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Estimates of the fertility of extensively managed Bonsmara bulls

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PREFACE

The experimental work described in this dissertation was carried out in the school of Agricultural and Food Sciences, University of Pretoria, Pretoria, from January 2006 to December 2007, under the supervision of Professor Edward C. Webb.

I declare that the thesis/dissertation, which I hereby submit for the degree MSC(AGRIC) PRODUCTION PHYSIOLOGY at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature ……..

Date ……..

Abstract

The aim of the present study was to determine if the breeding potential of 25-month old, extensively kept, Bonsmara beef bulls can be predicted from production data, spermatozoal characteristics and/or blood hormone concentrations at that age. A further objective of the study was to determine if any of the above mentioned criteria could be associated with the libido of these bulls. Forty-one Bonsmara bulls were included in an on-farm performance test (Phase D_1 growth test) for a period of 180 days. At an average age of 24.7 months, blood sampling took place (before and after GnRH treatment) and the bulls were subjected to a libido test, after which further blood samples were collected. Blood sample were analysed for cortisol and testosterone concentrations. The bulls were also subjected to an Overall Breeding Soundness Evaluation. This procedure involves an evaluation of the physical genitalia of the bulls, a measurement of scrotal circumference and semen evaluation. For purposes of statistical analyses the bulls were categorised into independent breeding potential categories according to the scores they obtained for the measured reproductive traits. The categories included scrotal circumference, spermatozoal morphology and motility and the overall breeding soundness category. A statistical analysis of the data was done by using the general linear models (GLM) procedure of the Statistical Analyses System (SAS version 8.2 BMDP). The production and growth measurements of the Bonsmara bulls did not differ between any of the high and low fertility categories and can not be used to predict the breeding potential of young bulls. The correlation between pre-weaning growth rate and the percentage morphologically normal spermatozoa was positive ($r = 0.33$; $P<0.1$), suggesting that relatively high growth rates before weaning may have a positive effect on potential fertility under normal extensive feeding conditions. By contrast, numeric differences in growth after weaning suggest that a high growth rate after weaning may have a negative effect on potential fertility. The results showed that the overall breeding soundness categories tended to be influenced by the pre-weaning growth rate ($r = 0.24$; $P>0.1$) and body lengths ($r = 0.18$; $P>0.1$) of bulls. Sampling time had a statistically significant effect on blood cortisol and testosterone concentrations for all of the breeding potential categories. Testosterone concentration increased significantly ($P<0.001$) after GnRH treatment. High plasma cortisol concentrations were associated with low plasma testosterone concentrations. High testosterone concentrations were associated with less spermatozoal morphological defects ($r = - 0.21$; $P>0.1$). The testosterone concentrations before GnRH treatment was higher for bulls with exceptional fertility ($P<0.05$), while testosterone concentration after GnRH treatment tended to be higher ($P<0.1$) for the bulls with acceptable fertility. This observation may be explained by the negative feedback system that operates between LH and testosterone secretion. The percentage spermatozoal defects were influenced to a greater extent by morphological abnormalities leading to reduced motility of the sperm than by any other abnormalities. From the results it seems that the semen morphology category is a better indicator of semen quality than the SC and semen motility categories. Overall breeding soundness classifications of bulls were largely influenced by spermatozoal

motility (P<0.001) and to a lesser extend by spermatozoal morphology and SC. None of the reproductive and production measurements showed a correlation with libido scores, implying that optimal bull reproductive evaluation should include the assessment of both breeding soundness and libido.

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Abbreviations

205 GI	205 day growth index
AD	Acrosomal defects
ADG	Average daily gain
AFD	Acrosome and flagellar defects
AM	Aberrant sperm movement
ANOVA	Analysis of variance
B. Indicus	Bos Indicus
BL	Body length
OBE	Overall breeding soundness evaluation
BFR	Bull to female ratio
B. Taurus	Bos Taurus
BW	Body weight
Cort	Cortisol concentration
Cort T_0	Cortisol concentration at starting time
Cort T_{150}	Cortisol concentration 150 minutes after GnRH treatment
Cort T_f	Cortisol concentration after sexual stimulation during the libido test
EE	Electro-ejaculation
FD	Flagellar defects
FSH	Follicle stimulating hormone
GLM	General linear model
GnRH	Gonadotrophin releasing hormone
H:L	Height to length ratio
I	Immotile sperm
i.m.	Intra-muscularly
K	Kilogram
KR	Kleiber ratio
LH	Luteinizing hormone
LSM	Least square mean
% PM	Percentage progressive movement
% AM	Percentage aberrant movement
% I	Percentage immotile
Morph	Spermatozoal morphology categories
Motil	Spermatozoal motility categories
% MN	Percentage morphologically normal sperm
ml	Millilitre
mRNA	Messenger ribonucleic acid
MN	Morphologically normal sperm
ng	Nanogram
NS	Non-significant
ND	Nuclear defects
N	Number of animals (sample size)
OBC	Overall breeding soundness category
OBC 1	Satisfactory potential breeders with exceptional fertility
OBC 2	Satisfactory potential breeders with acceptable fertility
OBC 3	Unsatisfactory potential breeders
PM	Progressively motile sperm
P	P value (significance level)
SAS	Statistical analyses system

SC	Scrotal circumference
SCC	Scrotal circumference category
SC 1	Scrotal circumference ≥ 340 mm
SC 2	Scrotal circumference < 340 mm
SC Ts:	Scrotal circumference after the growth performance
SC Te	Scrotal circumference at end of trial
S consist	Spermatozoal consistency
SE	Standard error of the mean
SH:	Shoulder height
SMPC	Spermatozoal morphology category
SMTC	Spermatozoal motility category
S marbling	Spermatozoal marbling
S motil	Spermatozoal motility
S motil 1	≥ 70 % motile sperm
S motil 2	< 70% motile sperm
S morph	Spermatozoal morphology
S morph 1	≥ 70% morphologically normal sperm
S morph 2	< 70% morphologically normal sperm
Std Dev:	Standard deviation
spp	Species
Test	Testosterone concentration
Test T_0	Testosterone concentration at starting time
Test T_{150}	Testosterone concentration 150 minutes after GnRH treatment
Test T_f	Testosterone concentration after sexual stimulation during the libido test
Total AFD	Total acrosome and flagellar defects
Total D	Total defects (normal, nuclear, acrosomal, and flagellar defects)
Total FD	Total flagellar defects
Total ND	Total nuclear defects
U_s	Kruskal Wallis test statistic

CHAPTER 1

1.1. TITLE

Estimates of the fertility of extensively managed Bonsmara bulls

1.2. AIM

The aim of the present study was to determine if the breeding potential of 25-month old, extensively kept Bonsmara beef bulls, could be predicted from production data, spermatozoal quality and blood hormone concentration. A further objective of the study was to determine if any of the above mentioned criteria could be associated with the libido of these bulls.

1.3. MOTIVATION

The contribution of a bull to reproductive efficiency and to the production of meat and milk is of immeasurable importance because each bull represents half of the genetic composition of its progeny. The percentage of calf crop weaned is the most important factor influencing the profitability of a cow-calve operation. It is believed that it is best to make your decision of breeding animals based on economically important traits because selecting for these traits can lead to improved production of the herd. During Phase D growth performance test, the bull's performance under natural grazing conditions is evaluated. Yearling beef bulls are typically selected as potential herd sires on their performance in a performance test Data are lacking on the relationship between performance test results and reproductive parameters but an unfavorable relationship is believed to exist (Ellis *et al.*, 2005; Ologun *et al*., 1981). At the Eastern Free State Veld Bull Club the animals' performance under natural grazing conditions are evaluated. Some of the traits that are evaluated include average daily gain (ADG), 205-day growth indices, and scrotal circumference (SC). Another trait that breeders are considering to evaluate as an additional measurement of potential bull fertility, is libido. To reduce the time, labor and aesthetic concerns associated with libido/serving capacity tests, it is necessary to develop an indirect method to assess bull sex-drive. An indirect method has the added advantage of allowing the assessment of bulls that normally do not respond well to libido tests.

Bull reproductive performance is influenced by one or more of the following three factors, semen characteristics, sex drive and mating ability, and the social interaction between bulls (Chenoweth, 1999). Perry *et al*. (1990) suggested that these traits could be used in a regression equation to predict the pregnancy rates of young bulls. Parkinson (2004) pointed out that literature lacks unanimity when attempting to associate single bull traits such as libido, sperm characteristics and scrotal circumference with fertility. A single test that reliably predicts bull fertility still needs to be developed (Chenoweth, 2004).

All aspects of the male reproductive system are under control of the endocrine system. Gonadotropins regulate the synthesis of reproductive steroids, which themselves control libido, spermatogenesis, and the action of the epididymis and accessory sex glands. Therefore, measuring certain circulating hormone concentrations might provide a straightforward way of assessing either libido or sperm output (Parkinson, 2004). The reproductive performance of prepubertal bulls can be predicted by testosterone response measurements taken as young as 17 months (Post *et al*, 1987b), and this can subsequently be used in a performance-testing scheme as an additional measurement of bull fertility, providing an additional selection tool when choosing breeding sires.

CHAPTER 2

2.1. INTRODUCTION

Reproductive performance is of fundamental importance in livestock production and economics. To this end, natural breeding is used most frequently in extensive situations (Burrow & Prayaga, 2004). Bull fertility can be defined as the ability to produce progeny and it is an integration of multiple biological and behavioral components. Multiple traits should be included in a fertility index used for the prediction of fertility in bulls, but the question arises as to which traits to include in the index (Chenoweth, 2004).

The word fertility refers to different factors when compared between natural breeding and artificial insemination situations. Therefore, the term 'breeding potential' is used in this review rather than the word fertility. Breeding potential refers to the potential reproductive capacity of a bull as influenced by whether or not the bull has reached puberty, the bull's spermatozoal quantity and quality (morphology and motility), scrotal circumference (SC), breeding experience (Landaeta-Hernandez *et al*., 2001), libido, mating ability (absence of physical and pathological tribulations) and the social interaction with other animals in their breeding pasture (Chenoweth *et al*., 1984).

This review centers around some of the factors affecting the breeding potential, and the expression of libido in young beef bulls, putting emphasis both on the behavioral constraints and the physiological mechanisms mediating the effects on breeding potential. In conducting this review, scientific studies have been referenced, but most of the literature on the sexual behavior of cattle is between 20 and 50 years old and there appears to be a need to conduct some meticulous hypothesis testing on this subject (Petherick, 2005).

2.2. SELECTING BEEF BULLS

Bourdon (2000) stated that before selecting a bull as a sire in your herd you should measure the performance (phenotype) of the animal. Performance-testing is defined as the systemic measurement of performance in a population. Seedstock producers commonly take part in performance-testing programs and the data they record are reported to breed associations.

The genotypic value of an animal represents the overall effect of an animal's genes on that animal's own performance for a trait. The breeding value is the part of the animal's genotypic value that can be transmitted from parent to offspring. Breeding values are not directly measurable but can be predicted from performance data and are thus referred to as estimated breeding values (EBV). The expected progeny difference (EPD) is the half of a parent's breeding value for a trait that is expected to be inherited form the parent. EPD's are commonly used to make comparisons among individuals (Bourdon, 2000).

The selection index is used as a method for genetic prediction and as a means of combining traits in order to select bulls in an economically optimal way (assuming that all animals come from genetically similar

contemporary groups). The best linear unbiased prediction (BLUP) is a method of genetic prediction that is particularly appropriate when performance data come from genetically diverse contemporary groups. The performance records used to calculate BLUP come from data that are regularly reported by individual breeders to breed associations (Bourdon, 2000).

Performance records of various economically important traits can be readily and accurately measured. During a Phase D growth performance test, the bull's performance under natural grazing conditions is evaluated. The following traits, which are of particular importance to a cow-calf producer, are measured.

