93.5	195
Calf birth weight (kg)	29.8[a]	4.1	17	40	28.4[b]	4.5	18	41
No. that calved (%)	136 (60%)[a]				128 (49%)[b]			
No. with dystocia (%)	46 (34%)[a]				42 (33%)[b]			

[1]Transverse diameter of the pelvis
[2]Vertical diameter of the pelvis
[3]Pelvis area
[a,b]Means and proportions with different superscripts between years of birth differ significantly ($P < 0.05$)

Pre-breeding BW was correlated with pre-breeding age, VD and TD (Pearson r = 0.44, 0.50 and 0.46, respectively; $P < 0.05$). Pre-breeding age was correlated with VD and TD and the latter two were also correlated (r = 0.28, 0.44 and 0.33, respectively; $P < 0.05$). Although correlations followed the same general trends within years of birth, coefficients were higher in the 2007 than in the 2006 cohort. Regression coefficients used to calculate BWPA, LBW and LBWPA are presented in Table 4.2.

Table 4.2: Regression coefficients used to calculate lean BW, BW adjusted pelvis area and lean BW adjusted pelvis area

Dependent variable	Independent variable	Used in calculation of	Age cohort	Regression coefficient (β)	R^2
Body weight (BW) (kg)	Body condition score (1-9)	Lean BW (LBW) (kg)	2006	9.4 kg/BCS	0.05
			2007	19.8 kg/BCS	0.18
Pelvis area (PA) (cm^2)	BW (kg)	BW adjusted PA (BWPA) (cm^2)	2006	0.23 cm^2/kg	0.17
			2007	0.34 cm^2/kg	0.45
PA (cm^2)	LBW (kg)	LBW adjusted PA (LBWPA) (cm^2)	2006	0.24 cm^2/kg	0.18
			2007	0.36 cm^2/kg	0.42

In the multiple regression model of PA (Table 4.3) both pre-breeding age and BW were positively associated with PA, low pre-breeding BCS was associated with larger PA (when adjusted for BW) and the random effect of sire was significant. The multiple regression model of calf BWT (Table 4.4) shows that the parity of the heifer's dam (1 vs 3 or more), the heifer's own BWT, the pre-breeding PA of the heifer, the age of the heifer at calving and the gender of the calf were all associated with calf BWT ($P < 0.05$, adjusted for year of birth) and the random effects of sire and AI bull were significant.

Table 4.3: Multiple regression model of factors associated with pelvis area (cm2).

Variable	Coefficient	SE	95% CI		*P*
Year of birth (2006 vs 2007)	-1.72	1.53	-4.72	1.29	0.26
Dam parity (1 vs. ≥3)	-1.60	2.34	-6.19	3.00	0.50
Dam parity (2 vs. ≥3)	-4.17	2.05	-8.19	-0.14	0.04
Pre-breeding age (d)	0.14	0.04	0.05	0.22	< 0.01
Pre-breeding BW (kg)	0.25	0.03	0.20	0.30	< 0.01
Pre-breeding BCS (≥6 vs. <6)	-3.44	1.38	-6.15	-0.73	0.01
Random effects (variance (SE); 95% C.I.):					
Sire	8.03 (5.12); 2.30, 28.04				
Residual	174.7 (12.3); 152.2, 200.6				

For every 1 kg increase in calf BWT the odds of dystocia increased by 37% after adjusting for year of birth, dam parity and PA (Table 4.5). Also, for every 1 cm^2 increase in PA, the odds of dystocia tended to decrease by 2% after adjusting for calf BWT, year of birth and dam parity ($P = 0.08$, Table 4.5). In this model the random effects of sire or AI bull were not significant.

Table 4.4: Multiple regression model of factors associated with calf birth weight (kg).

Variable	Coefficient	SE	95% CI		*P*
Year of birth 2006 vs. 2007	1.18	0.88	-0.53	2.90	0.18
Dam parity 1 vs. ≥3	1.90	0.93	0.07	3.72	0.04
Dam parity 2 vs. ≥3	1.32	0.85	-0.34	2.97	0.12
Own birth weight (kg)	0.21	0.06	0.09	0.32	< 0.01
Age at calving (d)	0.02	0.01	0.00	0.05	0.05
Pelvis area (cm^2)	0.05	0.02	0.01	0.08	< 0.01
Male vs. female calf	2.58	0.49	1.63	3.55	< 0.01
Random effects (variance (SE); 95% C.I.):					
Sire	1.78 (1.10); 0.53, 5.98				
AI bull	1.04 (0.88); 0.20, 5.46				
Residual	13.03 (1.34); 10.66, 15.95				

Neither the heifer's own BWT nor the occurrence of dystocia during her own birth was associated with dystocia at her first calving, even after adjusting for any of the other covariates. Heifers born from second parity cows were 2.63 times more likely to develop dystocia during their first calving compared to those born from first parity heifers (P = 0.02), and tended to be more at risk compared to heifers born from 3rd and greater parity cows (OR = 1.86, P = 0.08) after adjusting for year of birth. Heifers born from second parity cows in 2007 were significantly lighter pre-breeding than those born from 1st and 3rd and higher parity cows in the same year (mean±SD 278±28 kg vs. 290±26 kg, P = 0.02, and 298±40 kg, P < 0.01 respectively).

Table 4.5: Association between the outcome of an unassisted versus assisted birth for factors included in the logistic regression model.

Variable	**OR**	**SE**	**95% CI**		***P***
Year of birth 2006 vs. 2007	0.61	0.19	0.32	1.14	0.12
Dam parity 1 vs. ≥3	0.45	0.18	0.21	0.97	0.04
Dam parity 2 vs. ≥3	2.15	0.89	0.96	4.82	0.06
Pelvis area (cm^2)	0.98	0.01	0.96	1.00	0.08
Calf birth weight (kg)	1.37	0.06	1.25	1.51	< 0.01

Table 4.6: Accuracy of hypothetical culling after different PA ranking procedures for prediction of dystocia.

	Culling rate					
	10%			**20%**		
Ranking procedure	***Se***[1]	***Sp***[2]	***ROC-AUC***[3]	***Se***[1]	***Sp***[2]	***ROC-AUC***[3]
Pelvis area unadjusted	0.05^{a}	0.97^{a}	0.51^{a}	0.17^{a}	0.87^{a}	0.53^{a}
Pelvis area adjusted to 365d age using a fixed correction factor of 0.27cm^2/ d of age	0.11^{b}	0.92^{b}	0.52^{a}	$0.24^{a,b}$	0.86^{a}	0.56^{a}
Pelvis area:BW ratio	0.14^{b}	$0.94^{b,c}$	0.54^{a}	0.27^{b}	0.85^{a}	$0.56^{a,b}$
Pelvis area adjusted to BW by the linear regression coefficient	0.15^{b}	$0.97^{a,c}$	0.56^{a}	$0.26^{a,b}$	0.88^{a}	$0.55^{a,b}$
Pelvis area adjusted to lean BW by the linear regression coefficient	0.13^{b}	$0.95^{a,b,c}$	0.54^{a}	0.30^{b}	0.89^{a}	0.59^{b}
Model P-value	0.02	0.01	0.09	0.04	0.43	0.02

[1]Sensitivity
[2]Specificity
[3]Area under the receiver operating characteristic curve
[a,b]Se, Sp or ROC-AUC with different superscripts within a column differ significantly (P < 0.05)

The five different ranking procedures of PA data for prediction of dystocia resulted in significantly different *Se* for dystocia (P = 0.02 and P = 0.04 at 10 and 20% culling respectively). The *Se* of PA for dystocia was lower than that of all the adjusted PA variables at 10% culling, and also lower than BWPA and LBWPA at 20% culling rate (Table 4.6). The *Sp* of the different ranking procedures only differed at the 10% culling rate ($P = 0.01$), when the *Sp* of PA and BWPA for dystocia was higher than that of APA (Table 4.6). Overall, LBWPA predicted dystocia better than PA and APA at the 20% culling rate ($P < 0.05$, using ROC analysis, Table 4.6).

After hypothetical culling by PA, heifers that were retained were heavier, had a higher calving rate and calves tended to be heavier at birth compared to the culled heifers ($P = 0.08$), but the dystocia rate was not different (Table 4.7). Culling by APA also resulted in higher mean pre-breeding BW of retained heifers (Table 4.7) and a tendency of higher mean calf BWT at 10% culling (29.3 vs. 27.8 kg, $P = 0.10$). Culling by APA further resulted in a tendency towards a lower dystocia rate (Table 4.7).

Table 4.7: The effects that culling the lowest 20% of heifers would have on the retained heifers relative to those that were culled by using various ranking procedures.

Ranking procedure		n	Mean pre-breeding BW (kg)	Calves born (proportion of total bred)	Dystocia (proportion of calves born)	Unassisted births (proportion of total bred)	Mean calf birth weight (kg)
Pelvis area (PA) unadjusted	Retained	387	309.8**	226 (59%)**	73 (32%)	153 (40%)**	29.3*
	Culled	97	277.6**	38 (38%)**	15 (39%)	23 (24%)**	28.0*
PA adjusted to 365 d of age using a correction factor of 0.27 cm^2/d	Retained	389	308.0**	219 (56%)	67 (31%)*	152 (39%)**	29.2
	Culled	95	284.3**	45 (47%)	21 (47%)*	24 (25%)**	28.8
PA:BW ratio	Retained	385	299.3**	214 (56%)	64 (30%)**	150 (39%)**	29.2
	Culled	99	319.3**	50 (50%)	24 (48%)**	26 (26%)**	28.8
PA adjusted to BW by the regression coefficient of PA on BW	Retained	387	303.4**	219 (57%)*	65 (30%)**	154 (40%)**	29.3
	Culled	97	303.6**	45 (46%)*	23 (51%)**	22 (23%)**	28.7
PA adjusted to lean BW by the regression coefficient of PA on LBW	Retained	385	303.9	219 (57%)*	62 (28%)**	157 (41%)**	29.0
	Culled	99	301.2	45 (45%)*	26 (58%)**	19 (19%)**	29.5

**Values in columns within ranking procedure with different superscripts differ significantly ($P < 0.05$)
*Values in columns within ranking procedure tend to differ ($P < 0.10$)

Culling by PA:BW resulted in a significantly lower dystocia rate in retained heifers than in culled heifers, but retained heifers had lower pre-breeding BW than culls (Table 4.7). No differences in calving rate or calf BWT occurred after culling by PA:BW. Culling by BWPA or LBWPA resulted in a lower proportion with dystocia and a tendency towards a higher proportion of calves born in the retained heifers compared to culls, without affecting the pre-breeding BW or calf BWT (Table 4.7).

4.5. Discussion

In this study the effects of different ranking procedures of PA data used to hypothetically cull heifers before breeding were compared to determine the accuracy of each procedure to select against dystocia, as well as the side-effects of culling due to associations with fertility, BW and calf BWT. The study further investigated whether sire, own BWT of a heifer, or the parity of her dam were associated with dystocia.

The differences in numbers, BW, age, VD and calving rate between the 2 years of birth most likely occurred as a result of stricter pre-breeding culling based on BW in heifers born in 2006, which also accounted for the lower variability and weaker correlations in data of heifers born in 2006 (Tables 1 and 2). Despite the differences between the two birth cohorts, dystocia rates were similar. This, combined with a previous finding by Micke et al (2010a) that the association between pre-breeding PA and dystocia was not altered by differing levels of management after the application of pelvimetry, supports our assumption that the pooling of data in this study was valid.

Dystocia requiring surgical intervention had a low incidence in this study, supporting the binary classification used (Johanson and Berger, 2003, Zaborski et al, 2009, Citek et al, 2011). Pelvis area measured by the Rice pelvimeter is accurate when compared to carcass measurements (Kolkman et al., 2009) and moderately to substantially repeatable between and within veterinarians (Van Donkersgoed et al, 1993). Breed differences in pelvis conformation (Citek et al., 2011) support the use of PA rather than TD or VD for application of pelvic measures across breeds.

Based on the results of the multiple regression model of PA presented in Table 4.3, it is evident that BW and age have both joint and independent associations with PA. In other words, if a number of heifers of the same BW are compared, then the older of those will have larger PA, and similarly if a number of heifers of the same age are compared, then the heavier of those will have larger PA. However, adjusting PA in order to determine the relative size of the pelvis can be done either by adjusting to BW or to age, but not both simultaneously, due to the strong correlation between BW and age (Deutscher, 1988). From the results presented in Table 4.4.3 adjusting for BW appears most appropriate due to the stronger association with PA.

Similar to previous studies, calf BWT in our data was the single most important determinant of dystocia, whereas PA tended to be associated with dystocia only when adjusted for calf BWT (Van Donkersgoed et al., 1997, Cook et al., 1993, Wolverton et al., 1993, Price and Wiltbank, 1978b). The lack of statistical significance in the association of PA, APA or PA:BW with dystocia could have been a result of inadequate sample size, however BWPA and LBWPA were both significantly associated with dystocia after adjusting for calf BWT, further indicating that the adjustment by BW or LBW was the most appropriate.

The findings of the present study occurred in heifers measured as yearlings, with a dystocia rate of 33%, whereas in the study reported by Van Donkersgoed et al. (1993) the dystocia rate was 18% or 19% in heifers that were a year older than the heifers in our study. The reason for the difference in *Se* using PA or PA:BW reported in this study, and the one by Van Donkersgoed et al. (1993) may be that the relative threshold determining test positive (culling) status is changed if a similar culling rate is maintained while the prevalence changes (Dohoo et al., 2003a). For this reason a culling rate that suits the population tested should be applied: a lower culling rate is indicated in herds with a low risk of dystocia if high *Sp* is desired.

The consistently high *Sp* in both studies indicates that the test may be generally valid as a culling tool, where the incorrect culling of many "disease negative" animals is potentially economically more damaging than keeping "disease positive" animals in the herd (Chenoweth, 2005b). If BWPA was used to cull 10% heifers in the current study, 85% of dystocia cases would not have been predicted by the test, but only 3% of heifers that calved without assistance ("disease negative") were incorrectly culled by the test. For a test that is applied as a culling tool, this high *Sp* makes it useful, as long as it is assumed that the *Se* is poor, and that many unpredicted dystocia cases will still occur. This was not considered in the interpretation by Van Donkergoed et al (1993).