Weaning weight is an important production trait because it reflects the milk-producing ability of the dam and is a measure of the bull's genetic potential for early growth. The adjusted weaning weight, usually listed as 205-day weight (Kg), is the bull's actual weight at weaning corrected to a standard age of 205 days and adjusted to other known variables (e.g. dam age).

Average daily gain (ADG) is defined as the daily post-weaning weight increase over a period of time, measured in g/day.

The energetic efficiency of beef cattle is an economically important trait, and can be determined by use of the Kleiber ratio (KR) (Kleiber, 1947). The Kleiber ratio is defined as the metabolic growth efficiency ($W^{0.75}$). In cases where it is impossible to measure individual feed intake (e.g. Phase D tests), the Kleiber ratio gives an indication of the animal's feed/veld usage. The Kleiber ratio can thus serve as an indirect indication of feed conversion efficiency.

Shoulder height (mm) and body length (mm, measured form shoulder to pin bone) are used as indicators of cost-effective finishing in feedlot situations and are measured during performance tests.

The mentioned traits are moderately to highly heritable. Heritability is defined as the proportion of variation in a trait that is a result of genetic factors and is passed on to offspring. Traits with high heritability are easier to change though selection of breeding animals than traits with low heritability. Fertility is a difficult trait to measure directly, so it is generally indirectly measured with indicator traits (e.g. scrotum circumference).

Chenoweth (1999) stated that scrotal circumference (SC) is one of the most important measures of reproducing ability in bulls as it correlates with the number and quality of sperm produced. It is also positively associated with age at puberty of related females, thus favorably related to subsequent production, and because of it's high heritability it can be employed as a tool to improve the herds reproductive performance. Almquist *et al.* (1976) reported that because sperm production per unit of testis volume is a constant figure, testis size is highly correlated with daily sperm output. Scrotal circumference is an intermediate trait, indicating that both extremes are undesirable. A smaller SC indicates low fertility, while too large circumferences could indicate high fat deposits or inflammation of the testis (onchitis), which could lead to infertility. Scrotal circumference is also a good indicator of whether a yearling bull is pubertal. Nutritional and genetic effects influence the rate of

testicular growth. Puberty occurs when SC is between 28 and 30 cm. The genetic correlation between selection for high growth rate and SC is 0.5, indicating that selection for growth would lead to a significant increase in scrotal size, which can have a positive effect of sperm production and thus on male fertility (Burrow & Prayaga, 2004). It was also shown by Perry *et al.* (1991) that changes in liveweight are directly proportional to changes in SC of young tropical beef bulls. They reported that testicular growth reflects the pattern of ADG. Another study done by Smith *et al*. (1981) indicates that SC increases significantly as body weight increase ($r = 0.43$; $P<0.01$).

It is believed that it is best to make a decision on breeding animals based on economically important traits because selecting for these traits can lead to improved production of the herd. Yearling beef bulls are typically selected as potential herd sires based on their performance in a performance test (Ellis *et al*., 2005; Ologun *et al*., 1981). Ologun *et al*. (1981) conducted a study on yearling beef bulls to determine the relationship between superior production traits, such as ADG, final test weight after a 140-day performance test, and sex-drive. In this study it was found that productive traits such as ADG and final test weight are negatively correlated with serving capacity scores ($P<0.01$), reinforcing previous speculations that the sex drive of yearling beef bulls is not favorably related to production traits. A study done by Makarechian & Farid (1985) on yearling beef bulls revealed that a negative correlation ($r = - 0.47$; $P<0.05$) exists between fertility and preweaning average daily gain.

2.3. BULL FERTILITY

Reproductive performance is of fundamental importance in livestock economics and production. To this aid, natural breeding is used most frequently in extensive situations. The effective reproducing ability of both bulls and cows in the herd is of economic importance (Burrow & Prayaga, 2004). Fertility can be defined as the ability to produce progeny and it is an integration of multiple biological and behavioral components. Pregnancy rates after the breeding season are used as an overall indication of the fertility of an extensively managed herd. Herd fertility is a multi-factorial product of which some of the factors may be attributed to the female, the male or the environment, and most of these factors are interactive (Chenoweth, 2000). Parkinson (2004) argues that domestic cattle are not a highly fertile species because of their mediocre per-service calving rate of 50 – 60 %. Beef herds have to be managed in such a way as to prevent further loss of potential fertility and to increase the number of opportunities each animal has to conceive. Fertility is also time-sensitive, thus is it not surprising that literature lacks agreement when attempting to correlate single bull traits such as libido, sperm motility or SC with fertility. Due to these reasons, there is no single test that reliably predicts bull fertility (Hoflack *et al*., 2005, unpublished). Fertility refers to the reproductive success of an animal and when fertility is evaluated, factors such as calving percentage, conception rate, semen quality, SC, and mating ability (physical and structural breeding soundness) are included.

The success of the naturally breeding bull greatly influences herd reproductive rates (Hoflack *et al*., 2005, unpublished) due to the fact that a single bull is generally mated to several cows. The bulls used in natural mating make up 3 – 5 % of the herd. When a cow fails to conceive one calf is lost but when a subfertile bull is used there can be losses of 25 to 50 calves per bull (Molina *et al*., 2000). Breeding potential refers to the reproductive capacity of a bull as influenced by whether or not the bull has reached puberty, the bull's spermatozoal quantity and quality (morphology and motility), SC, breeding experience (Landaeta-Hernandez *et al*., 2001), libido, mating ability (absence of physical and pathological tribulations) and the social interaction with other animals in their breeding pasture (Chenoweth *et al*., 1984). Mating ability refers to the physical ability of a bull to complete a service (Chenoweth, 2000). A bull may be superior in one or several of these traits but it's fertility may be compromised by deficiencies in other traits (Chenoweth, 2000). The word libido refers to sexual drive, revealed through behaviors such as mate seeking, detection, courtship, and mating. It is the willingness and eagerness of a male animal to mount or to attempt service of a female (Chenoweth, 1981).

A study was done by Perry *et al.* (1990) to develop a regression equation that could be used to predict the pregnancy rates of young bulls. They reported that the most important traits to be incorporated into fertility indices are testicular volume, GnRH-induced LH levels, libido score, and body weight. The components of the breeding soundness examination, semen quality and ADG were excluded from their indices. For the bulls assessed at an age of 580 days, the amount of variation in pregnancy rates influenced by these indices were (r^2 = 0.58).

2.3.1. Sexual development

Puberty can be defined as the age at which an ejaculate contains ≥ 50 million sperm with ≥ 10% motile sperm. This occurs after SC has reached 280 mm (Brito *et al*., 2006). The cell motility and sperm concentration per ejaculate greatly increases from puberty to maturity but at puberty the ejaculate can initiate a pregnancy (Evans *et al.*, 1995). Brito *et al.* (2006) stated that an early rise in gonadotropin secretion between 2 and 6 month of age leads to a transient rise in LH concentrations (Evans *et al*., 1995), allowing testicular hypertrophy and the establishment of spermatogenesis. This early rise in gonadotropins occurs because of an increase in pulse frequency. LH pulse frequencies are known to be greater in early-maturing than in late-maturing bulls. After the early rise, the gonadotropin concentration declines, but LH concentrations start to increase to adult levels (Evans *et al*., 1995) and this is the period during which testicular growth accelerates (Brito *et al*., 2006). Evans *et al.* (1995) pointed out that faster-maturing bulls show greater pituitary-hypothalamic activity early in life, as seen by the greater observed LH secretion. However no differences in secretion patterns of testosterone were observed between the two maturity types. In the postnatal bull calf, the serum testosterone levels are low, whereas the androstenedione concentrations are high. The testosterone concentrations start to increase from 5 month of age.

It was concluded that GnRH secretion plays a significant role in the sexual development of young bulls. Thompson *et al.* (1994) cited a study demonstrating that testosterone concentration could be a useful predictor of whether puberty has been reached because the concentration rapidly rises from 4 nmol/L to 9.7 nmol/L at puberty. Because of its fluctuating concentration a single measurement is unreliable. The peripheral venous concentrations of 17-hydroxy-progesterone and testosterone begin to fluctuate synchronously just prior to the onset of puberty at about six months of age. The steroid precursors of testosterone have peak serum concentrations after puberty, associated with peak concentration of serum testosterone (Thibier, 1976).

A study was done by Perry *et al.* (1991) to depict the development of hormonal responses in tropical beef bulls. The animals where injected with GnRH every 2 weeks from 16 month age up to 27 month age. Blood samples were obtained twice, at 30 and 150 minutes, after each GnRH administration. It was found that testosterone values increases with age, proportionally to SC and ADG. These authors argued that peripubertal bulls could have a lower competence for pituitary sensitizations. Circulating LH levels develop in a sine-wave pattern. A peripubertal surge is followed by a drop and then a recovery to a lower level than initially. This lower level is distinctive to a bull and is caused by the negative feedback on LH due to the increased level of testosterone.

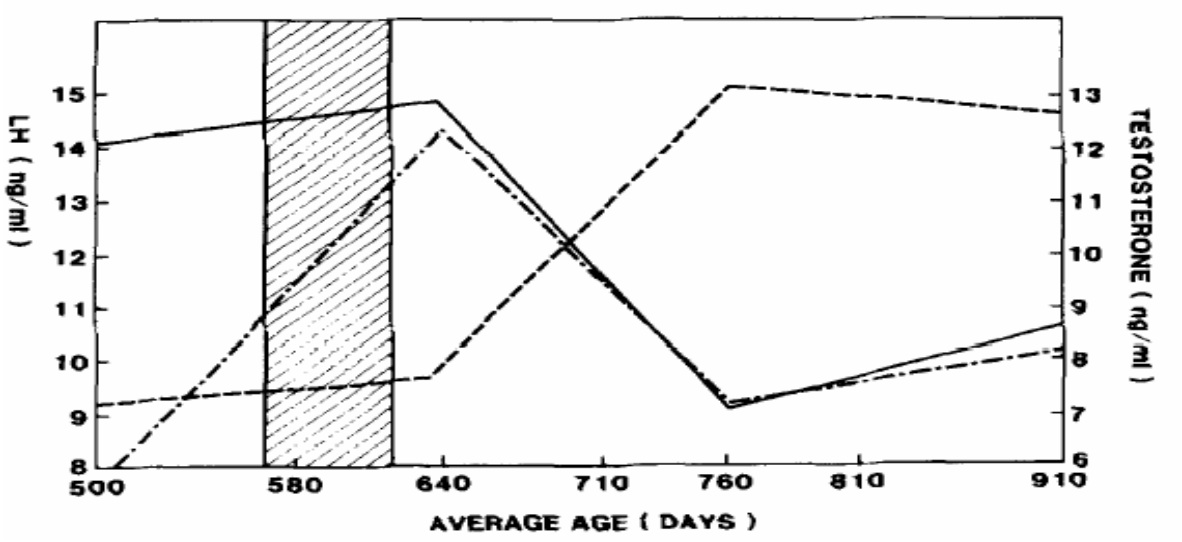

Figure 1 Peripheral levels of LH measured at 30 (---) and 150 (_._._.) minutes, and testosterone measured at 150 (- - -) minutes following GnRH administration (1µg/Kg). Window represents average ages at which first satisfactory ejaculate was obtained (565d) and first service occurred (619d) (Perry *et al.*, 1991).

2.3.2. Physical examination – Overall Breeding Soundness Examination

Chenoweth (1999) stated that a breeding bull's foremost duty is to impregnate all available females as early in the breeding period as possible and for this he needs good eyesight and musculo-skeletal conformation, as well as the ability to produce and deliver sufficient numbers of fertile spermatozoa into the female tract. Ellis *et al*. (2005) stated that yearling beef bulls are an important component of natural mating schemes and that the principle method employed to assess the reproductive potential of, and thus select bulls, is by overall breeding soundness examinations (OBE). An OBE is a quick, reliable and cost- effective method for screening and classifying bulls in terms of potential fertility and thereby minimizing the use of subfertile bulls (Hoflack *et al*., 2005, unpublished). Parkinson (2004) pointed out that physical examination of the genitalia of a bull can be used to assess potential fertility and this involves palpation of the penis, prepuce, accessory sex glands, scrotum and testis, measurement of SC and semen evaluations. The SC and spermatozoal motility and morphology are positively correlated and interrelated parameters (Ellis *et al.*, 2005).