When PA was used as culling procedure, it resulted in an increased proportion of unassisted births in retained heifers, but this was paradoxically as a result of significant differences in calving rate, and not dystocia rate. It also tended to increased calf BWT at a 20% culling rate in retained heifers relative to culls due to its correlation with frame size, which would likely offset the benefit of larger PA (Andersen et al, 1993, Laster, 1974). Price and Wiltbank (1978b) showed that Angus heifers had lower BW and calf BWT, smaller PA and less dystocia than their Charolais counterparts, and this can possibly be explained by the fact that the BWT of the calf as proportion of the dam's BW increases with increasing BW of the cow (Holland and Odde, 1992). Because PA is associated with calving rate, it may also be associated with age at calving, which may result in a confounding effect of PA on dystocia (Andersen et al., 1993, Zaborski et al., 2009). However in this data age at calving was not associated with dystocia (Table 4.5). Although culling based on PA was not effective in decreasing the dystocia rate in our data, it is currently widely used, and the use of PA as culling tool may have a positive side-effect of selecting for heifers with improved fertility.

The association of PA with fertility outcome is in agreement with findings that bulls with larger PA have shown improved libido (Singh et al., 2010) and that Jersey heifers with larger PA reached puberty at a lower BW and earlier age (Ramin et al., 1995), and needs further investigation. Further support for the relationship between PA and hormonal changes around puberty is the independent associations of age and BW with PA, being similar to what has been described for age at puberty (Yelich et al., 1995, Pineda et al, 2003, Pence et al., 2007, Holm et al., 2009). Lesmeister (1976) demonstrated that TD development that was induced by progesterone and estradiol implantation in heifer calves precedes VD development. Other studies reported a biphasic growth pattern of the reproductive organs of heifers from birth to puberty (Desjardin and Hafs, 1969, Honaramooz et al., 2004), with the first phase before 6 months of age under FSH stimulation, and the second phase preceding puberty under LH stimulus (Day et al., 1987). It is possible that pelvis development follows a similar biphasic hormone stimulated pattern, and we hypothesise that inadequate LH levels due to lower BW resulted in smaller VD as well as more of the 2007 heifers not achieving oestrus during the breeding season compared to those born in 2006.

Using a fixed correction factor of 0.27 cm^2/d of age to adjust PA to 365 d increased the *Se* but decreased the *Sp* of culling, without reducing the negative side effects such as the birth of heavier calves and selection of heavier heifers, compared to using PA (Table 4.6, Table 4.7). There was a much weaker association with calving rate than when PA was used, and as a result the proportion of unassisted births only increased significantly at the 20% culling rate. The reason may be that the correction factor was inappropriate for the study population, or that adjusting for age did not provide a measure of PA relative to frame size. Larger framed heifers likely to give birth to heavier calves, but that are younger at the time of examination, will be favoured by this procedure. Taylor et al. (2008) showed that large framed heifers reproduce less efficiently over the long term than their small- and medium framed counterparts in extensive systems of Southern Africa, making selection based on unadjusted PA potentially inappropriate.

Culling by PA:BW resulted in a significant decrease in dystocia rate in the retained heifers compared to the culls, but there was no effect on calving rate, probably due to the fact that this procedure resulted in retained heifers being lighter than culls, and therefore less likely to become pregnant during the restricted breeding season. These findings are similar to those previously reported using this method (Basarab et al., 1993a, Van Donkersgoed et al, 1993), and it is likely that the relative numerical value of BW compared to PA over-emphasises the former in the ranking procedure, resulting in heavier heifers being culled. Although this procedure resulted in the best *Se* at 5% culling rate in our data, it is not recommended due to its negative association with BW.

Adjusting PA to the median BW by the regression coefficient of PA on BW significantly increased *Se* at the 10% culling rate (Table 4.6), and appeared to cull heifers more efficiently for dystocia than for fertility (Table 4.7). The BW of retained and culled heifers, as well as the BWT of their calves was similar, indicating that this adjustment of PA data effectively avoided accidental culling based on frame size.

The negative association between BCS and PA was only significant when adjusted for BW; in other words, for any given BW, PA was larger in heifers with lower BCS, reasoned to be due to a larger frame size. This assumption formed the basis of our adjustment of LBWPA. Lean BW was firstly determined in an attempt to represent the frame size of the heifer, which was then used to adjust PA in order to have a measure of PA relative to frame size. Although the negative association of PA with BCS was similar for TD, it was not the case for VD, and in a study of Belgian Blue cows between 2 and 10 y of age that were measured before slaughter and compared with carcass measurements this association was not evident (Kolkman et al., 2009). Another possible explanation for the negative association between BCS and TD may be that higher levels of endogenous steroid hormones may lead to lower BCS and increased TD, particularly at a young age (Lesmeister, 1976). In the current study of the effects of different ranking procedures of PA as culling tool for dystocia, BWPA and LBWPA performed similarly, except that LBWPA appeared to be more accurate at higher culling rates, while BWPA appeared more accurate at lower culling rates. Further research is needed to clarify the effect of BCS on PA, in order to validate either BWPA or LBWPA.

There was no evidence from the present data that a heifer's own BWT was negatively associated with the occurrence of dystocia at the time of her first calving. In a herd such as this with very uniform animals (Schoeman and Jordaan, 1998), and where low BWT bulls had been used to control the incidence and severity of dystocia for >25 y (R. J. Wood, Johannesburg Water, personal communication, 2007), such an association would most likely have been demonstrable if it existed. In fact, in the present study heifers born from second parity cows were more at risk of developing dystocia, despite the fact that heavier BWT bulls sired them compared to daughters of first parity cows. The reason for this increased risk was not related to BWT, but was most likely related to the fact that heifers born from second parity cows in 2007 were lighter pre-breeding than those born from 1st and 3rd and higher parity cows in the same year.

We recommend that current practices using PA, APA or PA:BW ratios to rank heifers for culling should be revised due to their associations with frame size and calf BWT, and that ranking heifers based on their BW adjusted PA or lean BW adjusted PA should be considered. Since our data failed to demonstrate an association between own BWT and dystocia, it is further recommended that the use of bulls with low BWT or low BWT EPD and adequate nutrition of developing heifers, can be combined with culling by BW adjusted PA in sustainable management programmes for dystocia in beef heifers.

4.6. Conclusions

It is concluded that ranking by PA is a valid culling tool for yearling heifers, with a consistently high specificity for dystocia. Adjustment of PA data to median BW or LBW within age group improves its accuracy and avoids undesirable side-effects.

CHAPTER FIVE

ULTRASONOGRAPHIC REPRODUCTIVE TRACT MEASURES AND PELVIS MEASURES AS PREDICTORS OF PREGNANCY FAILURE AND ANESTRUS IN RESTRICTED BRED BEEF HEIFERS

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5.1. Abstract

Previous reports have shown that reproductive tract score (RTS) can predict reproduction outcomes in seasonally bred beef heifers, although the accuracy can vary. Some ultrasonographic measures of the female reproductive tract and pelvis area have also been associated with reproductive outcome in young heifers. The objectives of this study were to determine which transrectal ultrasound or pelvis measures taken at a single examination are independent predictors of reproductive failure, and if the RTS system can be optimised with this information. In this observational study 488 year-old beef heifers in 2 birth cohorts were followed from prior to first breeding until confirmation of pregnancy. A single pre-breeding examination included BCS, RTS, ultrasound measures of the reproductive tract (length and diameter of the left and right ovaries, presence and diameter of a CL, largest follicle diameter and left uterus horn diameter) and transverse and vertical diameters of the pelvis. Additional farm records including dam parity, sire, birth weight and birth date, weaning weight, weaning date, pre-breeding BW, AI dates and semen used were available. Breeding consisted of 50 d of AI, followed 5 to 7 d later by a 42 d bull breeding period. Pregnancy failure was defined as the failure to become pregnant after the AI and bull breeding periods while anoestrus was defined as the failure to be detected in oestrus during the 50 d AI period. From the pre-breeding data and farm records independent predictors of pregnancy failure and anoestrus were identified using step-wise reduction in multiple logistic regression models. Pre-breeding age was the only consistent independent predictor of pregnancy failure and anoestrus in both cohorts of this study ($P < 0.05$). BCS, uterus horn diameter, absence of a CL, largest follicle of less than 13 mm and pelvis area (PA) were the pre-breeding examination variables that remained in prognostic models ($P < 0.1$). Combining either the model based on the three remaining ultrasound measures or RTS with PA provided more accurate prognostic models for pregnancy failure and anoestrus than using RTS alone ($P < 0.05$). It is concluded that ultrasound measures have prognostic value for pregnancy failure in restricted bred yearling heifers as a result of their association with anoestrus, and that smaller pelvis area has additional prognostic value for poor performing heifers.

Keywords: beef cattle, fertility, heifer selection, pelvis area, reproductive tract score, ultrasonography

5.2. Introduction

The ability to select young heifers that will reproduce effectively in a seasonal breeding system has advantages over the alternative approach of waiting until reproductive failure occurs (Chenoweth, 2005a, Cushman et al., 2013). Reproductive tract score (RTS) predicts anoestrus and pregnancy failure in heifers independently of age, BW and BCS, and is a valid selection tool to enhance reproductive performance of herds (Andersen et al., 1991, Pence et al., 2007, Holm et al., 2009, Archbold et al., 2012b). However, oestrous cycle stage and proportion of heifers in anoestrus affect the accuracy of RTS, the complexity of the RTS system affects its repeatability, and other tests with potential to improve RTS are available (Rosenkrans and Hardin, 2003, Holm et al., 2009, Archbold et al., 2012a).

The ultrasonographic presence of a corpus luteum (CL) has been used to mark the onset of puberty, has substantial repeatability, is more accurate than blood progesterone determination and is a predictor of reproductive outcome in seasonally bred cows and heifers (Lean et al., 1992, Johnston et al., 2009, Mee et al., 2009, Archbold et al., 2012a). Ovary size is associated with antral follicle count (AFC), which in turn is associated with follicular reserve and fertility, whereas AFC is not affected by oestrous cycle stage (Cushman et al., 2009, Ireland et al., 2011). Maximum follicle diameter is correlated with uterus, cervix and vaginal diameter, and increases in the 10 weeks prior to first ovulation in heifers, due to increased LH pulse frequency (Desjardins and Hafs, 1969, Day et al., 1984, 1987, Bergfelt et al., 1994, Honaramooz et al., 2004, Cushman et al., 2009). Larger pelvis area has been associated with early onset of puberty in Jersey heifers and improved libido in bulls (Ramin et al., 1995, Singh et al., 2010).

The objective of this study was to determine which individual transrectal ultrasound or pelvis measures taken at one point in time before breeding are independent predictors of reproductive failure in seasonally bred beef heifers, and whether this knowledge can be used to optimise RTS.

5.3. Materials and methods

This was an observational study of 488 uniquely identified Bovelder beef heifers born in either 2007 (n = 259) or 2008 (n = 229) (2007 and 2008 cohorts) that were followed from just prior to their first breeding season to confirmation of pregnancy. The farming system, breed and location have been described previously (Paterson et al., 1980, Schoeman and Jordaan, 1998, Holm et al., 2008, 2009). Farm data collected included the following: birth weight and birth date, parity of dam, sire, bull allocated and first to fourth AI day numbered from the mating start date (MSD).

Heifers were weighed either 22 d (2007 cohort) or 27 or 24 d (2008 cohort) before the MSD (pre-breeding BW), and a single pre-breeding examination was performed 7 d (2007 cohort) or 27 or 24 d (2008 cohort) before the MSD. During the pre-breeding examination heifers were restrained individually in a chute, and the following data were collected in the same order by one experienced veterinarian: Firstly BCS was determined using a 9-point scale (Marston, 2005). This was followed by RTS by transrectal palpation using a 5-point scale (Andersen at al., 1991), then followed by transrectal ultrasonographic measurements of the reproductive tract (DesCôteaux et al, 2009), using a real-time digital ultrasound imaging system set in B-mode with a variable frequency linear probe set at 5 MHz (SIUI CTS-900V, Shantou Institute of Ultrasonic Instruments, Shantou, China). The inter-polar length of the left and right ovaries, the diameter of the left and right ovaries at the deepest point (2008 cohort only), the presence and diameter of a CL, the diameter of the largest follicle and diameter of the left uterus horn near the base (UD) were recorded (Monteiro et al., 2012). Finally internal vertical diameter (VD) and transverse diameter (TD) of the pelvis were measured by transrectal placement of a caliper type pelvimeter (Rice pelvimeter, Lane Manufacturing, Denver, Colorado) (Deutscher, 1988, Van Donkersgoed, 1997). Farm management and staff were blinded to all the measured pre-breeding data throughout the trial, except for the pre-breeding BW.

The MSD was 15 October of each year and breeding consisted of 50 d of continuous oestrus observation by visual inspection, and once daily AI of all heifers identified in oestrus during the preceding 24 h by the same inseminator. Five to 7 d after each 50-day AI season all heifers were joined with bulls in a single multi-sire group at a heifer:bull ratio of 30-35:1 for 42 days. Pregnancy diagnoses were performed by transrectal palpation (Sheldon and Noakes, 2002) 138 d or 165 d after MSD (2007 and 2008 cohorts respectively).

For the purpose of regression models and survival analysis, BCS was categorised into 2 categories (<6 and ≥6) and RTS 1 and 2 (pre-pubertal), and RTS 4 and 5 (post-pubertal) were combined (Stevenson et al, 2008). Diameter of the largest follicle was used either as a continuous variable or was dichotomized using various cut-offs (7, 8, 9, 12, 13 and 14 mm). Pelvis area (PA) was calculated as the product of the TD and VD, and standardised values of PA (SPA) as well as uterus diameter (SUD) were calculated within birth cohort using the following formula:

$$x^* = (x - x_{minimum}) \div (x_{maximum} - x_{minimum})$$

If a heifer was not detected in oestrus it was assumed that she remained prepubertal until the end of the 50-day AI season, and was defined as anestrus, whereas pregnancy failure was defined as a negative pregnancy test at the end of the AI and bull breeding periods.