The United States Society for Theriogenology gives recommendations on the scoring for breeding soundness. The breeding soundness score is based on a combination of the separate scores (Table 9) for SC, spermatozoal motility and spermatozoal morphology (Parkinson, 2004). Scrotal circumference must be adjusted based on bull age. Chenoweth *et al*. (1984) performed a study to investigate the effect of age on reproductive parameters in bulls, and found that the average SC of yearling bulls is less ($P<0.01$) than that of older bulls. The SC of bulls must be adjusted for by age when animals of different ages are compared.

The classification categories as described by Chenoweth (1999) are as follows. Satisfactory bulls equal or surpass the minimum thresholds for SC, sperm motility and sperm morphology. They do not show any genetic, infectious or other problems or faults that could compromise breeding or fertility. Unsatisfactory bulls are below one or more threshold and are not likely to improve their status. These bulls show genetic faults or irretrievable physical problems that would compromise mating and fertility. Any bull that does not fit into either of the above-mentioned categories and which could benefit from a retest are placed in the classification deferred category. These bulls may show immature sperm profiles as well as substandard spermatozoal quality capable of improving, commonly seen with peripubertal bulls. Bulls must be pubertal to be eligible for OBE classification.

A study was done by Chenoweth *et al*. (1988) to determine the relationship between OBE and the sex drive of bulls. They found that the factors exerting the principal effects on OBE classification were qualitative seminal characteristics. Spermatozoal motility and SC did not influence the classification of the bulls in that study but a study by Tomky *et a*l. (1979) showed that SC strongly affected the OBE score. Chenoweth *et al*. (1988) found that there was no significant relationship ($r = -0.16$ to $r = 0.24$) between the sex drive and breeding soundness traits of beef bulls. They suggested that, for these reasons, bull evaluation should include the

assessment of both breeding soundness and sex drive. Farin *et al*. (1989) suggested that the bulls that would impregnate more females during a limited breeding season cannot be identified by the breeding soundness score due to the poor correlation (r = - 0.22) between percentage of normal spermatozoa and pregnancy rate.

A temporal constraint of the OBE is that it assesses only the current physical, testicular and seminal status and cannot accurately predict the future reproductive performance of an individual. During the breeding period, factors such as season, climatic temperatures, disease and injury, physiological stress, and nutritional imbalances are the causes of changes in SC and seminal quality, and thus the OBE score of the bull (Ellis *et a*l., 2005).

2.3.2.1. Mating ability

Mating ability refers to the physical capabilities such as smell, sight, body condition (as indicator of nutritional level) and forward movement, necessary to successfully breed a cow (Perry & Patterson, www.muextension.missouri.edu/xplor/).

2.3.2.2. Scrotal circumference (SC)

Coulter & Foot (1979) reported a relationship between spermatozoal quality parameters (number of sperm and percentage of morphologically normal sperm) and SC. The SC of all bulls older than two years should exceed 33 – 34 cm and, after that age, the SC is breed dependent (Parkinson, 2004).

Table 1 Minimum scrotal circumference requirements for bulls to pass a breeding soundness evaluation, by age (Chenoweth *et al.*, 1992)

Age (months)	<15	15-18	18-21	21-24	>24
SC (cm)	30	31	32	33	34

There are discordances regarding the usefulness of SC measurements in beef cattle (Smith *et al*., 1981). Quirino *et al.* (2004) found genotypic and phenotypic correlations between libido and SC to be 0.43 and - 0.20, respectively, indicating that SC decreases with an increase in libido score. In a study done by Smith *et al.* (1981), it was reported that there was no significant relationship between SC and fertility in two-year-old beef bulls. This conclusion was made after they found low correlations between OBE scores and individual spermatozoal measurements.

2.3.2.3. Spermatozoal characteristics

Parkinson (2004) stated that, according to spermatozoal quality standards, a minimum of 30% motility and 70% morphologically normal sperm is acceptable during an OBE. Literature on the relationship between spermatozoal quality and fertility is contradictory. Some authors reported that none of the commonly assessed parameters of spermatozoal quality are reliably or consistently related to fertility (Smith *et al.*, 1981) while

others reported that percentage of morphological normal or abnormal sperm are related to conception rate. The quality of a bull's spermatozoa may even change over time (Perry & Patterson, www.muextension.missouri.edu/xplor/). Substandard nutrition, extreme environmental conditions and diseases can reduce spermatozoal quality. Soderquist *et al.* (1996) performed a study on the influence of season, age, breed, and stressful treatment on sperm characteristics, and concluded that any factor influencing sperm abnormalities will influence bull fertility. Despite the discrepancies, spermatozoal quality is warranted as a component of a OBE. Spermatozoal quality is determined by the volume of the ejaculate and by the motility and morphology of the sperm cells.

Rectal massage (RM) was the first method used to collect spermatozoal samples from bulls (Palmer *et al*., 2005). Another method that can be used is electro-ejaculation (EE). In recent years, the stress and pain associated with EE has become an animal welfare concern. A study was done by Palmer *et al.* (2005) to compare the effectiveness of EE and RM for spermatozoal collection. It was reported that the percentage of motile and live sperm was lower in samples collected by RM as opposed to EE and this could affect breeding soundness classification. The genetic correlation between libido and spermatozoal defects is r = 0.43, indicating that a bull that obtains the best libido score should have the lowest amount of spermatic defects (Quirino *et al.*, 2004).

Sperm morphology is calculated by evaluating the number of normal spermatozoa in a sample ejaculate stained with an eosin-nigrosin dye. Some semen ejaculates may show as few as 5% abnormal sperm, while others may approach 100%. Fertility is usually not affected until the level of abnormal sperm exceeds 20 to 25%. Abnormal sperm do not show progressive motility so, as the percentage of abnormal sperm increases, the progressive motility percentage decreases (Bearden *et al.*, 2004).

Parkinson (2004) stated that sperm abnormalities are classified according to their effect upon fertility. Abnormalities are classified according to the origin of the defect into primary (originating in the testis during spermatogenesis) and secondary (originating in the epididymis during sperm transport) abnormalities (Perry & Patterson, www.muextension.missouri.edu/xplor/). Major defects include abnormalities of the head and midpiece and proximal cytoplasmic droplets, while minor defects comprise looped tails, detached sperm heads and distal cytoplasmic droplets.

Morphologically normal spermatozoa consist essentially of a head, which is an ovoid structure made up of a nucleus (containing the paternal heredity material), and a 40-50 μ long tail (which principle function is locomotion). The head is covered anteriorly by an acrosome and posteriorly by the post nuclear cap. The tail is differentiated into the midpiece (thickened region that supplies energy to the sperm), the mainpiece (longest part of tail) and the endpiece. Movement is accomplished by a whiplash type of wave movement generated at the anterior end of the midpiece connecting the head. Any abnormalities in the head or tail of the spermatozoa will

affect the fertilizing ability of the spermatozoon (LaRey, 2002). A list of the most common sperm cell abnormalities are given below.

- Teratoid spermatozoa can be identified by a tail that is completely coiled and superimposed onto the sperm head. In some cases only the bend midpiece lies superimposed on the head and the principle piece projects away form the abnormal form. The mitochondrial sheath appears swollen and disrupted. Where spermatogenesis has been severely disrupted, this defect may occur at a rate of between 1 and 25%. Sperm cells with this abnormality have no fertilizing capabilities (Barth & Oko, 1989).
- Macroephaly suggests a sperm head that is larger than normal size. This defect rarely occurs in large numbers (5 – 7 %) and results in an excess of genetic material. These sperm cells may not be able to fertilize an ovum (Barth & Oko, 1989).
- The pyriform shape is the most common abnormality of the sperm head. It is identified as a pear-shaped head with a rounded acrosomal region and a narrow post-acrosomal region. Pyriform heads seem to appear whenever the normal testicular function is disturbed (e.g. heat regulation of the testis affected by an endocrine imbalance). Environmental influences may affect hormonal and metabolic events of the Sertoli cells and lead to formation of a rounder sperm head during spermatogenesis. Pyriform sperm should have no problem in penetrating the zona pelucida as long as the acrosome is intact and there are no other defects affecting the motility of the spermatid (Barth & Oko, 1989).
- Diadems, or nuclear vacuoles, are described as craters that occur in the nuclear region of the sperm head. Bane and Nicander (as cited by Barth & Oko, 1989), described diadems to appear as sparkling round and white spots, in the form of a string of beads, at the acrosome-postacrosomal sheath junction. Diadems were reported to occur in bulls that had disrupted spermatogenesis due to illness, injury or abnormal environmental conditions.
- Detached heads, or normally shaped loose heads, are commonly found in semen of bulls exhibiting normal fertility. It can be caused by testicular hypoplasia, testicular degeneration and inflammation, or a temperature increase of the testis (Barth & Oko, 1989). Large numbers of loose heads (more than 40%) can lead to reduced fertility due to the inability of the sperm cell to be propelled foreword.
- The knobbed acrosome is a refractile- or dark staining area, or peculiar thickening at the tip of the sperm head (Barth & Oko, 1989). This defect occurs if the spermatogenesis cycle has been disturbed, and can lead to reduced motility of the sperm cell if it occurs together with other abnormalities.

- When the tail of the sperm cell is in the form of a small stump at the base of the sperm head, it is referred to as stump tail defect (Barth & Oko, 1989). This defect may be of genetic origin.
- A Pseudodroplet is recognized as a local thickening somewhere along the midpiece. The incidence of this defect can vary between 7 and 26% and the frequency of occurrence increases with age (Barth & Oko, 1989).
- Mitochondrial aplasia occurs when a small gap is present in the mitochondrial sheath and this can cause a separation of the principle piece from the midpiece. The movement of the principle piece leads to a breakage at the point of the gap in the mitochondrial sheath (Barth & Oko, 1989).
- Corkscrew defects are identified as an irregular distribution of mitochondria along the mitochondrial sheath due to progressive degeneration of the testis.
- The Dag defect is recognized as coiling and folding of the midpiece, where the axis of the main fold is in the distal part of the midpiece. The ejaculate usually contains less than 5% of this defect.
- The midpiece reflex is the most common tail abnormality in bull semen. It is identified by a J- shape in the distal portion of the midpiece. The defect is classified as a minor defect and occurs at levels as high as 25 % in semen of bulls with normal fertility. The sperm cell's movement is restricted to backward direction due to the bending of the tail, and the spermatozoon is thus unable to penetrate the zona pelucida (Barth a& Oko, 1989).
- A bent midpiece differs from the midpiece reflex in that the tail does not fold (Barth & Oko, 1989).
- Principal piece defects usually involve coiling or bending of the principle piece (Barth & Oko, 1989). Motility is impaired and this has a negative effect on fertility.
- A type of end piece defect, the abaxial tail defect (Barth & Oko, 1989), occurs when the tail is attached to the head at an angle. This defect is usually accompanied by accessory tails.
- Parkinson (2004) pointed out that cytoplasmic droplets are indications of immature sperm. Sperm are released from the seminiferous epithelium with their residual cytoplasm in the form of a droplet just behind the head, and this is known as a proximal droplet. Proximal droplets are serious abnormalities that can substantially impair fertility. These droplets are common in the first few ejaculates of peripubertal bulls, but their percentage rapidly declines to normal levels after puberty. Puberty in the bull has been defined as the age at which an ejaculate contains 50 million spermatozoa with a minimum motility of 10%. The number of sperm cells per ejaculate increases greatly beyond these values as the bull matures (Evans *et al*. 1995). During passage of sperm through the epididymis, the droplet first migrates to the distal end of the midpiece (distal droplet), and then is lost entirely as the sperm matures.