Correlations were estimated using Pearson's correlation routine for normally distributed data and Spearman's correlation for other data. Independent proportions were compared using the Fisher exact test and means and medians were compared using ANOVA with the Tukey-Kramer multiple comparison test and Kruskal-Wallis one-way ANOVA respectively.

Multiple linear regression models (for length of the longest ovary, diameter of the largest follicle, UD and PA) and logistic regression models (for absence of a CL, absence of a follicle ≥13 mm, anoestrus and pregnancy failure) were constructed using a backward elimination process (Dohoo, 2003b) with $P < 0.20$ for initial inclusion and $P_{Wald} < 0.10$ for retention in models. Predictors that were considered included year of birth, dam parity (1, 2 or ≥3), pre-breeding BW (kg), growth rate (kg/d), age (d) and BCS category at examination, presence or diameter (mm) of the CL, diameter (mm) of the largest follicle or presence of a follicle of at least 7, 8, 9, 12, 13 or 14 mm, SUD, SPA and length of the longest or shortest ovary (mm), or combined length of the two ovaries (mm), or ovary length difference (mm).

Finally, independent pre-breeding examination predictors of anoestrus and pregnancy failure were combined into different prognostic models in order to estimate which models provided the best predictions of the outcomes. Areas under the receiver operating characteristic (ROC) curves (ROC-AUC) of prognostic models for anoestrus and pregnancy failure were compared using the algorithm of DeLong et al. (1988).

Data analysis was done using NCSS 2007 (NCSS, Kaysville, UT, USA) and STATA 11.1 (StataCorp, Texas, USA).

5.4. Results

The age (mean±SD) of heifers at pre-breeding weighing (384±28.8 d) and at MSD (407±28.7 d) were similar between age cohorts (P = 0.74 and P = 0.27 respectively), but heifers born in 2007 were examined at an older age than those born in 2008 ($P < 0.01$, Table 5.1). Heifers born in 2007 were significantly heavier pre-breeding than those born in 2008 ($P < 0.01$, Table 5.1) and BCS (median, interquartile range (IQR)) was also higher in the 2007 cohort (6, 5-6 and 5, 5-6 respectively, $P < 0.01$). Based on RTS, more heifers were post-pubertal (RTS 4 or 5 = 247/488) compared to pre-pubertal or pubertal (RTS 1 or 2 = 102/488, RTS 3 = 139/488) ($P < 0.01$), and the proportions were similar between birth cohorts (P = 0.87). Pelvis and ultrasound measures of the reproductive tract are reported in Table 5.1. The UD differed between the two birth cohorts (Table 5.1). Further, in the 2008 cohort sampling day was associated with right ovary length and diameter, and with UD ($P < 0.05$, Table 5.1). The left ovaries had shorter inter-polar length than that of the right (23.7 and 25.5 mm respectively, $P < 0.01$), but the diameter of the left and right ovaries for heifers born in 2008 did not differ (14.0 and 14.4 mm respectively, P = 0.26).

Age at examination, pre-breeding BW, BCS, length of the longest ovary, diameter of the CL and UD were all positively correlated with each other ($P < 0.05$). The diameter of the largest follicle was positively correlated with the length of the longest ovary and the length of the shortest ovary ($P < 0.05$). Reproductive tract score was most markedly associated with the length of the longest ovary, the length of the shortest ovary and the absence of a CL (Table 5.2). It was also associated with the diameter of the CL and the diameter of the largest follicle, less so with UD and the absence of a follicle ≥8 mm but not associated with the absence of a follicle ≥13 mm (Table 5.2).

In the multi-variable models, weaning weight and age at examination were independently associated with presence of a CL, whereas only pre-breeding BW was independently associated with presence of a follicle ≥13 mm (Table 5.3). Pre-breeding BW, age at examination and presence of a CL were independently associated with UD, and pre-breeding age, presence of a CL and largest follicle diameter were independently associated with the length of the longest ovary (Table 5.3). Dam parity 1 (vs. >1), weaning weight, pre-breeding BW, age at examination, BCS and presence of a CL were all independently associated with PA (Table 5.3).

Table 5.1: Pre-breeding measures and reproductive outcomes per year of birth and per sampling day.

	Sampling day		
	d -7 (n = 259)	**d -27 (n = 134)**	**d -24 (n = 95)**
	Born 2007	**Born 2008 (n = 229)**	
Age at examination (d)[1]	401±31^{a} [311-449]	383±27^{b} [308 – 453]	
Age at mating start date (d)[1]	407±31^{a} [317 – 455]	408±27^{a} [331 – 479]	
Pre-breeding BW (kg)[1]	292±36^{a} [195 – 392]	272±34^{b} [184 – 349]	
Vertical pelvis diameter (cm)[1]	12.9±0.9^{a} [11 – 16]	12.6±1.1^{b} [8 – 15]	
Transverse pelvis diameter (cm)[1]	11.0±0.9^{a} [8.5 – 13]	10.6±1.0^{b} [8 – 13]	
Largest follicle diameter (mm)[1]	10.7±2.8^{a} [4 – 18]	11.1±2.4^{a} [4 – 17]	
Proportion with CL	101/259 (39%)^{a}	56/229 (24%)^{b}	
CL diameter (mm)[1]	21.9±4.5^{a} [11 – 30]	20.7±4.4^{a} [11 – 30]	
Left ovary interpolar length (mm)[1]	24.3±5.7^{a} [13 – 43]	23.1±4.7^{b} [12 – 36]	
Left ovary diameter (mm)[1]	N/D[2]	14.0±4.2 [5 – 40]	
Right ovary interpolar length (mm)[1]	25.8±5.6^{a} [13 – 42]	24.1±5.1^{b} [14 – 43]	26.5±6.0^{a} [11 – 42]
Right ovary diameter (mm)[1]	N/D	14.0±4.2^{a} [8 – 30]	15.1±4.1^{b} [8 – 29]
Left uterus horn diameter (mm)[1]	15.3±2.6^{a} [10 – 24]	12.2±1.9^{b} [7 – 17]	11.7±1.5^{c} [8 – 15]
Proportion with pregnancy failure	56/258 (22%)^{a}	38/219 (17%)^{a}	
Proportion with anestrus	51/259 (20%)^{a}	50/229 (22%)^{a}	

[1]Mean±SD [minimum and maximum]
[a,b,c]Means or proportions in rows with differing superscripts differ significantly (*P* < 0.05)
[2]Not done

Table 5.2: Different ultrasonographic measures of the reproductive tract per reproductive tract score (RTS) category.

Ultrasound variable	**RTS 1**	**RTS 2**	**RTS 3**	**RTS 4**	**RTS 5**
Longest ovary length (mm)[1]	20.7±4.3^{a}	23.0±3.0^{b}	25.6±3.2^{c}	28.4±3.5^{d}	33.0±4.5^{e}
Shortest ovary length (mm)[1]	16.8±2.9^{a}	19.1±2.6^{b}	21.3±3.2^{c}	22.8±3.6^{d}	23.0±4.0^{d}
Absence of a CL [2]	15/15 (100%)^{a,b}	87/87 (100%)^{a}	127/138 (92%)^{b}	82/120 (68%)^{c}	19/127 (15%)^{d}
CL diameter (mm)[1]	-	-	18.0±4.7^{a}	18.9±3.9^{a}	22.7±4.1^{b}
Largest follicle diameter <8 mm [2]	5/15 (33%)^{a}	12/87 (14%)^{a,b}	16/138 (12%)^{b}	8/120 (7%)^{b}	12/127 (9%)^{b}
Largest follicle diameter <13 mm [2]	14/15 (93%)^{a}	69/87 (79%)^{a}	105/138 (76%)^{a}	82/120 (68%)^{a}	93/127 (73%)^{a}
Largest follicle diameter (mm)[1]	8.7±3.3^{a}	10.4±2.5^{b}	10.7±2.5^{b}	11.4±2.5^{c}	11.1±2.7^{b,c}
Uterus horn diameter (mm)[1]	12.7±3.0^{a}	13.4±2.6^{a}	13.6±2.9^{a}	14.0±2.9^{a,b}	14.2±2.6^{b}

[1]Mean±SD
[2]Proportion of the total number of heifers in each RTS category (%)
[a,b,c,d,e]Values in rows with different superscripts differ significantly (*P* < 0.05)

Table 5.3: Summary of the multiple logistic- or linear regression models of selected pre-breeding measures.

Predictor	Outcome				
	Presence of a CL[1]	Largest follicle ≥ 13 mm[1]	Uterus diameter (mm)[2]	Longest ovary length (mm)[2]	Pelvis area (cm^2)[2]
Dam parity >1 (vs 1)	#	#	#	#	-4.15 (-7.63, -0.66)
Wean weight (10kg)	1.16 (1.08, 1.25)	#	#	#	0.89 (0.05, 1.74)
Pre-breeding BW (10kg)	#	1.08 (1.02, 1.14)	0.06 (-0.01, 0.13)	#	2.35 (1.61, 3.09)
Age at examination (w)	1.13 (1.06, 1.20)	#	0.07 (0.01, 0.12)	0.09 (0.00, 0.18)	0.58 (0.16, 1.00)
BCS at examination ≥6	#	#	#	#	-3.25 (-5.92, -0.59)
Presence of a CL	N/a	N/a	0.58 (0.14, 1.01)	6.38 (5.56, 7.21)	7.64 (5.00, 10.30)
Largest follicle diameter (mm)	N/a	N/a	#	0.31 (0.16, 0.45)	#
Year of birth 2007	1.55 (1.00, 2.40)	#	3.13 (2.72, 3.55)	#	3.68 (0.78, 6.59)

[1]Odds ratios (95% C.I.) of independent predictors (*P* <0.10) in logistic regression models
[2]Regression coefficients (95% C.I.) of independent predictors (*P* <0.10) in multiple regression models
N/a: Not analysed
#Not an independent predictor (*P* >0.10)

Pre-breeding age was the only consistent independent predictor of pregnancy failure and anoestrus in both cohorts of this study ($P < 0.05$, Table 5.4). Body condition score, absence of a CL, largest follicle <13 mm, SUD and SPA were the pre-breeding examination variables that remained in multivariable models ($P < 0.1$, Table 5.4). The prognostic model for anoestrus using the three remaining pre-breeding ultrasonographic measures of the reproductive tract in combination with SPA (US + SPA model) yielded an ROC-AUC of 0.81 (Table 5.5, Figure 1).

Table 5.4: Summary of logistic regression models for pregnancy failure and anoestrus in the two birth cohorts separately and combined.

Predictor	Outcome					
	Pregnancy failure[1]			Anoestrus[1]		
	2007 cohort	2008 cohort	Combined data	2007 cohort	2008 cohort	Combined data
CL absent	3.00 (1.38, 6.50)	-	1.69 (0.94, 3.05)	15.07 (3.46, 65.61)	3.10 (0.84, 11.46)	6.13 (2.32, 16.21)
Largest follicle <13 mm	2.54 (1.05, 6.10)	-	2.07 (1.10, 3.90)	-	3.75 (1.34, 10.53)	2.13 (1.09, 4.16)
Standardised uterus diameter	-	0.32 (0.08, 1.26)	0.43 (0.18, 1.02)	0.34 (0.09, 1.26)	-	0.39 (0.15, 1.01)
Standardised pelvis area	-	-	-	-	0.06 (0.01, 0.31)	0.15 (0.05, 0.48)
Age at onset of breeding (w)	0.89 (0.83, 0.96)	0.86 (0.78, 0.95)	0.88 (0.83, 0.93)	0.87 (0.80, 0.94)	0.89 (0.80, 0.99)	0.90 (0.84, 0.96)
BCS <6	-	-	-	2.96 (1.39, 6.30)	6.42 (1.79, 23.06)	2.90 (1.66, 5.08)

[1]Odds ratios (95% C.I.) of independent predictors (*P* < 0.10)

Table 5.5: Logistic regression model for prediction of anoestrus using independent transrectal ultrasound measures of the reproductive tract in combination with pelvis area (n = 487).

Predictor	Coefficient	OR[2]	95% CI		*P*
Constant	-2.13	0.12	0.03	0.41	< 0.01
CL absent	1.97	7.15	2.75	18.57	< 0.01
Largest follicle < 13 mm	0.81	2.25	1.19	4.27	0.01
Rescaled uterus diameter	-0.96	0.38	0.16	0.94	0.04
Rescaled pelvis area	-2.77	0.06	0.02	0.18	< 0.01

[1]Area under the receiver operating characteristic curve = 0.81
[2]Odds ratio

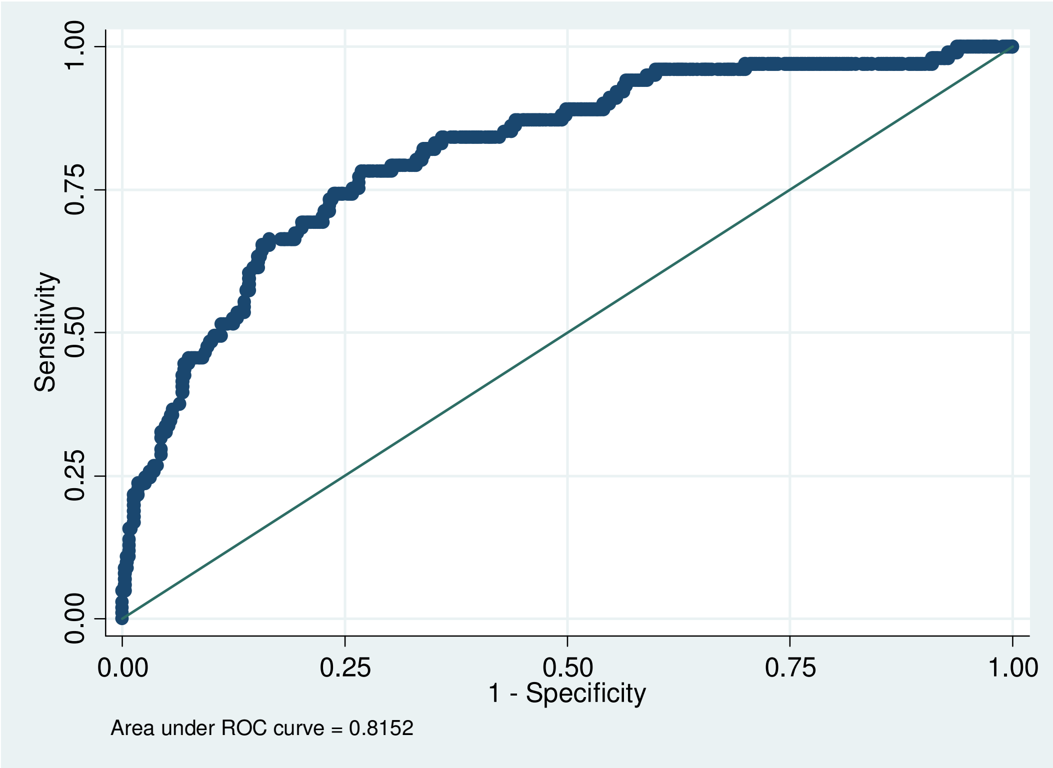

Figure 5.1: Receiver operating characteristic (ROC) curve for anoestrus using the model based on the ultrasonographic absence of a CL, absence of a follicle ≥13 mm, standardised uterus diameter and standardised pelvis area (Table 5.5).