Progressive motility is the most important individual quality test, because fertility is highly correlated with the number of motile sperm. Sperm motility is an indication of the percentage live spermatozoa in an ejaculate and can range from 0 to 80%. Samples with less than 40% motility would lead to decreased pregnancy rates. Progressively moving sperm are moving from one place to another in a straight line. Circular and reverse movements are associated with tail abnormalities, while vibrating or rocking movements are associated with ageing (Bearden *et al*., 2004). According to Perry & Patterson (www.muextension.missouri.edu/xplor/), sperm motility can be calculated as the percentage of spermatozoa that show forward movement in a sample ejaculate. The procedure used to calculate this is to put a droplet of diluted semen on a microscope slide and observe the number of spermatozoa with forward movement in relation to those with other than forward movement.

Table 2 Recommended thresholds for gross motility (Chenoweth, 1999a)

Mass activity (Gross Motility)	**Rating**
Rapid swirling	Very Good
Slower swirling	Good
Generalized oscillation	Fair
Sporadic oscillation	Poor

Table 3 Recommended thresholds for percentage progressive motility (Chenoweth, 1999b)

Percent progressive motility	**Rating**
>70 %	Very Good
50-69 %	Good
30-49%	Fair
<30%	Poor

Parkinson (2004) stated that assessing single parameters of spermatozoal quality does not allow the prediction of the absolute fertility level, thus there is a quest to find other characteristics of spermatozoal quality more closely related to fertility. Two areas that have proven rewarding as laboratory indicators of fertility include (i) the interaction of glycosaminoglycans with the sperm during acrosome reaction and (ii) the ability of sperm to fertilize oocytes *in vitro* fertilization regimens. Differential fluorescence staining and flow cytometry may also be a useful measure of fertility. The long term value for the prediction or fertility for all these tests still need to be established, so they have not yet been adopted for routine use in the field. Gabor *et al.* (1998) argued that measuring the testosterone response to GnRH injection may give an indication of the spermatozoal quality in adult bulls. The authors reported a moderate correlation of 0.58 between plasma testosterone concentration and the total number of spermatozoa.

2.3.3. Sexual behavior

The most common fertilization procedure used in beef cattle operations worldwide is natural mating, even though artificial technologies for cattle breeding are readily available. Therefore the reproductive capabilities of bulls are of great importance where natural mating is employed (Chenoweth, 1999). According to Chenoweth (1981) the sexual behavior of the bull can be differentiated into two components: firstly, libido, defined as the willingness and eagerness of a male animal to mount and to attempt to service a female; and secondly, mating behavior, defined as the behavior of the male animal in the period immediately before, during and after service.

The sight of mounting activity is the first sign that attracts bulls toward females in the pasture and sight is thus said to be the major singular sense used by bulls to detect receptive females. The tendency of females in estrus to form a mobile sexually active group facilitates the identification of receptive females (Chenoweth, 2000). Pheromones also play a large role in allowing bulls to detect receptive females but this mechanism requires close physical contact. A pheromone is a chemical substance that is secreted from an exocrine gland or is found in an excretory product such as urine. Pheromones from one individual exert an effect upon another individual of the same species. The bull test the receptivity of females by mounting attempts, chin resting and by licking and sniffing around the perineal region and this is known as the Flehmen response. During the Flehmen response fluids are transferred to the vomeronasal organ where they are assessed for pheromone activity (Chenoweth, 2000). Bulls spend more time in courtship than in mounting activities (Molina *et al*., 2000).

Chenoweth (1999) pointed out that bull sex drive is a measurable trait and testing procedures rely upon the following findings: libido has a genetic component; bulls are polygamous and distribute their services among receptive females; competition among bulls and pre stimulation of bulls increases their sexual response.

2.3.3.1. Libido and Serving Capacity tests

Hoflack *et al*. (2005, unpublished) stated that to avoid selection of bulls that are unable or reluctant to serve a cow, a bull should be tested for serving ability prior to the commencement of the breeding season. Parkinson (2004) pointed out that the mating ability of bulls at pasture is, by itself, not a very good indicator of mating ability and that libido should be assessed during a designated test period. Petherick (2004) stated that, even though the intensity of libido is exceedingly difficult to measure, it is essential for breeding cattle enterprises to have some indication that bulls are likely to mate. Many investigations have been conducted regarding the relationship between measures of libido and bull fertility and the results are equivocal (Petherick, 2005). Parkinson (2004) pointed out that the relationship between libido test scores and pregnancy rates achieved is not clear-cut. Positive correlations were reported by Blockey (1989), while negative relationships were reported by Godfrey & Lunstra (1989) and Bertram *et al.* (2002). Chenoweth (2000) suggested that a major

problem when attempting to demonstrate that a single trait, such as libido, has a consistent, fundamental influence on herd fertility is that fertility is a multi-factorial trait influenced by both male and female factors. Male factors include the OBE components and these do not appear to be genetically linked with behavioral traits such as libido. Bulls with superior breeding activity can be identified with libido and serving capacity tests, but an OBE is required to identify differences in ability to impregnate a female during service (Chenoweth, 1999). Quirino *et al.* (2004) reported the heritability of libido to be 0.34 ± 0.10. This heritability indicates that libido can be increased by genetic selection and should therefore be included in the bull fertility index.

Female libido can be assessed through the expression of estrus and the associated behavioral patterns such as soliciting, mounting, and standing to be mounted, the swollen appearance of the vulva and vaginal mucus. A variety of tests exist for libido assessment in bulls, determining the sexual responsiveness of bulls to females. These tests use different measurements, such as the number of mounts and/or services during a set period of time (Blockey, 1981a, Landaeta-Hernández *et al*., 2001); reaction time (Hoflack *et al.,* 2005, unpublished; Chenoweth, 1981, Landaeta-Hernández *et al*., 2001); counts and durations of interest, such as sniffing at the vulva and time spent with females (Chenoweth *et al*., 1979, López *et al*., 1999); and scores assigned according to various combinations of these measurements (Chenoweth *et al*., 1979, Chenoweth, 1981, Landaeta-Hernández *et al*., 2001). The ideal test for bull sex drive should be rapid, highly repeatable, and predictive of reproductive performance, and it should also be aesthetically acceptable (Chenoweth, 1999).

A serving capacity test can be defined as the number of services a bull achieves in a pasture-mating period under stipulated conditions and includes aspects of both libido and mating ability (Blockey, 1981a; Chenoweth, 2000). In a serving capacity test, four to six pre-stimulated bulls are admitted into a pen, together with two restrained females. The test continues for 30 minutes, during which the number of services and mounts achieved by each bull is recorded (Parkinson, 2004). Bertram *et al. (*2002) pointed out that differences are reported in the value of using a serving capacity test score obtained by a beef bull as a trait to increase fertility. The serving capacity tests were developed as a method to rate bulls on their sexual behavior and to identify bulls with structural and serving problems.

In a libido test, a single bull is sexually pre-stimulated by observing other males mate a female that is exhibiting signs of estrus. The bull is then allowed into a 22m x 22m pen with a dirt floor where a non-estrus restrained female is held in a service crate (Boyd & Corah, 1988). The bull is kept in the pen for exactly ten minutes, during which time his reactions and movements are recorded. Libido is then scored according to predetermined criteria that take into account the number and vigor of mating attempts, together with a subjective

assessment of overall sexual interest. Sexual performance of a bull is rated according to the following subjective "libido scoring system" devised by Chenoweth (1986):

0. Bull shows no sexual interest
1. Sexual interest shown only once (sniffing at perineal region)
2. Positive sexual interest in female on more than l occasion
3. Active pursuit of female with persistent sexual interest
4. One mount or mounting attempt with no service
5. Two mounts or mounting attempts with no service
6. More than two mounts or mounting attempts with no service
7. One service followed by no further sexual interest
8. One service followed by sexual interest, including mounts or mounting attempts
9. Two services followed by no further sexual interest
10. Two services followed by further sexual interest, including mounts, mounting attempts or further service

Chenoweth *et al.* (1979) argued that the libido score method is the most advantageous for assessment of sex drive in yearling beef bulls because libido testing requires less test exposure time (10 minutes) and is more repeatable than the serving capacity scoring method.

Chenoweth *et al*. (1988) reported that bulls that obtained a high libido score serviced up to 3 times more cows ($P<0.01$) during the testing period than did bulls of medium libido. Similar results were reported by Farin *et al*. (1989), in that the correlation between libido and total number of services was $r = 0.45$ ($P<0.01$). The number of mounts by high-libido bulls is also reported to be less ($P<0.05$) than that observed by the medium-libido bulls (Chenoweth *et al*., 1988), and a correlation of $r = -0.03$ (Farin *et al*., 1989) exists between libido and number of mounts. It was concluded that the bulls which service more estrus synchronized heifers could be identified by means of a libido score (Farin *et al*., 1989). Blockey (1981a) pointed out that it would be advantageous to pregnancy rates, time of conception, length of calving season, and homogeny of calves at weaning, if one used bulls with greater libido scores.

Landaeta-Hernandez *et al.* (2001) demonstrated that there is a need to perform more than two repetitions of the libido test to obtain an acceptable level of variance. They reported that a repetition of up to four times is needed for determining the libido of young bulls even though the identification of the top-scoring bulls during the first test is comparatively accurate. These findings could diminish the commercial relevance of a libido test because it is labour and time consuming. It was also stated that most of the studies done on the assessment of bull libido disregard the evaluation of the test procedure itself. This leads to the perplexity of unraveling aspects such as individual variability, learning, training, and test accuracy. Unless methods used to appraise bull sex

drive have sufficient accuracy and repeatability, the results they produce will lack predictability and be inconsistent. Landaeta-Hernandez *et al.* (2001) argued that the low repeatability of test procedures and the necessity to perform a number of repetitions to reduce the environmental variance reduces the practicality of the libido test for routine application.

Entwhistle & Fordyce (2003) suggested that the quantitative (services per unit time) and qualitative (interest, achievement of normal services) aspects of assessing sex drive should be emphasized according to the type of bull that is being used, the age of the bull, the breed of bull, and to the type of breeding system it is used in. Young bulls can then be ranked for libido as part of a selection or culling process by using the quantitative testing procedures. Petherick (2004) argued that the many inconsistencies that exist between libido tests make the interpretation of results very difficult. The test conditions (such as test duration), the difference of management of bulls during the mating period, and the number of bulls and females used influence the sexual activity of a bull. In disagreement to the results obtained by Chenoweth. (1984). Parkinson (2004) argued that the repeatability of a libido score can be poor, especially for yearling virgin bulls. This low repeatability can be attributed to the effect that rearing condition, sexual experience (Boyd & Corah 1988), and exposure to estrus females has on the score a bull obtains during a libido test. To reduce the time, labor and aesthetic concerns associated with libido/serving capacity test it is necessary to develop an indirect method to assess bull sex drive. An indirect method has the added advantage of allowing the assessment of bulls that normally do not respond well to libido tests. Attempts have been made to link blood testosterone levels to bull sex drive (Chenoweth, 1999) but the results obtained up to date are not conclusive.

2.3.3.2. Mating behavior

Bailey *et al.* (2005) argued that existing methods used to determine the sexual behavior of bulls may be impeded due to the fact that they require the use of restrained, non-estrus females that do not represent natural mating stimuli. Libido testing has been criticized as being somewhat unaesthetic in nature. Objections have been raised on the grounds of the welfare of the female animals that are used in a libido test. There are guidelines in the form of codes of practice giving recommendations of the type of cows that can be used, the frequency of use, and the criteria for discontinuing use of individual cows (Parkinson, 2004). An immobile, inverted, U-shaped object is believed by some researchers (Chenoweth 1981; Price *et al*., 1987) to be the strongest stimulus inducing copulatory behavior in bulls. Blockey (1981a) said that when differentiating between bulls of high, medium and low sex drive, the bulls must be given the opportunity to express their maximum sex drive. A study by Sambraus (1968) reported that during the estrus period a cow varies noticeably in its desire to be mounted. A lengthy period of courtship is needed to induce the cow to stand for mounting in early and late estrus and after each service because the cow's desire to be mounted is lower than in mid-estrus. The number of services bulls

could achieve in the short test is reduced due to this courtship activity. It was concluded that restraining estrus heifers during a serving capacity test increased the serving activity of the bulls four-fold. Bulls spent less time courting restrained heifers than unrestrained heifers. It was also stated that when heifers are immobilized it might stimulate bulls into greater serving activity since immobility is the characteristic that is believed to encourage a bull to mount an estrus cow. Opposing results where reported in a study where libido was tested with heifers in estrus and heifers not in estrus and a significant positive correlation (r = 0.67) was obtained between the two tests (Chenoweth *et al.*, 1979).