The US + SPA model, and the model combining RTS with SPA (RTS + SPA model) provided more accurate predictions of pregnancy failure and anoestrus than using RTS alone ($P < 0.05$, Table 5.6). The SPA model and the model using ultrasonographic absence of a CL, absence of a follicle ≥13 mm and SUD (US model) tended to predict anoestrus better than RTS ($P = 0.09$ and $P = 0.06$ respectively). The SPA model predicted anoestrus better than RTS in the 2007 cohort ($P = 0.05$, Table 5.6), and tended to predict pregnancy failure better than RTS in the combined data ($P = 0.09$). In the 2007 data the US model tended to predict pregnancy failure better than SPA ($P = 0.07$).

Table 5.6: Areas under the receiver operating characteristic curves (ROC-AUC) of different predictive models for pregnancy failure and anestrus in the two birth cohorts separately and combined.

Model	Pregnancy failure			Anoestrus		
	2007 cohort	2008 cohort	Combined data	2007 cohort	2008 cohort	Combined data
RTS 1+2, 3, vs 4+5	0.59[a]	0.63[a]	0.60[a]	0.72[a]	0.69[a]	0.71[a]
SPA[1]	0.62[a,b]	0.63[a]	0.62[a]	0.74[a,b]	0.78[b]	0.76[a]
Ultrasound[2]	0.70[b,c]	0.64[a]	0.65[a,b]	0.77[a,b,c]	0.76[a,b]	0.76[a,b]
RTS + SPA	0.63[a,b]	0.65[a]	0.64[a,b]	0.79[b,c]	0.80[b,c]	0.79[b,c]
Ultrasound + SPA	0.71[c]	0.67[a]	0.68[b]	0.81[c]	0.83[c]	0.81[c]

[1]Standardised pelvis area
[2]Ultrasonographic absence of a CL, absence of a follicle ≥13mm and standardised uterus diameter
[a,b,c]ROC-AUC values in columns with differing superscripts differ significantly ($P < 0.05$)

5.5. Discussion

In this study individual ultrasonographic measures of the reproductive tract, and pelvis measures were compared as pre-breeding predictors of pregnancy failure and anoestrus in seasonally bred beef heifers, and this information was used to determine if the current RTS system can be optimised.

Despite good heritability of age at puberty (AP) (h^2 = 0.43, Brinks, 1994 and h^2 = 0.52, Johnston et al, 2009), environmental factors from foetal development to puberty can influence the phenotypic expression of reproductive potential (Honaramooz et al., 1999, Holm et al., 2009, Johnston et al, 2009), which may have led to different levels of pubertal development achieved by the MSD in the two birth cohorts in this study. However the anestrus- and pregnancy failure proportions were not different and we assumed that considering year of birth as a potential covariate in models would adequately adjust for any differences between the years.

The difference in UD between the two birth cohorts in this study may have occurred as a result of a true biological difference in the younger group of heifers in 2008 (Honaramooz et al., 2004), or may have been a systematic measuring error that occurred during sampling (Melendez et al, 2004). The relatively small UD reported in the 2008 cohort of the present study compared to the 2007 cohort as well as previous reports (Andersen et al., 1991, Honaramooz et al, 2004) indicates that a systematic error was more likely to have occurred in the 2008 cohort than in the 2007 cohort. The possibility of such an error to occur justifies the use of a rescaled value of the raw data in analyses, and we assumed that the relative uterus diameter within age cohort in the present study, rather than the absolute diameter provided a better indication of the relative pubertal development stage of an animal in a group. We assumed the same for PA data. Monteiro et al. (2013) showed that ultrasonographic thickness of the endometrium before the onset of breeding in Nellore heifers was associated with reproductive outcomes, being a similar and possibly more appropriate measure.

The difference in length between the left and right ovaries could either have been a true biological difference (Petskol, 1941), or it could also have been a systematic measuring error due to the operator using the same hand, and the alignment of the ultrasound probe being different on the left and right ovaries (DesCôteaux et al., 2009). Honaramooz et al. (2004) could not demonstrate a difference in ultrasonographic size between the left and right ovaries, however the largest follicle on the right side was 1 mm larger than on the left side in their data. The fact that there was a numerical difference in ovary diameter between the two sides in the 2008 cohort, that was not significant, is not useful to support either of the two hypotheses. It may be that an adjustment for the side of the largest ovary may improve the ability of the length of the longest ovary to predict reproductive outcomes, but due to obvious confounding by the size of the largest follicle and the presence of a CL on the predictive ability of ovary length, this was not investigated any further.

Age at examination was associated with UD and PA independent of BW, confirming that the development of the reproductive system is a function of both age and BW, and that age and BW when puberty is reached varies between animals, even in a uniform group such as the study population (Holm et al., 2009). The age range of the study population fell in or just after the second phase of rapid development of the reproductive tract (Desjardin and Hafs, 1969, Honaramooz et al., 2004), and as such a lot of variance could be expected due to the proximity to puberty. The results of this study may therefore not necessarily be extrapolated to heifers in other age ranges.

5.5.1. Individual independent predictors of reproductive outcomes

None of the ovary length variables were independently associated with reproductive outcomes in this study. However, two significantly independent predictors of reproductive outcomes, largest follicle ≥13 mm and the presence of a CL at the time of examination, were both also independent predictors of ovary length, and we conclude that the effect of ovary length on reproductive outcome is confounded by the presence of ovarian structures. Cushman et al. (2009) and Ireland et al. (2011) suggested that the size of the ovaries may give a reflection of antral follicle count, which is associated with fertility in young adult cattle. Antral follicle count was not considered as an input variable in the current study, however we assumed that either longest-, shortest- or combined ovary length would provide a reflection of antral follicle count after adjustment for the size of the largest follicle and the size or presence of a CL.

The diameter of the largest follicle was not correlated with UD as was the case in the study of Honaramooz et al., 2004, and was only independently associated with the length of the longest ovary, both which appear in this study to be confounded in their prognostic value for reproductive outcomes. After testing several cut-off points to dichotomise the diameter of the largest follicle, <13 mm was the only predictor of anoestrus and pregnancy failure in this study, which is in agreement with the observation by Honaramooz et al. (2004) that the maximum follicle size increases prior to puberty from 10 to 12 mm. We conclude that heifers in the current trial that did not have a CL, and also had a largest follicle diameter <13 mm were at risk of being too far from puberty at the time of examination to show oestrus during the 50-day AI season, or to become pregnant during the breeding season. None of the other follicle size cut-offs tested had any significant associations in this study, indicating that whether dominance of a follicle has occurred, or not (using a cut-off between 7 and 9 mm, Wiltbank, 2002) did not have prognostic value for reproductive outcome in our study.

Previous findings indicating the superior ability of ultrasonography to detect the presence of a CL (Lean et al., 1992) are supported by this study due to the fact that significant proportions of heifers with ultrasonographically visible CLs were assigned RTS 3 or 4. These CLs were smaller than those of heifers with RTS 5, and were most likely not easily palpable, however the tendency of the ultrasound model to have a better predictive value for anoestrus and pregnancy failure when compared to RTS is likely partly as a result of the better sensitivity of ultrasound to detect a CL. In the current study the absence of a CL not only predicted anoestrus, but also pregnancy failure. Keeping in mind that the total breeding season length was 90 days, this can be partly explained by the fact that the first few ovulations after puberty have decreased fertility (Byerley et al., 1987, Rawlings et al., 2004), which will further decrease the ability of heifers that reach puberty after the MSD to become pregnant during a restricted breeding season.

Although not validated against oestrus or pregnancy outcomes during the breeding period following examination, Archbold et al. (2012a) estimated the sensitivity of ultrasonography to determine pubertal status to be reduced during pro-oestrus and met-oestrus, due to the relatively poor ability to visualise the regressing corpus albicans and the corpus haemorrhagicum respectively. We therefore assume that the reason why the absence of a follicle ≥13 mm remained an independent predictor in our models was either that some heifers that were pubertal at the time of examination had their first oestrus in the few days after the examination, or that in post-pubertal heifers a CL was not detected due to stage of the oestrous cycle. Due to the fact that some heifers may have been at stages of the follicular wave before divergence of the dominant follicle at the time of examination (Wiltbank, 2002) the absence of a follicle ≥13 mm cannot completely rule out cyclicity, but improves the predictive ability when a CL is not present.

5.5.2. Optimising the RTS system for improved accuracy

Due to the inaccuracy of transrectal palpation relative to ultrasonography to detect a CL, to distinguish between follicles <13 mm and ≥13 mm and to estimate the uterus horn diameter, transrectal ultrasonography tended to provide better prognostic models for reproductive failure than the current palpation model of Andersen et al. (1991). However the accuracy of RTS by palpation may be improved by putting more emphasis on the presence of a CL, the size of the largest follicle and the diameter of the uterus horn, and less emphasis on the absolute size of the two ovaries. Our data confirms that the operator assigning the RTS scores weighed the size of the ovaries relatively heavily in the scoring system, but this study further indicated that the size of the ovaries after adjusting for ultrasonographically visible structures on the ovaries was not an independent predictor of reproductive outcome, and should preferably not be emphasised.

Pelvis area, a measure previously used only to predict dystocia in heifers (Deutscher et al., 1988, Van Donkersgoed, 1997), had a strong and independent association with reproductive outcome in this study, and added significant prognostic value to models based on palpation or ultrasonography of the reproductive tract. This is in agreement with previous reports of associations between PA and reproductive outcomes (Ramin et al., 1995, Singh et al., 2010). Similar to uterus diameter, pelvis area has the potential to overcome the inaccuracy to predict pubertal stage at a single point in time caused by different stages of the oestrous cycle. We assume that the reason why pelvis area predicted reproductive outcome independent of other measures, and why it added significant prognostic value to models predicting reproductive failure, is because it develops gradually over time and is probably not significantly associated with the daily oestrous cycle stage.

The absence of a CL, being the best predictor of reproductive failure, was a particularly good predictor in the 2007 birth cohort, in which case heifers were examined closer to the MSD, and the proportion of heifers with a CL was also higher. In the 2008 cohort, when heifers were examined more than 3 w before the MSD, BCS, uterus diameter and PA were more important predictors of anoestrus and pregnancy failure. We suggest that emphasis should be placed on different predictors depending on the age of heifers at the time of examination, or depending on the proportion of heifers with CLs at the time of examination. When heifers are examined long before the MSD, or when only a small proportion of heifers have CLs, more emphasis should be placed on the relative diameter of the uterus horn and the relative PA, whereas when examination is done shortly before the MSD, or when a larger proportion of heifers have CLs, more emphasis should be placed on the absence of a CL and the absence of a follicle ≥13 mm diameter. Further research is needed, possibly using Bayesian modelling, to establish if different prognostic models should be applied based on herd status.

5.6. Conclusions

Transrectal ultrasonography of the reproductive tracts of beef heifers can provide prognostic models of pregnancy failure due to its association with anoestrus during a restricted breeding season. The ultrasonographic measures that remained independent predictors of pregnancy failure and anoestrus were the absence of a CL, absence of a follicle ≥13 mm, and relatively smaller uterus horn diameter.

Relatively smaller pelvis area (PA) can either replace, or add value to reproductive tract scoring by transrectal palpation or ultrasonography as predictor of poor reproductive performance in restricted bred beef heifers.

CHAPTER SIX

GENERAL DISCUSSION

6.1. Introduction

This thesis describes a series of four observational studies, using data of the Bovelder beef herd at Johannesburg Water's Northern Farm, to investigate the associations between pre-breeding examination data in heifers obtained prior to their first breeding season and reproductive outcomes from their first and subsequent breeding seasons. The purpose was to determine the value of reproductive tract scoring (RTS), pelvimetry and ultrasonographic variables as predictors of various reproductive outcomes, namely failure to show oestrus and become pregnant during a restricted breeding season, dystocia, time to calving and calf weaning weight. Pre-breeding examination variables were adjusted for potential confounders using multivariable linear, logistic and Cox regression models. This is the first report using these data analysis techniques on such a variety of pre-breeding examination data. Using this approach it was found that pelvimetry, previously intended only to predict dystocia outcome, had prognostic value for failure to become pregnant, independent of oestrous cycle stage, whereas the accuracy of RTS and ultrasonographic measures were affected by oestrous cycle stage. In fact, the association between pelvis area (PA) and pregnancy outcome may be stronger than the association of PA with dystocia (Chapters 4 and 5).

In the following discussion, the main findings from each of the studies are highlighted and discussed. Certain data from the studies are aggregated, summarised and further analysed. Some additional data collected during the studies but not included in previous chapters are also presented and analysed to provide further insights.

6.2. The value of RTS as pre-breeding management tool

In Chapter 2 the use of low RTS was validated as a predictor of reproductive failure in the first breeding season in South African beef heifers, but also in the second breeding season due to the association of RTS with days to calving during the first calving season. In Chapter 2 only one age cohort of heifers was investigated, of which 50% were randomised to a synchronisation programme in which a luteolytic dose of prostaglandin $F_{2\alpha}$ was injected into all heifers that had not been inseminated by day 6 of the breeding season (PGF6). Multivariable analyses demonstrated that RTS, despite being associated with pre-breeding BW and age, was associated with reproductive outcomes independently of pre-breeding BW and age. This supported the previous finding that RTS was an indirect measure of the trait for age at puberty that is independent of body weight (Pence et al., 2007). In other words, RTS has the ability to select animals in a group that have a lower critical BW and age needed for the onset of puberty to be triggered. This has an advantage over selecting for growth rate, which is genetically correlated with adult BW (Forni et al., 2007). In Chapter 2 it was also shown that RTS has a superior ability to select heifers that are likely to wean more and heavier calves than other selection methods. It also appeared that this selection would not result in a change in frame size of the herd, which is likely to have advantages due to the association between frame size and reproductive performance demonstrated in extensively managed beef cattle (Taylor et al., 2008).

When the other age cohorts (Chapters 3 to 5) were considered, with the exception of one age cohort, heifers with RTS scores 1 and 2 combined had consistently lower pregnancy proportions at the end of the 50-day AI season when compared to heifers with scores 4 or 5 (Table 6.1). The exception occurred in the age cohort (heifers born 2003) where 50% of the heifers were randomised within RTS category to oestrus synchronisation using a controlled internal drug release (CIDR) device containing 1.92 g of progesterone intra-vaginally for 8 days followed by prostaglandin (CIDR protocol), where there was no significant difference in pregnancy proportions between RTS categories. However, when only the heifers that were not synchronised were considered in the 2003 cohort, those with RTS 1 had a significantly lower pregnancy proportion than the rest of the group (Chapter 3). This is most likely explained by the induction of puberty which happened as a result of progesterone supplementation in those animals with low RTS that received a CIDR device (Claro et al., 2010). In other words, the use of reproductive technologies such as a CIDR device may mask the effect of pubertal stage on reproductive performance.

In an analysis of the combined data of all animals in this thesis that were followed to at least the first pregnancy test (n = 1191), heifers with lower RTS had a lower pregnancy proportion (Table 6.1), and heifers with RTS 1 or 2 calved significantly later than those with RTS 3, and also than those with RTS 4 or 5 (median days to calving from first day of the calving season; interquartile range: 26.5, 16 – 39; 23, 13 – 35; 20, 12 – 31; $P = 0.017$ and $P < 0.001$ respectively). This is confirmed in a Cox regression model of days to calving using the combined data, where heifers with RTS 4 or 5 were more likely to calve early than heifers with RTS 1 or 2 (HR 1.40; 95% CI 1.14, 1.72) and heifers with RTS 3 tended to be more likely to calve earlier than those with RTS 1 or 2 (HR 1.21; 95% CI 0.96, 1.51). These results confirm the value of RTS as selection tool in beef heifers as discussed in Chapter 2, over a number of years in this herd. In a recent report by Gutierrez et al. (2014), RTS was performed approximately 4 weeks prior to the onset of breeding in a total of 4041 Angus cross heifers (mean age 14.8 months). After a total breeding period of 85 d consisting of a combination of fixed time AI using progesterone impregnated CIDR devices and natural breeding, pregnancy rates were 80.5%, 85.9% and 92.0% for heifers with RTS categories 1-2, 3 and 4-5 respectively. The relatively long time from RTS recording to the onset of breeding and the relatively longer breeding period most likely resulted in less outspoken differences in pregnancy rates between different categories, when compared to the results of this thesis.

Table 6.1: Heifer pregnancy proportions by year of birth and RTS category at Johannesburg Water's Northern Farm (summarised data of the animals described in Chapters 2 to 5).

	Pregnancy proportion					
	2002*	2003**	2006	2007	2008	Combined
RTS 1-2	38%^{a} (33/86)	70%^{a} (74/105)	58%^{a} (49/84)	43%^{a} (23/53)	47%^{a} (23/49)	54%^{a} (202/377)
RTS 3	53%^{a} (43/81)	82%^{a} (49/60)	85%^{b} (44/52)	42%^{a} (30/71)	61%^{a,b} (38/62)	63%^{b} (204/326)
RTS 4-5	73%^{b} (76/104)	75%^{a} (40/53)	79%^{b} (70/89)	69%^{b} (92/134)	73%^{b} (79/108)	73%^{c} (357/488)
TOTAL	56% (152/271)	75% (163/218)	72% (163/225)	56% (145/258)	64% (140/219)	64% (763/1191)

*50% of heifers randomised within RTS category to a prostaglandin synchronisation protocol
**50% of heifers randomised within RTS category to a intravaginal progesterone device (CIDR) synchronisation protocol
^{a,b}Proportions in columns with different superscripts differ significantly ($P < 0.05$)

In Chapter 2 it was hypothesised that heifers with low RTS may have lower lifetime production due to a lower pregnancy rate during the first breeding season, but also due to later calving dates during the first calving season, which have been associated with repeated later calving dates in subsequent calving seasons (Lesmeister et al., 1973, MacGregor and Casey, 1999, Pence et al., 2007, Stevenson et al., 2008, Cushman et al., 2013). It was also hypothesised that the association of antral follicle count with the size of the ovaries (Ireland et al, 2011) may result in an association of RTS with fertility beyond cyclicity. However, in the Cox regression models presented in Chapter 3 it was found that the effect of RTS category on lifetime production of the cow was as a result of the reproductive outcome during the first breeding season only, in other words whether or not the heifer was already cycling at the start of the breeding season. In these data an association of RTS category with days to calving during subsequent calving seasons could not be shown. Although this appears to be in contrast with findings of Ireland et al. (2011) that antral follicle count is associated with fertility due to its association with follicular reserve, it may be possible that such relationships will only become evident later in the production life of the cows. In the data presented in Chapter 3, cows were followed until their 5th breeding season (6 years old), which is still before the age that antral follicle count starts to result in decreased fertility in cows (Cushman et al., 2009, Ireland et al., 2011). Annual culling of cows that failed to wean a calf had also reduced numbers significantly by that time, and it is likely that animals with relatively poor fertility were already removed from the system by then. This would mean that those few animals with initial low RTS scores that still remained in the herd represented "good" animals misclassified by RTS ("false positives"). In Table 3.4 it would seem that 31 of the initial 102 animals classified as RTS 1 or 2 were in fact good animals, because they became pregnant during the second breeding season, and subsequent performance after the second breeding season was not different from other RTS categories.

In further analyses of the interaction between RTS category and synchronisation by prostaglandin $F2_{\alpha}$ (PGF6) in heifers born in 2002 and a progesterone impregnated CIDR device in heifers born in 2003, for heifers with RTS 1 and 2, there was no differences in the case of the PGF6 protocol, whereas in the CIDR protocol median days to calving was decreased and mean weaning weight of the calves increased by synchronisation, but the pregnancy and weaning rates did not differ (Table 6.2). Heifers with RTS 3 tended to have higher pregnancy and weaning rates (P = 0.121 and P = 0.149 respectively), and had decreased median days to calving by PGF synchronisation, but the mean weaning weight did not differ. In the case of the CIDR protocol synchronisation tended to decrease days to calving for heifers with RTS 3 (P = 0.075), but weaning weight was once again not different (Table 6.2). Heifers with RTS 4 and 5 weaned heavier calves with the PGF6 protocol while the pregnancy and weaning rates did not differ, and the CIDR protocol tended to decrease the days to calving for heifers with RTS 4 and 5 (P = 0.108) but no other differences were significant (Table 6.2).

These findings make biological sense as PGF acts on the CL and will be ineffective when a CL is not present (as is expected to be the case in heifers with low RTS) (Noakes, 2001). The effect of PGF6 on heifers with RTS 3 is somewhat surprising, and is most likely due to stage of the cycle (met-oestrus or early di-oestrus at the time of RTS, and responsive to

PGF by day 6), or due to luteal tissue that was not palpable (in other words misclassification of RTS), or possibly due to some other unknown effect of PGF.

Table 6.2: Reproduction and production outcomes by RTS category and synchronisation protocol in heifers born in 2002 and 2003.

RTS	Pregnancy proportion		Median days to calving		Proportion calves weaned		Mean calf BW at weaning	
	Synchronised	*Control*	*Synchronised*	*Control*	*Synchronised*	*Control*	*Synchronised*	*Control*
PGF6 protocol (2002)								
1-2	37%[a] (16/43)	40%[a] (17/43)	33[a]	25.5[a]	21%[a] (9/43)	26%[a] (11/42)	199[a]	200[a]
3	63%[a] (25/40)	44%[a] (18/41)	9.5[a]	28.5[b]	38%[a] (15/39)	22%[a] (9/41)	226[a]	225[a]
4-5	77%[a] (40/52)	69%[a] (36/52)	14[a]	17[a]	65%[a] (34/52)	52%[a] (27/52)	218[a]	212[b]
Total	60%[a] (81/135)	52%[a] (71/136)	14[a]	20[b]	43%[a] (58/135)	35%[a] (47/136)	217[a]	212[a]
CIDR protocol(2003)								
1-2	71%[a] (42/59)	61%[a] (28/46)	15[a]	21.5[b]	53%[a] (31/59)	50%[a] (23/46)	223[a]	203[b]
3	73%[a] (22/30)	83%[a] (25/30)	15[a]	25[a]	60%[a] (18/30)	73%[a] (22/30)	218[a]	209[a]
4-5	71%[a] (15/21)	72%[a] (23/32)	15[a]	25[a]	67%[a] (14/21)	63%[a] (20/32)	215[a]	206[a]
Total	72%[a] (79/110)	70%[a] (76/108)	15[a]	24[b]	57%[a] (63/110)	60%[a] (65/108)	220[a]	206[b]

[a,b]Proportions, medians or means with different superscripts between synchronised and control groups differ significantly ($P < 0.05$)

The CIDR device provides an artificial source of progesterone, the withdrawal of which is likely to induce first ovulation in prepubertal heifers, which explains its significant effect in heifers with low RTS scores (Claro et al., 2010). Although days to calving tended to be decreased by CIDR synchronisation in heifers with RTS 3 to 5 this was not enough to lead to increased weaning weight, probably because heifers with RTS 3 to 5 performed well with or without synchronisation. Apart from this, evidence also exists that the high dose of progesterone (1.92 g) in the CIDR device may have negative effects in pubertal heifers with a CL (Dias et al., 2009, Peres et al., 2009, Butler et al., 2011). The findings presented in Table 6.2 indicate that RTS is potentially a useful tool to predict synchronisation response. Allocating heifers with RTS 1 and 2 to a CIDR programme and heifers with RTS 3 – 5 to the PGF6 protocol would likely lead to the most cost beneficial oestrus synchronisation.

Although Monteiro et al. (2013) did not demonstrate a similar beneficial effect in heifers synchronised with a CIDR device followed by natural breeding, versus heifers only bred by bulls in heifers with RTS 1 or 2, the relatively long breeding period used in their study (85d) possibly diluted the effect of the CIDR device on pre-pubertal heifers. This is a potential area of future research.

6.3. Accuracy of RTS

In the data of the five age cohorts combined, RTS 1 had a Se of 11% and a Sp of 96% for prediction of reproductive failure (Table 6.3). The low Se is due to many other factors leading to reproductive failure, and that are not explained by RTS. However, a prognostic test with a consistently high Sp such as RTS for reproductive failure, can be considered a useful culling tool because not many "good" animals will be culled when the test is applied. When the different age cohorts reported in these studies are considered, and RTS 1 assumed as culling threshold in every cohort seperately, the Sp remained above 95% except in the cohort where 50% of the heifers were synchronised using a CIDR device. In this cohort the Sp was most likely reduced due to the number of "good" animals (the demoninator in the calculation of Sp) being artificially increased by the induction of puberty in pre-pubertal heifers (Claro et al., 2010).

In Chapter 3 some factors that may affect the accuracy of RTS were investigated. It was concluded that the proportion of heifers in anoestrus, and the stage of the oestrous cycle at the time of scoring may affect the accuracy of RTS. It was therefore hypothesised that repeating RTS on low scoring animals may improve its accuracy due to the fact that animals that were misclassified with low RTS, and that were at a stage of the oestrous cycle that was likely to reduce the accuracy of RTS (met-oestrus or early di-oestrus), would be at a stage of the oestrous cycle (mid- to late di-oestrus) less likely to result in misclassification. The reason for misclassifications in met-oestrus and early di-oestrus appears to be the poor sensitivity of transrectal palpation for detecting a corpus haemorrhagicum or small CL during the first few days after oestrus (Fernández Sánches, 2008).

Figure 6.1 represents the suggested causal pathways from age at puberty (AP) to pregnancy failure during the first breeding season, and the ability of RTS to accurately predict 24-day anoestrus and pregnancy failure in the animals described in Chapter 3. Figure

6.1.A represents the situation for the 2002 cohort. The incidence of 24-day anoestrus was relatively low in this cohort, and it appears that anoestrus was mostly as a result of animals not having reached puberty at the onset of breeding, as indicated by the good ability of RTS 1, 2 and 3 to predict anoestrus in heifers relative to scores 4 and 5. Although other factors leading to reproductive failure seem to have existed in the first breeding season of the 2002 cohort, it appears from our analyses that RTS had the ability to predict pregnancy failure not only due to the failure to cycle during the first 24 d of the breeding season, but also due to other, unknown factors. It may be reasoned that these other factors may simply be the occurrence of anoestrus for the entire 50-day breeding season; however, in further modelling it was found that RTS predicted pregnancy failure also after adjusting for anoestrus during the entire 50-day AI season. The other factors leading to pregnancy failure in this breeding season could not be determined, making it difficult to speculate about the association between RTS and fertility which lies beyond cyclicity. However, associations between size of the gonads, AFC, and fertility outcomes have been described previously (Ireland et al., 2009). Potential confounding due to different levels of oestrus observation accuracy (in particular sensitivity) or other unmeasured factors between the two years cannot be ruled out.