Bailey *et al.* (2005) said that reports that bull serving capacity has little relationship with fertility may be attributed to the fact that the temporal changes in the expression of sexual behavior between males and females are not fully evaluated. The temporal expression of a bull's sexual behavior varies with the type of female stimulus. Quirino *et al*. (2004) pointed out that some bulls may show temporary deficiencies in libido due to environmental influences or because stimuli were inadequate, e.g. females not fully in estrus. Many investigators have suggested that the sexual behavior expressed by a bull is purely due to an innate or inherent sexual libido. The study done by Bailey *et al.* (2005) reported that a group of unrestrained, sexually receptive females induce greater sexual responsiveness in bulls than sequentially pairing them with individual females. They argued that other practices such as using restrained, non-estrus females or even restrained male cattle in serving capacity tests seems questionable. The aforementioned method of using restrained male cattle lead to decreased variation in stimulation and can lead to a faulty assumption that stimulation condition is insignificant in terms of regulation of sequential expression of copulatory behavior in the bull. In a study done by Barth *et al.* (2004), it was reported that some bulls will not copulate with restrained females but they readily displayed normal copulatory behavior during pasture mating. It has also been demonstrated that *Bos Indicus* bulls fail to display intense sexual behavior in small pen tests due to the presence of investigators and the use of restrained, non-estrus females (Chenoweth, 1981). It was also reported by Bailey *et al.* (2005) that bulls repeatedly service individual females until the female's sexual receptivity becomes attenuated and/or bulls approach sexual satiety.

Bertram *et al*. (2002) suggested that the time taken during a serving capacity test could be reduced by using a restrained estrus female. They conducted a serving capacity test using restrained estrus females and examined the females the next day. The reported that 76% of the females revealed no visible evidence of trauma to their reproductive tract and only 0.06 % showed slight hemorrhage. It was concluded that the serving capacity test results in only minor trauma to the female reproductive tract and that the repeatability of sexual behaviors is greater when using restrained females rather than unrestrained cows.

Petherick (2005) reported that, in general, bull to female ratio (BFR) has a minimal effect on libido expression and fertility. Even though data on this subject are limited, mating activity has been reported to increase with the number of females in estrus and, as BFR increases, the amount of females mounted decreases. Chenoweth (1999) pointed out that single-sire mating is more efficient than multi-sire mating but the individual capabilities of bulls have greater impact on herd fertility than BFR.

Petherick (2005) pointed out that cattle in groups interact and develop relationships with each other, one form of relationship being a dominance hierarchy or social order. Whenever groups of bulls are used during the mating season, one animal sires most of the calves. Dominance is of little importance when yearling or two-year-old bulls are used and the difference in the number of calves sired does reflect underlying differences in bull's inherent fertility. Bulls should be allowed to establish a social hierarchy for some time before they are exposed to cows in a group mating system (Parkinson, 2004). It has been reported by López *et al*. (1999) that the sexual behavior of some other males can be inhibited by the presence of a more dominant male and Chenoweth (1999) pointed out that dominant bulls sire the majority of calves in a multi-sire group. In the presence of multiple sexually active bulls, the sexual activity of other bulls is stimulated, but high levels of aggression can also occur and interfere with sexual activity. Physical injuries to the bulls can occur due to social interaction and these can affect the mating ability of the bull and thus the observed libido (Petherick, 2005). Bulls tested for libido with other bulls close by may not show their true potential due to dominance–subordinance relationships, but this can be overcome by the presence of several females in estrus at the same time during the test (Petherick, 2005).

Blockey (1981b) determined social dominance order by confining a group of bulls in a 20m x 20m pen and forcing the bulls to compete for individual space. All instances of a bull clearly avoiding another, or retreating when bunted were recorded. Dominance is said to be related to neither libido nor fertility. In situations where bulls of mixed ages are used the dominant bull may sire a disproportionately large number of calves. This can result in low conceptions rates when the dominant bull is sub-fertile (Parkinson, 2005). Ologun *et al*. (1981) stated that there may be a genetic influence on the dominance value a bull obtains during a social dominance test. They reported that there is a negative correlation between dominance value and libido score in yearling beef bulls.

There are a few complicating factors in the relationship between dominance and libido. The first is that the method used to determine the dominance hierarchy in a test situation may not reflect the situation during paddock mating (Godfrey & Lunstra, 1989). The interaction between age and dominance is a second complication. The length of time that the bull has spent in the herd tends to determine the dominance of that bull and this is closely associated with the bull's age. Bulls that have been reared together and are of a similar age are less likely to fight, reducing bull abrasion and injuries (Ologun *et al.*, 1981).

There is not much agreement between results of studies done on the effect of age on libido. Petherick (2005) pointed out that older bulls become more competent in serving capacity tests and in any courtship behavior, and that the libido score of a bull will increase with bull age. Perry *et al.* (1991) reported higher libido in older bulls than younger ones. This is thought to be due to the effect of sexual experience. Older, more experienced bulls have a greater ability to determine the optimum time for mating than younger bulls and they do not need to spend time and effort determining the receptivity of females by repeatedly mounting them. It was also reported by Bertram *et al*. (2002) that libido scores increased from 3.3 ± 1.2 to 7.5 ± 0.7 ($P< 0.01$) in two-year-old *B. Taurus* bulls after allowing them to obtain sexual experience during a 24-hour period. Landaeta-Hernandez *et al.* (2001) reported that during a two-month consecutive testing period, the libido scores of *B. Taurus* bulls increased from 4.9 to 7.0. They reported high positive correlations between the last libido score (eight) and the sixth ($r =0.67$, $P<0.0001$) and seventh scores ($r =0.84$, $P<0.0001$), demonstrating that a learning process occurred during the trial. They pointed out that the bulls obtaining the top score at the beginning of the trial invariably obtained the top score throughout.

Mating ability is said to have a learning component (Chenoweth, 1999). Price & Wallach (1991) reported that the serving capacity tests administered to virgin beef bulls younger that 18 months may give an underestimate of the mating potential of some of the animals. They found that most bulls might start to mount females from 9 months of age, but only accomplish their first ejaculation by 15 months of age. Ellis *et al.* (2005) also pointed out that bulls show a progressive continuum of physical and sexual maturation between 12 and 24 months of age, and are particularly vulnerable to factors affecting fertility during this period. Landaeta-Hernandez *et al.* (2001) reported that the main determinants of libido are environment and learning rather than genetics or intrinsic effects due to the low repeatability they obtained for libido tests scores of yearling bulls. López *et al*. (1999) reported that a learning component may be involved in the sexual performance of a young bull (15-month old), and for these bulls physical contact with an older bull may be required. Blockey (1981b) argued that young bulls may not be considered as potential competitors for older bulls. Boyd & Corah (1988) argued that yearling virgin bulls with no previous sexual experience must be given a pre-test session to acclimatise them to the test environment. This is achieved by allowing a bull to be prestimulated by watching the mating activity of other bulls, followed by exposure to estrus females for a period of 10 minutes or until the bull has successfully completing one service. The serving capacity test was conducted one week later. They subjected the bulls that obtained low serving capacity scores to a sexual experience school in which the bulls are exposed to estrus females for a period of 4 days, at a bull to female ratio of 1:2. The serving capacity score of 85% of these bulls increased to the medium or high serving capacity category during a second test, indicating that sexual experience does have an effect on sex drive.

Silva-Mena *et al.* (2000) reported that both serving capacity score and libido score are highly correlated to age (r = 0.78, P<0.01 and r = 0.56, P< 0.05, respectively). On the other hand Chenoweth *et al.* (1984) found no age-related changes in the libido score of bulls. López *et al*. (1999) cited a study by Blockey (1981b), also stating that sexual experience does not change the innate serving capacity of bulls and that virgin bulls exhibit their inherent serving capacity by two years of age.

2.3.3.3. Other factors affecting libido

Breed differences in reproductive traits such as sheath measurements, SC and spermatozoal quality are well documented. These traits are often correlated with fertility and are likely to be confused with the effect of libido on fertility. Petherick (2005) pointed out that genetics largely affects the libido, as well as the inherent fertility of individual bulls. Evidence of differences in sex-drive between beef and dairy herds has long been reported (Chenoweth, 1999). Verification is presented by Petherick (2005) that Afrikaner bulls and their crosses achieve the highest libido scores, Brahman and Brahman crosses the lowest, and European genotypes show intermediate libido scores. Petherick (2005) postulated that Zebu/Brahman bulls are selective and shy breeders, with a tendency to only mount females that are in full estrus, and they generally do not perform well in a pen test to assess libido (Bertram *et al.*, 2002) or show mating when observed at pasture. Bulls of one genotype/phenotype appear not to associate with, or show sexual interest in, cows of a different genotype/phenotype (Petherick, 2005; Chenoweth, 1981). There are also inherent fertility differences between individual bulls (Petherick, 2005) and it was shown in multi-sire mating herds that some bulls sired 70% of the calves while others sired as little as 4%. A number of reports indicate that although *B.Indicus* bulls show lower and more variable sexual responses during libido tests than *B.Taurus*, this apparent reduced libido does not result in poorer fertility.

Ologun *et al.* (1981) conducted a study on yearling beef bulls to determine the relationship between superior production traits, such as ADG and final liveweight after a 140-day performance test, and sex drive. Yearling beef bulls are typically selected as potential herd sires on their performance in a performance test. Previous studies revealed that a negative relationship exists between high growth and feeding levels, and sex drive of bulls (Zoder *et al.*, 1969; Flipse & Almquist, 1961). In this study, it was found that ADG and final liveweight are negatively correlated with serving capacity scores (P<0.01). Their observations reinforced previous observations that the sex drive of yearling beef bulls is not favorably related to production traits.

Brito *et al.* (2006) argued that nutrition may have a major impact on sexual development in bulls through the effect it has on gonadotropin secretion. They reported that sexual development and the hypothalamic-pituitary-testis axis are regulated by nutrition through effects on the GnRH pulse generator in the hypothalamus,

and through the effect that this has on LH secretion. Perry *et al.* (1991) pointed out that the testosterone–LH feedback mechanism in bulls has been shown to be influenced by underfeeding and is associated with decreased testosterone and LH release as well as decreased testicular growth.

Cattle are not normally considered to be seasonal breeders, but they are subject to seasonal influences on reproduction associated with ambient temperatures, feed availability, and parasite loads (Chenoweth, 2000). Petherick (2005) stated that extremes of thermal environments and climates can operate to reduce the expression of libido. During high temperatures, *B. Taurus* bulls show greater discomfort leading to disinterest in estrus females than *B. Indicus* bulls. Using *B. Indicus* bulls in temperate environments can also have adverse consequences on fertility.

Conflicting observations exist regarding the seasonality of plasma LH and testosterone concentration in bulls. Perry *et al.* (1991) reported higher GnRH-induced testosterone levels in the spring as opposed to the winter and predicted that this is due to a seasonally induced increase in testicular sensitivity to GnRH-induced LH secretion. Peirce *et al*. (1987) stated that a negative correlation has been observed between LH concentration and mean daily temperature and this is in observance with the lower mean LH levels found in heat-stressed bulls. Seasonal changes in circulating LH and testosterone occur in adult bulls and are higher during the winter months ($P<0.05$). Landaeta-Hernandez *et al.* (2001) reported a moderate repeatability of 0.64 for libido tests over a period of two months. This indicates that environmental influences play a large roll and must be considered when interpreting libido score results.