Table 6.3: Accuracy of RTS 1, or RTS 1 and 2 combined as predictor of failure to become pregnant during the 50 day AI season per age cohort.

Age cohort	2002*	2003**	2006	2007	2008	Combined
Pregnancy failure proportion (incidence)	43.9%	25.2%	27.6%	43.8%	36.1%	35.9%
50-day anoestrus proportion (incidence)	5.2%	4.1%	14.7%	19.7%	21.8%	13.6%
	RTS 1 as predictor of pregnancy failure					
Proportion with RTS 1	5.9%	10.6%	11.1%	2.7%	3.7%	7.0%
Sensitivity	9.2%	21.8%	27.4%	2.7%	3.8%	10.7%
Specificity	96.7%	93.3%	95.1%	97.2%	96.4%	95.7%
Positive predictive value	68.8%	52.2%	68.0%	42.9%	37.5%	58.2%
Negative predictive value	57.6%	78.0%	77.5%	56.2%	63.9%	65.7%
	RTS 1 and 2 combined as predictor of pregnancy failure					
Proportion with RTS 1 or 2	31.7%	48.2%	37.3%	20.5%	22.4%	31.7%
Sensitivity	44.5%	56.4%	56.5%	26.5%	32.9%	40.9%
Specificity	78.3%	54.6%	69.9%	84.1%	83.6%	73.5%
Positive predictive value	61.6%	29.5%	41.7%	56.6%	53.1%	46.4%
Negative predictive value	64.3%	78.8%	80.9%	59.5%	68.8%	69.7%

*50% of heifers randomised within RTS category to a prostaglandin synchronisation protocol
**50% of heifers randomised within RTS category to a intravaginal progesterone device (CIDR) synchronisation protocol

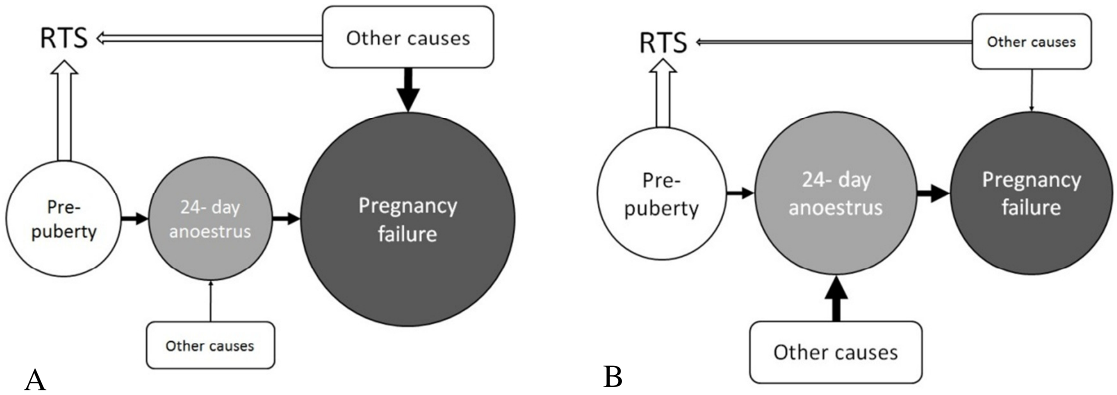

Figure 6.1: Flow diagrams indicating how the proportions of heifers with anoestrus and pregnancy failure due to other causes than anoestrus influenced the accuracy of RTS in the cohorts of heifers born in A: 2002 and B: 2003 (Chapter 3). Relative size of circles and thickness of arrows indicate relative importance of factors in each cohort.

Figure 6.1.B represents the situation for the 2003 cohort (Chapter 3), where RTS did not predict pregnancy failure as accurately as in the 2002 cohort. Also, in this cohort RTS did not predict pregnancy failure after adjusting for anoestrus. It is therefore concluded that other factors leading to animals failing to show oestrus during the first 24 d of the breeding season, and that were not measurable by RTS, existed in this cohort. These may have included management factors such as nutrition and oestrus detection sensitivity, as well as other environmental factors such as weather (Larson, 2005). However in this cohort there was a very strong association between 24-day anoestrus and pregnancy failure, and it appears that there were very few other factors leading to pregnancy failure except for heifers that were not observed in oestrus. For these two reasons RTS could not predict pregnancy failure as accurately as in the heifers born in 2002. Due to the experimental model of AI that relied on visual oestrus observation used in this trial, once again lower sensitivity of oestrus observation accuracy in the 2003 cohort could have resulted in a weaker association between RTS and anoestrus, and a relatively stronger association between anoestrus and pregnancy failure.

However, in Chapter 3 only two age cohorts with differing proportions of anoestrus were studied, and the unexpected inverse relationship between the proportion of heifers in anoestrus and the proportion of heifers with low RTS may have confounded these results. Further analysis of the data presented in Table 6.3 where accuracy data of RTS 1 and RTS 2 as threshold were combined to represent different cut-off levels (or culling rates) of the test, showed that the sensitivity and specificity were not dependent on the true prevalence of pregnancy failure or failure to show oestrus, but were highly dependent on the culling rate determined by the proportion of heifers with either RTS 1, or RTS 1 and 2 (Figure 6.2). This inverse relationship between Se and Sp for changes in test threshold level is a typical characteristic of diagnostic tests (Thrusfield, 2007). The green box in Figure 6.2 indicates the culling level (or test threshold) that resulted in the most appropriate accuracy of the test: when the test threshold exceeded the lowest 10% of group of animals tested, it resulted in a Se above 20%, whereas if the threshold exceeded 30% the Sp dropped below 80%, and would result in an unacceptably high level of false positive test results ("good" animals being culled).

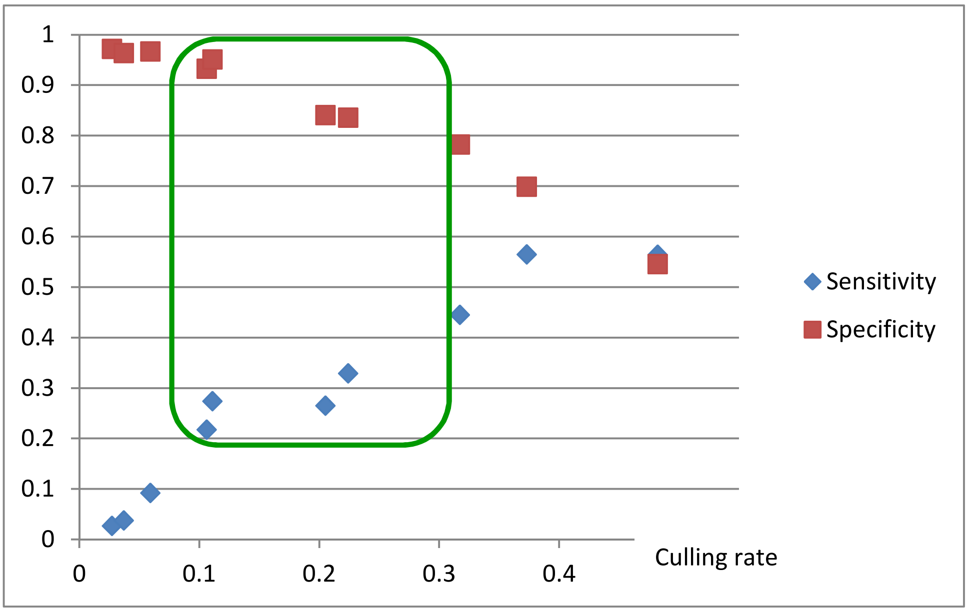

Figure 6.2: The relationships between culling rate and sensitivity and specificity when either RTS 1 or RTS 2 is used as culling threshold, based on the data presented in Table 6.3. The green rectangle indicates the most appropriate range of culling rates.

Normally in diagnostic tests the true prevalence of a condition will not affect the test Se and Sp, but will affect the predictive values of the test (Thrusfield, 2007). In the case of the data presented in Table 6.3, for every 1% increase in true prevalence of pregnancy failure, the negative predictive value decreased on average by 1% ($P < 0.01$, $r^2 = 0.93$) and the positive predictive value increased by 1.4% ($P = 0.01$, $r^2 = 0.90$). Negative predictive value is the important measure of accuracy of a culling test, because the test negative animals are the ones that are retained after application of culling, and therefore RTS will be more valuable in herds of heifers with relatively low levels of pregnancy failure. However it was interesting to note that the culling rate also had a weak association with the negative predictive value (β = 0.13, P = 0.04) when adjusted for the true prevalence of pregnancy failure, whereas this was not the case for the positive predictive value (P = 0.47). The practical implication of this would be that in herds where a high prevalence of reproductive failure is expected, a higher culling rate should be applied in order to improve the negative predictive value.

6.4. Repeatability of RTS

Rosenkrans and Hardin (2003) found the RTS system to be moderately or substantially repeatable between and within veterinarians, respectively, using multicategory Kappa statistics (Kappa values 0.46 and 0.64 respectively), and also to distinguish between prepubertal (RTS 1, 2 or 3) or post pubertal (RTS 4 or 5) heifers (Kappa values 0.58 and 0.72 respectively). In our own investigations (unpublished data, not from this study), estimating the repeatability of RTS between experienced, blinded veterinarians with only theoretical training in the RTS system resulted in only slight agreement between and fair agreement within veterinarian (Table 6.4).

Table 6.4: Kappa values for agreement beyond chance of RTS (3 categories: 1 or 2; 3; 4 or 5) between and within three experienced, blinded veterinarians after examination of 56 18-month old Bonsmara heifers (unpublished data).

	Veterinarian 1	Veterinarian 2	Veterinarian 3
Veterinarian 1	0.31		
Veterinarian 2	0.14	0.33	
Veterinarian 3	0.23	0.13	0.37

The overall Kappa value within all 3 veterinarians was 0.35. However, in our study the prevalence of heifers with either low (1 or 2) or high (4 or 5) RTS was below 15%, most likely resulting in instability of the Kappa statistic (Dohoo, 2003a). In the study by Rosenkrans and Hardin (2003) the prevalence of pre-pubertal and post-pubertal heifers was not given but the same group of heifers was examined 3 times at different ages, making it likely that a more even distribution amongst RTS categories was present in their data than was the case in our data where RTS was measured only on one occasion. It is therefore possible that the study design of Rosenkrans and Hardin (2003) favoured higher Kappa values due to the fact that only a very small group of heifers was examined, followed over time. This is not the way that RTS is implemented in veterinary practice, indicating a need for further research required to investigate factors that may be present under field conditions, affecting the repeatability of RTS.

6.5. The use of relative pelvis size to predict calving ease

As discussed in Chapter 4, the association between pelvis area and calf birth weight is well documented, and was also evident in the data of heifers born in 2006 and 2007 (Figure 6.3). This association, in combination with the well-established fact that calf birth weight is the most important factor associated with dystocia, results in the inability to successfully prevent dystocia by culling heifers with small pelvis area (Chapter 4). Selection based on unadjusted pelvis area is, however, currently practised by veterinarians in southern Africa (Van Zyl, 2009, personal communications with D. Midgley, C. Nel, D. van Zyl, W. Reisinger and J. Wessels, 2013), generally at a culling rate in excess of 25%. In the data presented in Chapter 4, when 30% of heifers were culled based on unadjusted PA, this would have resulted in the culled heifers being 32 kg (10.2%) lighter than the retained heifers ($P < 0.001$), and the calves of the culled heifers tending to be 1.1 kg (3.7%) lighter at birth than those of the retained heifers ($P = 0.08$). What was of particular importance in the data of Chapter 4 was the fact that pelvis area, and not pre-breeding body weight, was independently associated with calf birth weight, and that the association between pre-breeding body weight and calf birth weight was in fact confounded by the association between pelvis area and calf birth weight. This indicates that culling after ranking by unadjusted PA data is even more likely than culling after ranking by BW, to result in an increase in calf BWT, and probably results in an increase in frame size. Increasing frame size may have negative effects on the reproductive performance of extensively managed beef cattle (Taylor et al., 2008), which indicates a possible negative effect of using unadjusted PA as culling tool in extensively managed beef cattle.

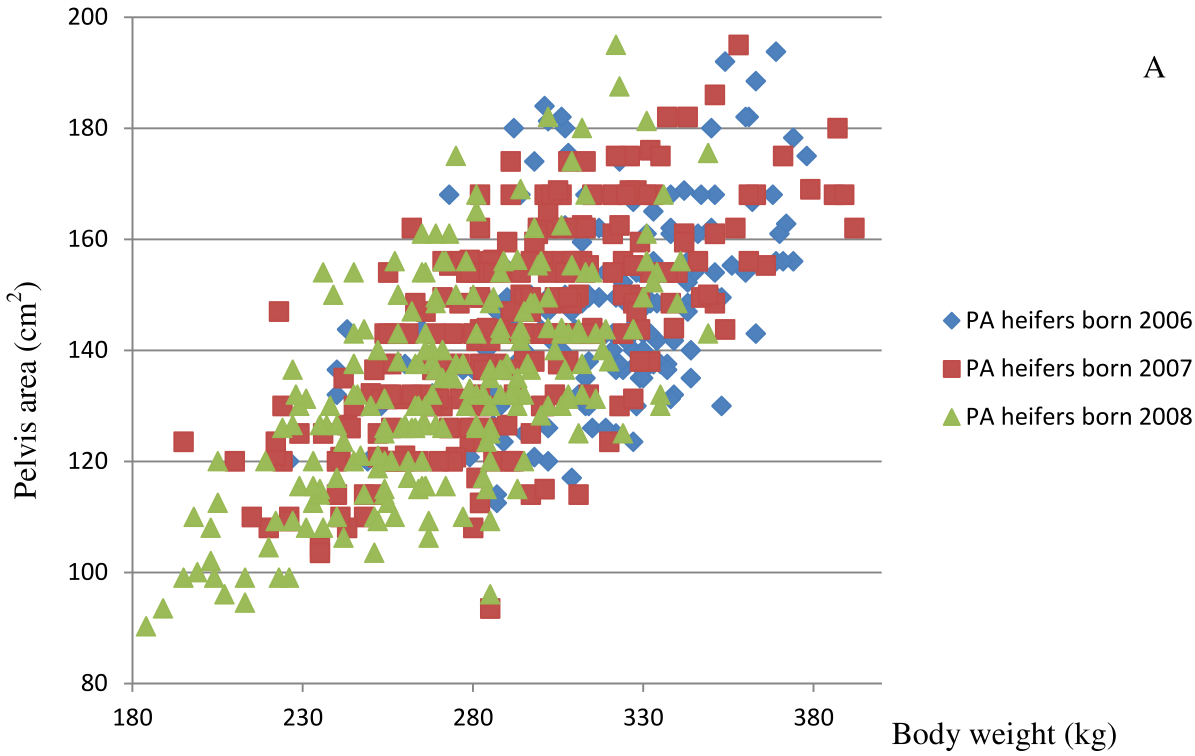

Figure 6.3(A): Scatter plot showing the associations between pelvis area (PA) (cm^2) and body weight in heifers born in 2006, 2007 and 2008 (n = 713).