2.3.3.4. Frequent oversights in libido testing

Chenoweth (1999) pointed out that there are many precautions to be taken when testing bulls for libido and mating ability. Bulls must be handled quietly during the test to prevent excessive restlessness. Testing of bulls immediately following other procedures such as electroejaculation (EE), vaccination, and parasite control measures should be avoided. The bulls should not be tested during periods of adverse weather conditions such as extreme heat, cold or wind. Testing should be done in groups of similar ages to reduce the effects of social dominance. Inadequate stimuli due to females not being in full estrus is another setback. From an ethical stance, the females used during such a test should be closely monitored for signs of stress or injury, and be replaced if these become evident. Prior to testing, all the participating animals should be tested for venereal diseases and all precautions should be practiced to prevent these diseases from spreading.

2.3.4. Hormones as indicators of bull fertility

Swenson & Reece (1993) stated that physical and chemical changes arising from both within and outside the body must be responded to by the various body functions. They described hormones as compounds that are synthesized and secreted by specialized endocrine cells and transported via the bloodstream to exert a regulatory effect on a different group of target cells. Hormones are divided into two classes, the soluble peptide hormones (binding with receptors on the plasma membrane, which exert effect by way of intracellular enzymes) and the insoluble steroid hormones (interacting with nuclear receptors, which affect protein synthesis through stimulation of mRNA).

Diverse humoral and neural means are employed in the regulation of hormone secretion. Some hormones are secreted in response to the concentration of chemical compounds, or ions, in extracelllular fluids, others respond to neural stimuli, and still others are controlled by hormones from another endocrine gland. Most hormonal control systems are regulated by feedback mechanisms. In negative feedback, the target cell response inhibits the regulating signal. For example, GnRH stimulates secretion of LH, which, in turn stimulates synthesis and secretion of testosterone. An increased blood concentration of testosterone inhibits the hypophyseal secretion of LH, either directly or by inhibiting the hypothalamic secretion of GnRH (Swenson & Reece, 1993).

Parkinson (2004) pointed out that the endocrine system regulates all the functions of the male reproductive system. The synthesis of reproductive steroids is controlled by the gonadotropins. The reproductive steroids control libido, spermatogenesis, and the action of the epididymis. Contradictory results have been found regarding whether or not the measurement of circulating hormone concentrations can be used to predict or assess libido, sperm production, and the breeding potential of bulls.

2.3.4.1. Gonadotropin-releasing hormone (GnRH)

Swenson & Reece (1993) said that the hormone output from the adenohypophysis is both humoral and neural and is centered in the hypothalamus. Gonadotropin releasing hormone (GnRH) is a decapeptide synthesized in the neurosecretory cells of the hypothalamus and secreted into the primary capillary bed of the pituitary gland in a coordinated, pulsatile manner. At the gonadotrophe cells of the pituitary gland, GnRH binds to specific cell surface receptors, and this triggers the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), and *de novo* synthesis of LH and FSH (D'Occhio *et al*, 2000). Follicle stimulating hormone and LH are classified as glycoprotein hormones, which regulate the production of sex gametes and the secretion of hormones from the gonads (Swenson & Reece, 1993).

When measuring the plasma concentrations of the glycoprotein hormones, more constant data can be generated by administrating exogenous GnRH to bulls, serving as an exogenous stimulation of the reproductive

endocrine axis. By doing this the problems associated with the pulsatile secretion of both LH and testosterone can be overcome, and less variable hormone data can be generated (Parkinson, 2004). Some of the GnRH agonists that are commercially available include Buserelin, native GnRH, and Fertirelin. Marked differences exist between these various analogues in relative potencies to release LH and FSH in cattle. GnRH agonists have a higher affinity for GnRH receptors and have a longer half-life in circulation. These properties allow them to be used at substantially lower doses than natural sequence GnRH (D'Occhio *et al*, 2000). GnRH-induced effects can be indirect through their induced release of LH and FSH, or they may have a direct effect on reproductive tissues. GnRH and its analogues are very reliable in altering the endogenous secretion of LH. The variability of LH response to GnRH among animals is due to the physiological state of the animal at the time of injection (Thatcher *et al.*, 1993).

2.3.4.2. Luteinizing Hormone

Swenson & Reece (1993) pointed out that the hormones produced by the testis are either from the Leydig cells or the Sertoli cells. Testosterone, which is important for the development and maintenance of spermatogenesis, sex drive and male characteristics, is produced and secreted by the Leydig cells under control of LH. Luteinizing hormone, secreted from the adenohypophysis, is under the control of episodically released GnRH and is released in an episodic fashion, ranging from four to five releases per 24 hours. A critical minimum frequency of LH releases is required for the secretion of testosterone. After LH has bound to specific receptors on Leydig cell membranes, cyclic-AMP is activated, leading to a hormone cascade and mobilization of hormone precursors. Cholesterol is converted to pregnenolone and then the biosynthetic pathway involves either Δ5-intermediates (pregnenolone, 17α-hydroxypregnenolone, dehydroepiandrosterone and 5-androstenediol) or Δ4-intermediates (progesterone, 17α-hydroxyprogesterone and 4-androstenedione) to the production of testosterone. The Δ4- pathway predominates in the bull (Thompson, 1994).

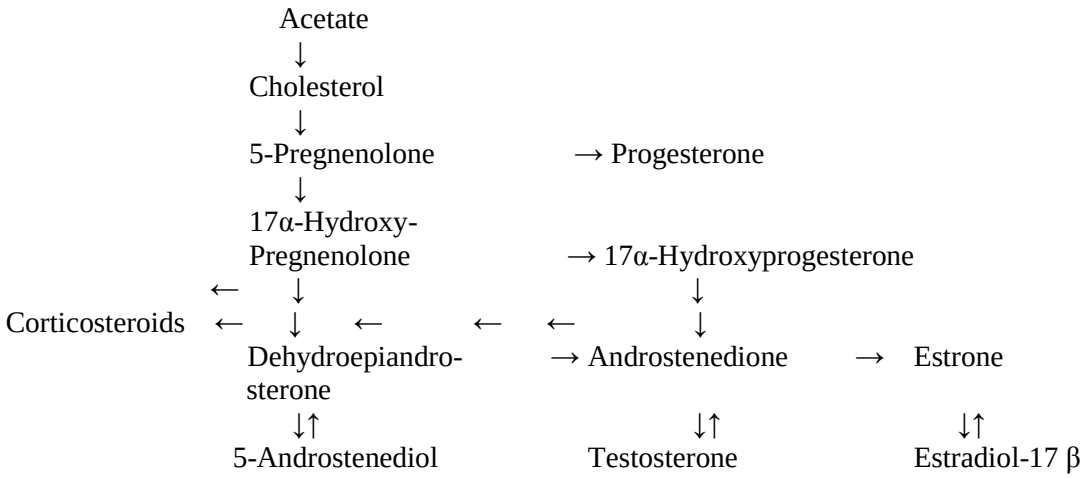

Figure 2 The synthetic pathway for biologically active steroids from acetate precursors (Swenson & Reece, 1993).

Removal of LH leads to a great reduction in the size of the Leydig cells and to cessation of testosterone secretion. A sensitive negative-feedback system operates between LH and testosterone secretion. Increased levels of testosterone can be observed 30 to 60 minutes after increased LH secretion, and the elevated testosterone level lasts for one to several hours. Levels of testosterone barely above physiological amounts can inhibit LH release and inhibit further testosterone synthesis. The negative feedback inhibition of LH secretion by testosterone is followed by a resultant decline in testosterone secretion (Swenson & Reece, 1993). Mean serum LH levels of bulls and non-estrus cycling females were shown by Mori *et al.* (1974) to be similar at 1.29 ng/ml.

Post *et al.* (1987a) postulated that differences between LH statuses in animals can be determined by administrating enough GnRH to elicit maximum LH responses. A dose of 1 μg/Kg live weight was needed to elicit the maximum LH response in 17-month old beef bulls. Larger doses of GnRH only extended the duration of the response. The repeatability of LH responses was very low (0.08) and the error variation very high. The animals used in the study did not differ greatly in LH responses. The optimum time to measure the LH response peaks was found to be one hour post-injection. The LH responses to GnRH in animals that differ appreciably in live weight can not be compared to each other unless adjustments are made for the weight differences. Byerley *et al.* (1990) reported that the LH response to GnRH varies over years and this further limit the use of the hormone as an endocrinological analyst of reproductive potential of beef bulls.

2.3.4.3. Testosterone

Swenson & Reece (1993) stated that testosterone is a steroid hormone which is produced by the Leydig cells from a steroid precursor (progesterone produce by the adrenal glands (Thompson *et al*., 1994). It moves into the seminiferous tubules of the testis by either simple or facilitated diffusion. Spermatogenesis requires high intratesticular concentrations of testosterone, but the largest proportion rapidly moves into the blood vascular system. The presence of various plasma proteins, such as sex-hormone-binding globulin, is important for testosterone secretion and transport. Here it is important for the development and maintenance of libido, secretory activity of the male accessory organs and development of body features associated with the male phenotype. Behavioral characteristics influenced by testosterone include the aggressiveness of male animals.

Swenson & Reece (1993) pointed out that some steroids can be metabolized to other biologically active compounds in the animal's body cells. Many tissue cells that are targets for androgens contain the enzyme 5α-reductase that converts testosterone to 5α- dihydroxytestosterone, a more biologically active compound. This enzyme is absent in young animals to prevent the premature androginization in response to the presence of testosterone.

Sitarz *et al*. (1976) reported that the daily gains of feedlot cattle are related to the testosterone concentration of a single blood sample. Apart from being indicative of growth rates, testosterone levels are indicative of reproductive potential of beef bulls. Testosterone is secreted in an episodic fashion that makes it difficult to assess normalcy of testosterone production and thus Leydig cell function. Testosterone production can be declared normal when the particular testosterone value obtained is at least equal to values observed at the lowest point of the episodic cycle of about 3.5 nmol/L of plasma. Testosterone values can be as high as 34.67 nmol/L of plasma (Swenson & Reece, 1993). Three hours after an increase in the LH concentration in the blood, there is a pulsatile release of testosterone. These pulses occur three to four times daily at six to eight hour intervals. Only after the testosterone concentration has decreased to basal levels will there be another increase in LH level, leading to the next testosterone release. Individual bulls show random sequential changes in plasma testosterone and there is no evidence of diurnal discrepancy (Nancy *et al*., 1977). A study was done that demonstrated the variation in testosterone levels during a normal pulsatile secretion. After an increase in LH levels, testosterone concentration increases for 40 minutes and stays elevated. After 30 minutes of elevated levels, the concentration decreases to one half the pulsatile peaks over a period of 1.5 to 2.5 hours. A bulls that is accustomed to capture and breeding has an average testosterone value of 14 to 24 nmol/L. (Thibier, 1976).

Parkinson (2004) pointed out that the pulsatile pattern of testosterone secretion creates a problem for elucidating its relationship with reproductive performance. Low-frequency blood sampling has an arbitrary chance of being collected during periods of episodic secretions and there is thus difficulty in associating daily testosterone concentrations with other parameters of reproductive performance. The high frequency sampling

necessary for proper characterization of testosterone concentrations in bulls is not practical in most bull management systems. Post *et al.* (1987a) pointed out that the effect of restraint during sequential blood sample collection over a period of adequate length may have an inhibitory effect on testosterone secretion. Nancy *et al.* (1977) also pointed out that when evaluating normal temporal variation in testosterone concentration, sequential collection of blood from bulls unfamiliar to restraint and capture cannot be used due to the negative effect that stress has on blood testosterone and LH levels. The release of corticoids during stress can directly or indirectly cause a decrease in testosterone concentration in the blood. This problem can be overcome by exogenous GnRH providing exogenous stimulation to the reproductive endocrine axis (Post *et al.*, 1987a).