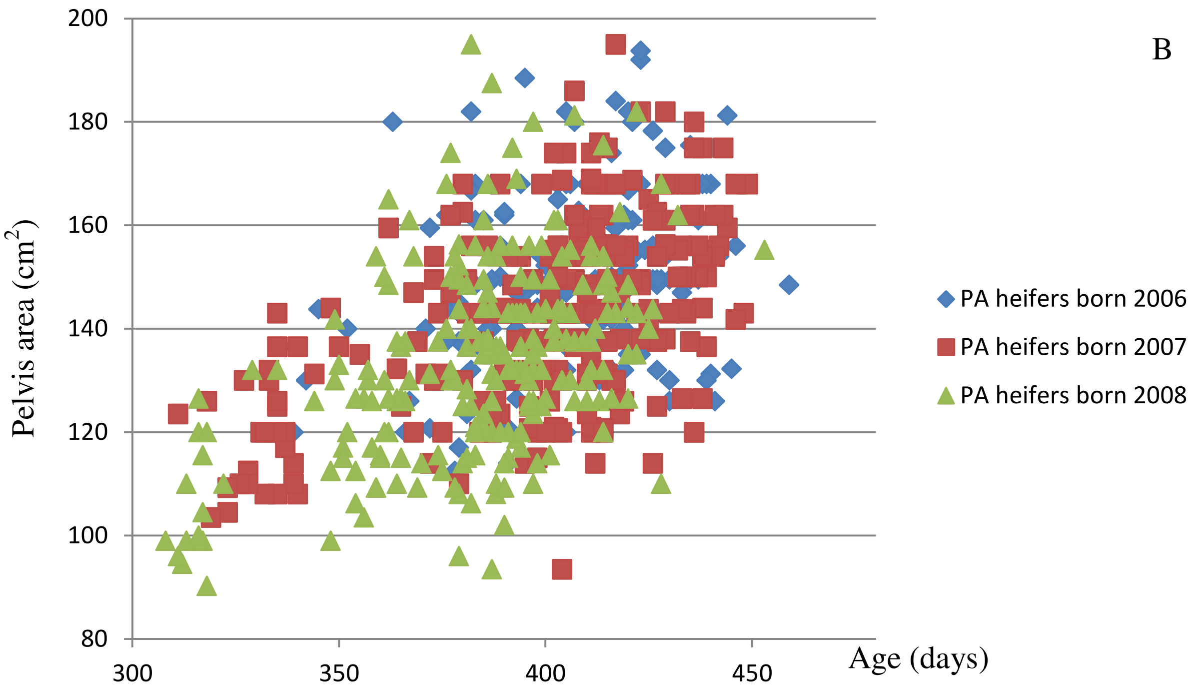

Figure 6.3(B): Scatter plot showing the associations between pelvis area (PA) (cm^2) and age in heifers born in 2006, 2007 and 2008 (n = 713).

Taylor et al. (2008) found that, although calves of large frame sized heifers may grow faster, they also have higher birth weight, and large frame sized heifers reproduce less efficiently than medium and small framed heifers during subsequent breeding seasons under extensive conditions. Using unadjusted PA as selection tool, in particular when a high culling rate is used, is therefore likely to increase the genetic frame size of the herd over time, with resultant negative effect on reproductive efficiency. In the data presented in Chapter 4, pelvis area was more significantly associated with body weight than with age, similar to previous reports (Laster, 1974, Van Donkersgoed, 1997). Adjusting pelvis area for age using a fixed correction factor of 0.27 cm^2 per day of age (as recommended by Deutscher, 1988) before applying hypothetical culling, resulted in an outcome very similar to using unadjusted pelvis area. In Chapter 4 it was shown that adjusting for BW, or better adjusting for lean BW in an attempt to represent frame size, resulted in the best accuracy of PA data as a test to predict dystocia. Sensitivity of pelvimetry as predictor of dystocia increased with increasing culling rate, but this happened at the cost of specificity, and using unadjusted pelvis area to cull 30% of heifers, as currently often practiced in South Africa, resulted in a sensitivity of 33% in the data presented in Chapter 4. This is similar to the Se of using lean body weight adjusted pelvis area at a culling rate of 20%, the latter having a specificity of 89% compared to 80% of unadjusted pelvis area applied at a 30% culling rate. This means that a much better end result could be obtained when adjusting PA data to lean BW. High sensitivity for dystocia cannot be achieved by pelvimetry due to the poor ability to predict calf birth weight (Cook et al., 1993). However, high specificity can be maintained at relatively lower culling rates, and by adjusting pelvis area data to the BW of the heifers within contemporary groups. High specificity is an essential property of a culling tool in order to avoid good animals being culled.

6.6. Adding ultrasonography to pre-breeding examination of beef heifers

In Chapter 5 it was shown that ultrasonography can predict reproductive outcome due to its prediction of cyclicity, similar to that of RTS. Although ultrasonography only predicted reproductive outcome significantly better than RTS in one age cohort in this study (Table 5.6), it may be possible that ultrasonography can partly overcome the inaccuracies of RTS at a single time due to stages of the oestrous cycle (Chapter 3), because it has better sensitivity in detecting luteal tissue in the ovaries (Lean et al., 1992, Johnston et al., 2009), and has shown good repeatability compared to that of RTS (Rosenkrans and Hardin, 2003), although the repeatability of the two methods for the purpose of RTS has not been compared in the same study. The ability to accurately measure the diameter of the uterus, or the thickness of the endometrium as was done by Monteiro et al. (2013), adds further prognostic value to the use of ultrasonography. In the model developed in Chapter 5 that uses ultrasonographic measures of the reproductive tract, the critical size of the largest follicle was determined to be 13mm, however Monteiro et al. (2013) points out that *Bos indicus* breeds are likely to have smaller dominant follicles, and the model may have to be adapted for heifers from these breeds. However, the way in which uterine diameter was standardised in Chapter 5 makes biological sense given the fact that similar breed differences may also exist in uterus horn diameter (Monteiro et al., 2013).

Adding pelvimetry to the ultrasound model to predict reproductive outcome seemed to improve the accuracy more consistently than replacing RTS with ultrasonography (Table 5.6), and it can therefore be assumed that due to the cost of performing ultrasound, it is likely that pelvimetry will add more value to RTS than ultrasonography. However, the repeatability of RTS by transrectal palpation, ultrasound, as well as pelvimetry needs to be compared in a single study using the same population of animals, before a final conclusion can be made.

6.7. Cost effectiveness of pre-breeding examination of beef heifers

To the knowledge of the researcher, the cost-effectiveness of culling based on RTS, pelvimetry or ultrasonography of the reproductive tract has not been determined. Due to many factors associated with reproductive performance that cannot be predicted by RTS or pelvimetry, and many other factors determining the income of a beef cattle operation, this type of investigation will require many assumptions and will likely not be highly repeatable. Nevertheless, a simple deterministic cost:benefit analysis based on the methods used by Holm et al. (2008), and using the associations of RTS category with reproduction and production outcomes obtained in this study, summarised in Tables 6.5 and 6.6, is presented in Appendix 1.

Table 6.5 shows the effect that culling at the threshold of either RTS 1 or RTS 2 would have on all the animals that were followed to at least the time of weaning of their first calves. In Table 6.5 it is shown that culling heifers with RTS 1 and 2 would result in the total kg of calves weaned per total kg of heifers at the start of the breeding season to be significantly increased in retained heifers, which is likely to result in an economic benefit.

Table 6.5: Summary of data of all animals followed to weaning by RTS category (2002, 2003, 2006 and 2007 cohorts).

RTS category	n	Mean heifer weight (kg) at start	No that Calved	Median days to calving	Calf mortality	No that weaned a calf	Mean weaning weight	Total kg calves weaned per kg heifers at start
3-5	625	313[a]	383 (61%)[b]	20[b]	125 (33%)[b]	258 (41%)[c]	210[a]	28%[c]
1-2	348	309[a]	173 (50%)[a]	26.5[a]	82 (47%)[a]	91 (26%)[a]	207[a]	18%[a]
2-5	897	311[a]	527 (59%)[b]	22[a,b]	191 (36%)[b]	336 (37%)[b]	209[a]	25%[b]
1	76	307[a]	29 (38%)[a]	25[a]	16 (55%)[a]	13 (17%)[a]	213[a]	12%[a]
Total	973	311[a]	556 (57%)[b]	22[a,b]	207 (37%)[b]	349 (36%)[b]	209[a]	24%[b]

[a,b]Proportions, medians or means with different superscripts within columns differ significantly ($P < 0.05$)

Due to the fact that the proportions of heifers with RTS 1 differed amongst birth cohorts, it would have been most appropriate to adapt the culling threshold applied in order to achieve a culling rate of between 10 and 30% (see above). In Table 6.6 different culling thresholds of RTS were applied per age cohort in order to achieve a culling rate in each birth cohort that lies between 10 and 30%. Due to the discrete nature of the RTS score it was not possible to apply exactly the same culling rate in each cohort. However, in practice this could be done since other criteria would also be considered when culling.

In the deterministic model used in Appendix 1 to calculate the cost benefit of culling based on RTS, it was found that the advantage of culling heifers that are less likely to reproduce based on RTS does not necessarily lead to sufficient financial benefit to warrant the veterinary expense of performing RTS. When RTS 1 is used as culling threshold in all cohorts, the nett present value (NPV) is –R 1,248.78 and the benefit:cost ratio is 0.94 (R 0.94 is returned for every R 1.00 invested in having RTS performed at a cost of R20.00 per animal). The lower Se caused by the lower culling threshold in the 2002, 2007 and 2008 cohorts when RTS 1 is used as threshold, probably reduced the cost benefit, and under these circumstances the break-even point for the cost of RTS is R 18.71.

Table 6.6: Summary of data of all animals followed to weaning when culling of the lowest 10 to 30% of RTS scores is applied (RTS culling threshold is 1 for 2003 and 2006 cohorts, and 2 for 2002, 2007 and 2008 cohorts).

Category	n	Mean heifer weight (kg) at start	No that Calved	Median days to calving	Calf mortality	No that weaned a calf	Mean weaning weight	Total kg calves weaned per kg heifers at start
Retained	781	313[b]	485 (62%)[c]	21[b]	181 (37%)[a]	304 (39%)[b]	210[a]	0.26[c]
Culled	192	302[a]	71 (37%)[a]	29[a]	26 (37%)[a]	45 (23%)[a]	202[a]	0.16[a]
Total	973	311[b]	556 (57%)[b]	22[b]	207 (37%)[a]	349 (36%)[b]	209[a]	0.24[b]

[a,b]Proportions, medians or means with different superscripts within columns differ significantly ($P < 0.05$)

It was expected that adapting the RTS threshold for culling in order to cull between 10 and 30% heifers in each age cohort would result in the best cost benefit due to the improved sensitivity compared to culling only heifers with RTS 1 in each cohort, and due to fewer good animals being culled unnecessarily (improved specificity) compared to culling heifers with RTS 1 and 2 in each cohort (Table 6.6). However, when culling was applied at RTS 1 for the 2003 and 2006 cohorts, and RTS 1 and 2 for the 2002, 2007 and 2008 cohorts, it resulted in a NPV of –R17,536.27 and a benefit:cost ratio of 0.10 in the model presented in Appendix A. Reasons for this relatively poorer cost benefit are not clear; however, the reason is likely to be the difference in calf mortality rates noted in Table 6.5 that was not present when RTS threshold differed between years to achieve a culling rate between 10 and 30% (Table 6.6). Although there is no clear biological explanation for the difference in calf mortality rates noted in Table 6.5, it is concluded from these analyses that the culling strategy with the higher sensitivity resulted in improved cost benefit in this study under the current market conditions.

The economic benefit of pre-breeding examination of beef heifers warrants further study, based on further data acquisition and preferably using a stochastic model to account for variation in outcomes and uncertainty in assumptions. Sensitivity analysis should then be done to identify those factors with the biggest influence on the cost:benefit outcome.