Thompson *et al.* (1994) did a study in which 13-month-old bulls were injected with exogenous GnRH and blood samples were taken immediately prior to injection (T_0), 30 minutes after injection (T_{30}), and two to three hours after injection (T_{150}). The serum testosterone concentrations were highly variable at T_0, ranging from 3.1 nmol/L to 66.3 nmol/L. At 30 minutes after GnRH injection, the testosterone concentration increased ($P<0.0001$) and continued to increase through T_{150}, reaching a value approximately two-fold greater than at T_0. Post *et al*. (1987a) also did a study on 17-month-old beef bulls to elicit the testosterone response to GnRH injection. They found that the dose of GnRH is not decisive in obtaining maximum testosterone responses and there is no need to adjust dose to the body weight of the animals used. They also found that the best possible time to assess the elevation of the testosterone response is two to three hours post-injection. The error variation in testosterone response was low and repeatability was high (0.74 to 0.78). It was concluded that the testosterone status of bulls can be determined by the analysis of a single blood sample obtained two to three hours after GnRH injection and that this may provide an endocrinological assessment to detect the reproductive potential of a bull. Post *et al.* (1987b) reported a similar ranking between libido and testosterone response to GnRH in beef bulls. Conflicting results was obtained by Byerley *et al*. (1990), in that there was no difference ($P< 0.1$) between high and low libido bulls in testosterone concentration following GnRH injection. Conversely, they found that the testosterone concentrations were elevated ($P<0.05$) in high libido bulls in the two hours prior to GnRH injection as compared to concentrations in low libido bulls. They postulated that low testosterone concentrations do not decisively imitate low libido, but the bulls with the lowest libido in a population may endure unsatisfactory testosterone production.

Malak & Thibier (1985) conducted a study to determine the effect that sexual stimulation has on testosterone and LH secretion in bulls. Studies done on other species (rat, sheep) showed an increase in these sex hormone secretions after sexual stimulation. They found no elevation in any of these hormones after subjecting the bulls to electroejaculation. They postulated that the presence of female pheromones may cause the elevated levels of testosterone and LH reported in other studies. The methodology of electroejaculation does not result in the physiological conditions involved in natural mating. Differences in somatic responsiveness to a threshold

level of testosterone accounts for differences in sexual activity between individual animals (Smith *et al.*, 1981; Blockey & Galloway, 1978; Chenoweth, 1981).

2.3.4.4. Other

Other hormones that have been related to aspects of reproductive performance include progesterone and inhibin. Progesterone is a steroid hormone found mainly in female animals where it is secreted by the corpus luteum and functions to maintain pregnancy (Swenson & Reece, 1993). In the male it serves as a steroid precursor for testosterone and is secreted by the adrenal glands and the testis (Thompson *et al*., 1994). Parkinson (2004) stated that the relationships between progesterone and libido, testosterone concentration and the percentage of abnormal sperm are all negative. Inhibin is a protein hormone secreted by the Sertoli cells and has the main function of being an FSH inhibitor at the level of the pituitary gland (Swenson & Reece, 1993). Inhibin concentration has been found to be positively correlated with both libido and spermatozoal quality (Parkinson, 2004).

2.4. PHYSIOLOGICAL STRESS AND THE ENDOCRINE SYSTEM

Grandin (1997) argued that both physiological and behavioral measurements (vocalization, kicking and struggling) should be considered when assessing stress. Heart rate, beta-endorphins, and cortisol concentrations are common physiological measurements of stress. Grandin (1997) stated that animals can be stressed by either physiological stressors such as restraint and handling, or by physical stressors such as hunger and thirst, injury, and thermal extremes. Although procedures such as restraint in a squeeze chute do not usually cause significant pain, fear can still be a physiological stressor in extensively raised cattle.

Minton (1994) pointed out that the degree of secretory activity of the adrenal gland gives an indication of conditions that are stressful. Plasma cortisol concentrations are thus indicative of stressful conditions. Cortisol synthesis and secretion from the adrenal cortex is regulated by adrenocoricotropic hormone (ACTH), which is secreted from the corticotropic cells of the adenohypophysis. The secretion of ACTH is, in turn regulated by corticotrophin releasing hormone, secreted by the hypothalamus. Elevated blood concentrations of ACTH result in increased adrenocorticol activity, leading to increased secretion of cortisol and resultant decreased adrenal cholesterol due to the increased cholesterol conversion to pregnenolone, which is the precursor for cortisol synthesis. During acute stress, the sympathetic nervous system is activated, but because of the relative difficulty in measuring plasma epinephrine when compared to measuring peripheral concentrations of cortisol, relatively few studies have characterized catecholamine responses to stress. Plasma epinephrine is also considered to be very variable.

Grandin (1997) argued that short-term stress from handling can be assessed by cortisol concentration and there are three categories of cortisol concentration. Baseline levels indicate procedures that were not stressful and the concentrations range between 8-17 nmol/L for *Bos Taurus* cattle. Normal levels of cortisol that occur during restraint in a head gate range between 99 and 174 nmol/L. Mean values larger than 256 nmol/L indicate that animals were experiencing extreme stress.

Moberg (1991) stated that increases in circulating concentrations of the adrenal glucocorticoids are used as indicators of stress because of the responsiveness of the adrenal axis to physiological stress. The adrenal axis is also known to regulate the gonadal axis, and this implies that the adrenal cortex modulates reproduction. Excessive blood cortisol concentrations have also been shown to inhibit LH release and to enhance the ability of the sex steroids (testosterone) to suppress gonadotropin secretion through negative feedback (Swenson & Reece, 1993). Daley *et al.* (1999) reported that stress-like concentrations of cortisol enhance the negative feedback potency of estradiol and reduce estrogen-dependant accumulation of GnRH receptors in pituitary tissue, leading to a decrease in LH pulse frequency. Glucocorticoids can act directly on the GnRH-secreting cells of the hypothalamus because they can readily penetrate the blood-brain barrier. Breen & Karsch (2004) reported that cortisol inhibits the pulsatile release of LH by suppressing the pituitary responsiveness to GnRH rather than by actually inhibiting hypothalamic GnRH release. Their findings demonstrated that a stress-like increment in cortisol inhibits pituitary responsiveness to relevant GnRH pulses. Management-related stressors have been shown to increase glucocorticoid concentrations and at the same time decrease the pituitary's responsiveness to exogenous GnRH. An inverse relationship has also been shown between the plasma concentration of glucocorticoids and the amount of testosterone secreted in response to exogenous LH. Regardless of these responses of the testis to the adrenal axis, there is no evidence that male fertility is severely affected (Moberg, 1991).

CHAPTER 3

3.1. MATERIALS AND METHODS

3.1.1. Location

During November and December 2006 the trial was conducted on 41 sexually inexperienced bulls residing at the Bonsmara stud farm of Mr. A.M. de Villiers. The farm is located 30 km south-east from the town Vrede, situated in the Eastern Freestate, South Africa. The exact coordinates of the farm is 29º54'54.51" S, 26º31'33.11" E at an elevation of 1635.3 m above sea level. This area receives the majority of its annual rainfall between mid-October to March and the average annual rainfall varies between 720 – 750 mm/ year. This area is defined as a highveld area, with indigenous vegetation falling into the grassveld class. *Themeda triandra* and *Heteropogon contortus* are the dominant grass species. Other naturally occurring grass species includes *Eragrostis spp*. and *Felecia spp.* The highveld is classified as a sourveld area where the forage produced will support animal performance only during the active growing season, which is in the summer (Maree & Casey, 1993). Mr. de Villiers compensates for the inadequacies of the forage in the grazing camps during the winter months by planting *Lolium spp* and *Eragrostis curvula*. He also provides all the animals on the farm with 500g per animal per day of a 43% protein lick in the winter months. The composition of the lick can be seen in Table 4. During the summer months all animals are provided with a P6-mineral lick (9g P6 per animal per day).

Table 4 Composition of winter protein licks

50%	Chicken litter
25%	Salt
10%	Urea
10%	Cottonseed oilcake
5%	Maize meal

The trial was performed during the summer months, the average daytime minimum and maximum temperatures being 5 and 23 ºC, respectively. During the trial period the bulls were grazed on a mixture of *Themeda triandra* and *Heteropogon contortus* pastures *ad libitum* and were supplemented with 8 Kg of bull-meal per head daily. The nutritional composition of the bull meal can be seen in Table 5.

Table 5 Composition of bull meal (g/Kg) according to Act 36/1947

Composition (g/Kg)	min	Max
Moisture		120
Protein	130	
Urea		5
Fiber		150
Fat	25	
Calcium (Ca)		10
Phosphorus (P)	4	
Ca:P	1.1	3.1

3.1.2. Animals

Sixty-six bull calves born on the farm in 2004 were available for use in the trial. All of these bulls were reared together from birth onwards. All bulls were included in an On-Farm Performance test, also known as a Phase D_1 growth test, for 180 days. For the duration of the performance test the bulls were given balanced bull ration at 1% of body weight. The ration consisted mainly of maize, hominy chop, cotton seed, molasses and a mineral complex. This test took place from mid October to mid April 2005 and involved the evaluation of post-weaning growth under controlled conditions. The bulls were weighed twice weekly and body measurements, including measurement of SC, were done regularly. After the growth performance test, the bulls were divided into 4 separate homogenous groups according to weight.

The ages of the bulls ranged from 23 to 26 months (mean = 24.7 ± 0.70 months) at the time the trial commenced (Table 6). The bulls were weighed prior to the onset of the trial (week 1). Weights ranged from 367.0 to 522.0 Kg (mean = 399.5 ± 27.4 Kg). The bulls were also evaluated for structural soundness and any bull that displayed structural faults were excluded from the trial. Specific emphasis was placed on SC. Any bulls that had a SC < 330 mm were also excluded from the trial because this is the minimum SC required to enable bulls with weighing from 400- 500 Kg to pass a breeding soundness evaluation. The 43 heaviest bulls were selected and allocated a number from 1 to 43 from the heaviest to the lightest bull. These 43 bulls represented three of the four social groups of bull calves born in 2004.

During the second week, a group of thirty non-pregnant intact, nuliparous heifers, also born during 2004, were selected to be used during the libido tests. All heifers were of similar weights to ensure that a homogenous sexual stimulant is used on all of the bulls during the libido tests. Eighteen of the selected heifers were treated with Crestar® (Intervet SA Ltd) to facilitate the onset of estrus. This treatment comprises of a 3 mg Crestar implant containing the progestagen, Norgestomet, implanted into the ear and a 2 ml estradiol valerate injection administrated intramuscularly. The treatment results in synchronized estrus on day twelve of treatment, two to three days after removal of the implant on the ninth day of treatment.

Table 6 Summary statistics of age (days), birth weight (Kg), BW (Kg), 205 GI (Kg), ADG (g/day), KR, SH (mm), BL (mm), H:L, ST (mm), SC Ts (mm) and SC Te (mm) for the 41 bulls used during the experimental period

Variable	N	Mean ± SD	Minimum	Maximum
Age	41	24.7 ± 0.7	23.1	26.1
Birth weight	41	34.9 ± 3.9	26.0	44.0
BW	39	399.5 ± 27.4	367.0	522.0
205 GI	35	103.5 ± 7.4	90.0	119.0
ADG	35	105.5 ± 12.3	90.0	131.0
KR	35	104.6 ± 10.8	90.0	130.0
SH	41	1170.3 ± 29.1	1100.0	1233.0
BL	41	1376.5 ± 34.7	1290.0	1433.0
H:L	41	1.2 ± 0.0	1.1	1.2
ST	41	14.1 ± 1.7	11.0	18.0
SC Ts	41	347.1 ± 18.9	316.0	387.0
SC Te	41	370.0 ± 25.9	330.0	420.0

BW: Body weights at time of test
205 GI: 205 day growth index
ADG: Average daily gain
KR: Kleiber ratio
SH: Shoulder height
BL: Body length
H:L: Height to length ratio
ST: Skin thickness
SC Ts: Scrotal circumference after the performance test
SC Te: Scrotal circumference at end of trial

During week four, twelve days after the Crestar treatment, the eighteen heifers were placed in a grazing camp along with the bulls, for a pre-test session of 24 hours, as described by Boyd & Corah (1988). The pretest session provided the bulls with some sexual experience as well as acclimatized them to the test conditions. Six heifers were assigned to each of the three groups of bulls used during the trial with a 3:1 male to female ratio.