6.8. Limitations of the study

The following factors resulted in weaknesses of this study:

- The fact that the study herd was a unique farming system utilising intensive grazing on irrigated pastures, breeding heifers at a young age and using a single breed of cattle means that the results of these studies are not necessarily valid in the general beef cattle population. In particular, important differences in reproductive tract and pelvis dimensions exist between *Bos Taurus* and *Bos Indicus* breeds (Deutscher et al., 1988, Monteiro et al., 2013). Further, in the experience of the researcher, when extensively managed beef heifers bred at 2 years of age are examined shortly following a long dormant season, the predictive value of RTS and pelvimetry for reproductive outcomes may be different from that reported in these studies (Tshuma et al., unpublished data).
- The conclusion made in Chapter 3 that RTS predicts long-term reproductive outcome only as a result of its association with cyclicity at the onset of the first breeding season, and the inability of the long-term study to demonstrate a significant association between days to calving in the first, and subsequent calving seasons, is in contrast with several studies on the same topic (Lesmeister, 1973, Cushman, 2013 and Perry and Cushman, 2013), even in a study using the same herd as the one in this thesis (MacGregor and Casey, 1999). It is possible that the small sample size resulted in inadequate power of the long-term study reported in Chapter 3, leading to a type II error, if RTS before the onset of the first breeding season was in fact associated with days to calving in subsequent calving seasons (Dohoo, 2003b).
- Antral follicle count was not recorded during ultrasonographic data capture, and the length of the ovary had to be used as a proxy for AFC; however, it was not an independent predictor of reproductive outcome in Chapter 5. Subsequent to data collection for this study, other researchers have emphasised the importance of AFC as an independent variable (Cushman et al., 2009, Ireland et al., 2011).
- Due to the complexity of factors affecting reproductive outcomes (especially when artificial insemination is used), the possibility exists that other confounding factors that the researchers were not aware of, may have biased the conclusions made from these results.

6.9. Practical applications of pre-breeding examination of beef heifers

Possible applications of pre-breeding examination data of heifers (RTS and pelvimetry) have been described (Andersen et al., 1991, Van Donkersgoed et al., 1997):

- Firstly, RTS can be used as a screening test to determine the pubertal status of heifers before the breeding season, whereas pelvimetry can be used to determine the maximum birth weight of calves that will likely prevent dystocia in order to select the most appropriate bulls.
- Secondly, both methods can be used as an indication of the nutritional requirements of heifers when sufficient time is allowed before the breeding season.
- Thirdly, the two methods can be used as selection tools for age at puberty or calving ease, respectively.

A fourth application of RTS, resulting from this thesis, is the selection of the most appropriate or most cost effective oestrus synchronisation protocol per individual animal in the herd.

This thesis has also shown that pelvimetry has a fourth application in its potential to select for reproductive outcome, and that ultrasonography can be used to replace RTS by transrectal palpation.

Culling policies should be implemented in beef herds in ways that undesirable side-effects do not occur as a result of culling in order to achieve improvement of another trait (Taylor et al., 2008). Genotype x environment interactions should also be kept in mind, and animals that are well adapted to a specific management system should be selected (Bourdon, 2000, Taylor et al., 2008, Beffa et al., 2009). In the case of pre-breeding examination of heifers, this, as well as the accuracy and repeatability of the selection method, the true prevalence of the condition selected against and the cost-benefit of the selection procedure, should be considered before implementing it in a herd. Table 6.7 summarises the possible applications of pre-breeding examination data that are likely to result in the most appropriate culling criteria in different herd scenarios, based on the findings of this thesis.

Generally when the overall reproductive performance of heifers based on history in the herd is good, a high culling rate will not be appropriate due to many "false positive" tests, in other words many animals culled that would have reproduced efficiently if not culled. Based on the findings in Chapter 3 and summarised in Figure 6.2 a culling rate below 10% will most likely result in an acceptable Sp in such herds. In herds with relatively poor reproductive performance a low culling rate may not be cost effective due to the lower Se, whereas in herds with a high incidence of dystocia, a low culling rate based on PA is also likely to result in inefficient culling due to the low Se at low culling rates.

Table 6.7: Applications of pre-breeding examination data for the purpose of culling that are likely to be most appropriate in different herd scenarios.

Frame size of cattle	Overall herd reproductive performance	Expected incidence of dystocia	Pre-breeding data to use for culling	Culling rate
Small	Good	Low	PA[1] and RTS[2] combined	< 10%
	Poor	Low	PA and RTS combined	10-20%
	Any	High	PA or APA[3]	20-30%
Medium	Good	Low	BWPA[4] and RTS combined	< 10%
	Poor	Low	BWPA and RTS combined	10-20%
	Any	High	BWPA or LBWPA[5]	20-30%
Large	Good	Low	PA:BW[6] and RTS combined	< 10%
	Poor	Low	PA:BW and RTS combined	10-20%
	Any	High	PA:BW	20-30%

[1]Pelvis area
[2]Reproductive tract score
[3]Age adjusted pelvis area
[4]Body weight adjusted pelvis area (see Chapter 4)
[5]Lean body weight adjusted pelvis area (see Chapter 4)
[6]Pelvis area to body weight ratio

In herds with small framed cattle using unadjusted PA or age adjusted PA will not be inappropriate, because increasing frame size of the cattle slightly by selection may improve the general efficiency of the herd due to improved growth rates (Taylor et al., 2008). On the other hand, when the frame size of the animals is large this may result in poor reproductive performance due to poor adaptation especially in dry and warm environments such as Southern Africa (Taylor et al., 2008, Beffa et al., 2009), in which case selection using PA:BW may be most appropriate due to the ability to select against large framed animals with poor reproductive abilities (Chapter 4). Also, in herds with large framed animals where dystocia has a high incidence, it is likely that dystocia occurs as a result of foeto-maternal disproportion due to calf birth weight that is disproportionately high large framed cows (Meiering, 1984, Holland and Odde, 1992, Zaborski et al., 2009). In such cases selection after ranking by PA:BW will result in large framed heifers, that are likely to give birth to heavy calves, to be culled.

In medium framed animals the ideal would be for a selection process that does not affect frame size, in which case BWPA or LBWPA will be most appropriate. Combining RTS or ultrasonography with pelvimetry in a selection process will be appropriate in herds where the aim is mainly to select for improved fertility, and not necessarily against dystocia (Chapter 5), in other words in herds where dystocia in heifers has a low incidence.

Body condition scoring should always be added to pre-breeding examination due to its association with reproductive outcome (Chapters 2 and 5), and due to its value of potentially improving adjustment of PA data (Chapter 4), at no additional cost when pre-breeding examinations are in any case performed.

CHAPTER SEVEN

CONCLUSIONS, RECOMMENDATIONS AND FURTHER RESEARCH QUESTIONS

7.1. Conclusions

The hypothesis that reproductive performance of beef heifers cannot be predicted before the onset of the first breeding season was rejected. It was concluded that pre-breeding examination of beef heifers is useful to identify animals with lower potential to reproduce successfully over the long term in a restricted breeding system. All the procedures investigated had consistently high specificity, making them valid culling tools.

Furthermore:

- It was concluded from that RTS is a predictor of heifer fertility and compares well with other traits used as a predictor of production outcomes.
- It was concluded that RTS is a valid culling tool to improve long-term reproductive success in a seasonal breeding system, by excluding heifers that are likely to fail to become pregnant or likely to calve late in their first calving season.
- It was concluded that the predictive value of RTS decreases with increasing prevalence of anoestrus and at certain stages of the oestrous cycle, and that RTS may predict pregnancy failure due to causes other than anoestrus.
- It was concluded that pelvimetry is a useful culling tool to aid in the management of dystocia in yearling heifers.
- It was concluded that adjustment of PA to median BW or LBW within age group improves its accuracy and avoids the undesirable side-effects of using unadjusted pelvis area.
- It was concluded that ultrasound measures of the reproductive tract (absence of a CL, absence of a follicle of at least 13mm and relatively small uterus diameter) have prognostic value for pregnancy failure in restricted bred yearling heifers as a result of their association with anoestrus.
- It was concluded that smaller pelvis area has additional prognostic value to RTS or ultrasound measures of the reproductive tract for reproductively poor performing heifers.

7.2. Recommendations

It is recommended that culling based on pre-breeding examinations of heifers can be applied before the onset of the first breeding season at culling rates ranging from 5 to 30%, with higher culling rates being more appropriate in poor performing herds.

It is recommended that body condition scoring and pelvimetry should always be included as part of pre-breeding examinations of beef heifers. Pelvimetry data should either be applied as unadjusted pelvis area in herds with inadequately small frame size, or as pelvis area adjusted to body weight or lean body weight within contemporary group in herds with adequate frame size, or as pelvis area to body weight or lean body weight ratio in herds with inadequately large frame size. Pelvimetry data should be applied in combination with ultrasonography of the reproductive tract, or RTS by transrectal palpation in herds with poor reproduction performance. Ultrasonographic measures of the reproductive tract to consider should include the presence of a corpus luteum, the presence of a follicle of at least 13 mm and the relative diameter of the left uterine horn. When ultrasonography is not available, the accuracy of reproductive tract scoring by transrectal palpation can likely be improved by repeating it in low scoring animals after 7 days.

The use of ultrasonography of the reproductive tract or RTS by transrectal palpation is recommended to improve the cost effectiveness of synchronisation programmes for individual animals within a herd.

7.3. Further research questions resulting from this work

These studies, being largely observational, have given rise to further research questions that can best be answered by hypothesis-driven trials in future. These research questions include the following:

1. Does repeating RTS after 7 days on low-scoring heifers improve the specificity for reproductive failure during a restricted breeding season?
2. Is RTS associated with fertility beyond its association with cyclicity in heifers?
3. Is the prognostic model for reproductive failure based on ultrasonographic measures of the reproductive tract and pelvimetry (Chapter 5) valid in other herds of heifers?
4. Are RTS, ultrasonography of the reproductive tract or pelvimetry cost beneficial to the beef cattle producer?
5. Can RTS, ultrasonography of the reproductive tract or pelvimetry add value to oestrus synchronisation programmes in beef heifers?
6. Do operator, operator fatigue, heifer age, breed, BW, pubertal stage, method of restraint or BCS affect repeatability of RTS, ultrasonography or pelvimetry using a Rice pelvimeter?
7. Are vertical diameter and horizontal diameter development of the internal pelvic opening of heifers associated with hormonal changes before and during puberty, ovarian development or antral follicle count?
8. Does AFC add any prognostic value for reproductive failure to a model consisting of the absence of a CL, the relative diameter of the left uterine horn and the absence of a follicle of at least 13 mm using transrectal ultrasound?
9. Do dietary changes that occur after estimation of reproductive potential using RTS or pelvimetry affect their association with reproductive outcomes?

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APPENDIX ONE

COST BENEFIT ANALYSIS OF RTS

In this deterministic cost:benefit model of RTS the cost that was considered was only the veterinary cost of performing RTS, which was estimated at R 20.00 per heifer. The other input variables that were modelled include a single value for the direct maintenance cost of heifers and cows which was estimated at R 5.00 per day (F Meyer, personal communication, 2013). Initial capital cost is the purchase value of pre-breeding yearling heifers, and the end capital value is the final resale value of 2.5 year old cows.Calculations are based on current market values (www.safeedlot.co.za) and are adjusted to a common time using the real interest rate (difference between bank lending rate and interest rate) which was estimated at 2% (F Meyer, personal communication, 2013). To simplify the calculations, interest was added once per 90-day or 180-day period. Some further assumptions were made, which include:

Value/kg BW at start of breeding (12 – 15 months old):	R14.00/kg
Time from start to pregnancy testing:	90 days
Body weight at pregnancy testing:	350 kg
Value/kg at pregnancy testing (16 – 19 months old):	R13.00/kg
Time from start to weaning:	540 days
Value at weaning (7 months):	R16.00/kg

Table 1: Partial farm budget without any selection by RTS

Day	Event	Unit value	No of animals	Total value
1	Start value	-R 4 340.00	973	-R 4 222 820.00
1	Culling (RTS 1 and 2)	R 4 340.00	0	R 0.00
1-90	Maintenance	-R 450.00	973	-R 437 850.00
1-90	Interest			-R 23 303.35
90	Culling (not pregnant)	R 4 550.00	417	R 1 897 350.00
91-270	Maintenance	-R 900.00	556	-R 500 400.00
91-270	Interest			-R 32 870.23
271-540	Maintenance	-R 900.00	556	-R 500 400.00
271-540	Interest			-R 38 202.94
540	Weaning 1	R 3 360.00	349	R 1 172 640.00
540	End value 1	R 5 040.00	556	R 2 802 240.00
540	Nett end value 1			R 116 383.48

Table 2: Partial farm budget when heifers with RTS 1 and 2 are culled before breeding starts

Day	Event	Unit value	No of animals	Total value
1	Start value	-R 4 340.00	973	-R 4 222 820.00
1	Culling (RTS 1 and 2)	R 4 340.00	348	R 1 510 320.00
1-90	Maintenance	-R 450.00	625	-R 281 250.00
1-90	Interest			-R 14 968.75
90	Culling (not pregnant)	R 4 550.00	242	R 1 101 100.00
91-270	Maintenance	-R 900.00	383	-R 344 700.00
91-270	Interest			-R 22 523.19
271-540	Maintenance	-R 900.00	383	-R 344 700.00
271-540	Interest			-R 26 195.42
540	Weaning	R 3 360.00	258	R 866 880.00
540	End value	R 5 040.00	383	R 1 930 320.00
540	Nett end value 2			R 151 462.64

From Tables 1 and 2 the Nett Present Value and the Benefit:Cost ratio of performing RTS and culling 10-25% heifers with lowest RTS can be calculated as follows:

Nett Present Value = Nett end value 2 – Nett end value 1 – cost of performing RTS

= R 15,619.16

Benefit:Cost ratio = (Nett end value 2 – Nett end value 1)/cost of performing RTS

= 1.80

The model shown in Tables 1 and 2 is however very dependent on certain input variables, in particular the financial input variables. For this reason a few break-even points could be calculated for culling of heifers with RTS 1 and 2 to remain profitable, when all other input variables remain constant:

Daily maintenance cost break-even point: R 4.84/animal/day

Lower maintenance cost means that performing RTS for culling purposes is not profitable anymore. It is possible that the daily maintenance cost may be less than R 4.84 per animal (F Meyer, personal communication, 2013) in which case performing RTS will not be cost beneficial.

Weaner price break-even point: R 16.82/kg

If a weaner price above R 16.82/kg is paid, performing RTS to cull heifers with scores 1 and 2 is not cost beneficial. However, when the weaner price increases, the price of yearling heifers also tend to increase, which will again improve the cost benefit of culling heifers with RTS 1 and 2.

Cost of RTS break-even point: R 36.05 per heifer

Under the circumstances modelled in Tables 1 and 2 the farmer can spend up to R 36.05 per heifer on RTS for the procedure to be cost beneficial.