The remaining twelve heifers where randomly allocated to six groups in pairs of two. Twelve days before the onset of the first libido test the six groups of heifers where treated with Crestar® one day apart. This resulted in two heifers showing estrus on each of the six consecutive days of week five during which the libido tests were carried out (Figure 3). Bailey *et al*. (2005) showed that using a group of unrestrained sexually receptive females induces greater sexual responsiveness in bulls compared to a situation where only one female animal is used.

Figure 3 Two heifers displaying mounting behavior as an indication of standing estrus.

3.1.3. Blood sampling

Blood samples for the analysis of testosterone and cortisol concentrations of the 41 bulls were collected during week two and week five. Blood samples where taken via tail venipuncture into a heparinized tube (10 ml). A basal blood samples was taken after which each bull was treated with 2.5 ml synthetic GnRH i.m. (Buserelin ®, Intervet SA Ltd.). As recommended by Post *et al*. (1987a) and Thompson *et al.* (1994) the second blood sample was taken 150 minutes after GnRH treatment. The blood samples were kept at 1ºC overnight and centrifuged the next morning to obtain the plasma needed for analysis. A third blood sample was taken from each bull directly after the sexual stimulation succeeding the libido tests throughout week five. No blood samples could be obtained form bull number 8 and 43 and consequently these two bulls were used as the teaser animals in this trial as mentioned below.

The Coat-A-Count® procedure was used to analyze testosterone concentration (Coat-a-Count, Diagnostic Production Corporation, USA). This procedure is a solid-phase radioimmunoassay, based on testosterone-specific antibody immobilized to the wall of a polypropylene tube. ^{125}I-labelled testosterone competes for a fixed time with testosterone in the sample for testosterone-specific antibody sites. The tube is then decanted, to separate bound from free, and counted in a gamma counter. The amount of testosterone present in the sample is determined from a calibration curve. Another Coat-A-Count® procedure was used to analyze the cortisol concentration (Coat-a-Count, Diagnostic Production Corporation, USA). This procedure is also a solid-phase radioimmunoassay, wherein ^{125}I-labeled cortisol competes for a fixed time with cortisol in the sample for

antibody sites. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radio-labeled cortisol. Counting the tube in a gamma counter then yields a number, which converts by way of a calibration curve to a measure of the cortisol present in the sample. The average testosterone and cortisol concentrations at different collection times are given in Table 13 (page 47).

3.1.4. Libido test

Forty-one bulls were randomly divided into six groups (A to F) with seven bulls in group A to E and six bulls in group F. The groups were assigned to successive weekdays from Monday to Saturday. Throughout week five these bulls were subjected to the libido test devised by Chenoweth (1986) with some modifications.

The tests were carried out in the morning to compensate for the possible effect that light intensity, heat and time of day may have on the expression of libido. For sexual stimulation before each test, as recommended by Boyd & Corah (1988), each bull was allowed to observe the mating activity of another bull for a period of ten minutes before being tested. This was accomplished by placing the bulls into a pen adjacent to the test pen. The first bull tested on each day was prestimulated by watching a teaser bull mount the heifers for a period of ten minutes (Figure 4). The teaser bull was of the same age and weight as the rest of the experimental bulls and had no previous sexual experience except for the pre-test session. Bull's number 8 and 43 were used as teaser animals. Previous studies by Godfrey & Lunstra (1989) and Blockey (1981b) reported that the sexual activity of bulls is enhanced by the presence and sexual activity of other males.

Blockey (1981b) argued that the majority of young virgin bulls will display their inherent serving capacity in their first test if they are adequately sexually stimulated before the onset of the libido test. Adequate sexual stimulation is defined as 10 minutes of mounting activity by a bull in an adjacent yard that can be clearly seen by the young bulls. The bulls should respond by mounting one another or by achieving penile erection while watching the mounting activity of the teaser bull.

Figure 4 Sexual stimulation of 25 month old Bonsmara bulls by watching the mating activity/ courting behavior of a teaser bull.

For the test, each bull was individually placed in a 20 m x 20 m pen with a dirt floor containing two non-restrained estrus heifers as recommended by Bailey *et al.* (2005). During a ten minute period a modified numerical system was used to record the sexual behavior of each bull and transform it into a score for overall libido performance. The numerical system is as follows:

1. Sexual interest shown only once
2. Positive sexual interest in female on more than l occasion
3. Active pursuit of female with persistent sexual interest (Figure 5)
4. Bull has an erection/ pre-ejaculation due to sexual excitement (Figure 6)
5. One mount or mounting attempt with no service
6. More than one mounts or mounting attempts with no service
7. One service followed by no further sexual interest
8. One service followed by sexual interest, including:
   a. Sniffing/ licking at perineal region and showing Flehmen
   b. Bull has an erection/ pre-ejaculation
   c. Mounting attempts with no service
9. Two services followed by no further sexual interest
10. Two services followed by further sexual interest, including:
    a. Sniffing/ licking at perineal region and showing Flehmen
    b. Bull has an erection/ pre-ejaculation
    c. Mounting attempts with no service

Figure 5 Bonsmara bull actively pursuing an estrus heifer.

Figure 6 Bonsmara bull demonstrating pre-ejaculation due to sexual stimulation.

Interest was defined as any form of sexual interest, including sniffing (Figure 7) or licking at perineal region and Flehmen (Figure 8).

Figure 7 Bonsmara bull showing sexual interest by sniffing at perineal region of and estrus heifer.

Figure 8 Bonsmara bull showing sexual interest in a heifer by performing the Flehmen response.

A Flehmen response was defined as flexing of the bulls' nostrils and retraction of the upper lip followed by investigation of the female's perineal region. A mount or mounting attempt was defined as both front feet lifting from the ground, culminating in physical contact with the female, penile seeking, or intromission without the ejaculatory thrust (Figure 9).

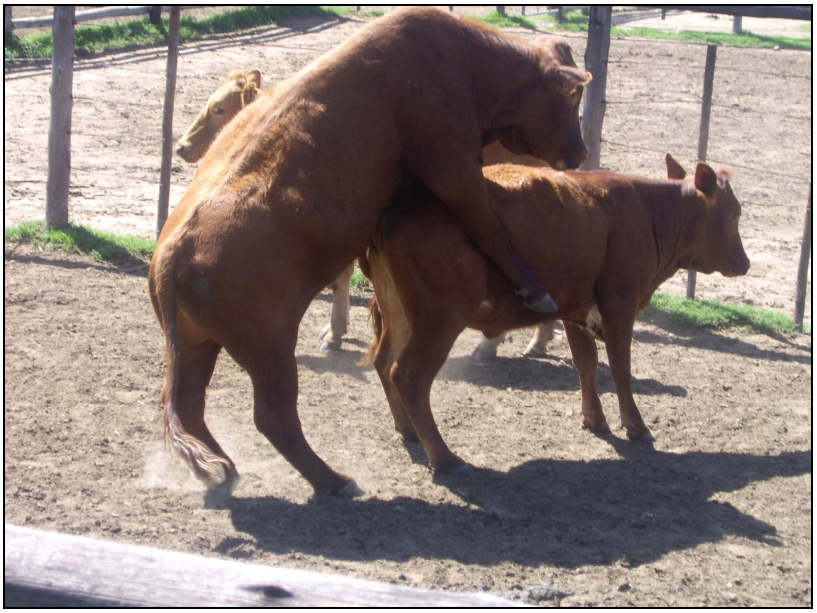

Figure 9 Bonsmara bull performing a mounting attempt with no intromission.

A service was a properly orientated mount on the posterior portion of the female followed by intromission and a deep ejaculatory pelvic thrust. Reaction time (time taken by the bull to reach each of the scores) was measured with a stopwatch. Care was taken to handle the bulls quietly to avoid anxiety.

The average libido score and time taken to reach each sore are given in Table 7. None of the bulls used during the experimental period obtained a score higher than 8b, being one service followed by sexual interest, including sniffing/ licking at perineal region and showing Flehmen and/or the bull having an erection/ pre-ejaculation.

Table 7 Summary statistics of the overall libido score and time (in seconds) to reach each of the numerators indicating libido

Variable	N	Mean ± SD	Minimum	Maximum
Overall libido score	41	4.7 ± 1.6	1.0	9.0
Time taken to reach 1	39	26.5 ± 38.0	4.4	174.7
Time taken to reach 2	20	75.1 ± 97.7	12.9	341.7
Time taken to reach 3	24	40.9 ± 39.1	12.5	206.9
Time taken to reach 4	30	105.4 ± 103.8	17.4	378.8
Time taken to reach 5	19	179.8 ± 148.3	20.0	494.6
Time taken to reach 6	8	359.8 ± 196.5	76.2	573.3
Time taken to reach 7	3	180.9 ± 199.1	40.1	408.7
Time taken to reach 8a	3	216.2 ± 203.5	51.4	443.6
Time taken to reach 8b	2	355.9 ± 110.9	277.5	434.3

3.1.5. Semen collection

During week eight, an Overall Breeding soundness Evaluation (OBE) was performed on all bulls. An overall score was given to the bull, which is derived from individual values for SC, spermatozoal motility and morphology. Final categorisation of bulls into exceptional, acceptable and unsatisfactory potential breeders is dependant upon this score (Chenoweth *et al*., 1988).

Scrotal circumference was measured by a qualified veterinarian. The testes were pulled down into the scrotum and a metal scrotal tape was placed around the greatest diameter of the scrotum. The average SC of the bulls can be seen in Table 6 on page 32. The testes and scrotum were manually palpated to ensure normal consistency and symmetry. The epididymis was also palpated for any detectable abnormalities. The prostate, ampullae, seminal vesicles and internal inguinal rings were palpated through the rectum for any detectable abnormalities. With the use of electro-ejaculation, the prepuce and penis were examined and spermatozoal samples were collected from all the bulls upon erection and ejaculation. This is a harmless procedure and yields spermatozoal of acceptable quality to be used in evaluating sperm morphology.

The sperm-rich fraction of the spermatozoal was collected into a conical test tube. A 4 mm drop of diluted spermatozoal was placed on a pre-warmed microscope slide, covered with a cover-slide, and evaluated for percentage of progressively motile sperm. The eosin–nigrosin staining method and microscopy at 1000x magnification were used to determine the percentage live sperm (Vilakazi & Webb, 2004). Eosin is a differential stain that only passes through non-living cell membranes, while the nigrosin is a background stain that makes the unstained live sperm heads visible. Spermatozoal characteristics were categorised as follows:

- semen colour was divided into three categories: Ivory (best), grey and white and
- semen marbling was categorized as being present (distinct and weak) or absent.

Sperm morphology was determined by evaluating the number of normal versus abnormal sperm per 200 sperm in the eosin-nigrosin stained smear with microscopy at 1000x magnification (Palmer *et al*, 2005). Spermatozoal morphology was evaluated for the percentage of normal sperm, percentage major defects (e.g. knobbed acrosomes, pyriforms, abnormal loose heads, dag defects, degenerative heads, mid-piece reflexes) and the percentage minor defects (e.g. normal loose heads, distal droplets, curled end-piece and loose acrosomes). Spermatozoal was evaluated according to the criteria given in Table 8.

Table 8 Semen morphology evaluation form

Bull identity number	
NUCLEUS:	Total
Teratoid	
Macrocephalic	
Pyriform	
Diadem	
Abnormal base	
Abnormal loose heads	
Double heads	
Rolled/ crested heads	
Tapered heads	
Narrow base	
Other abnormal head shapes	
Total nuclear defects:	
Percentage nuclear defects:	
NUCLEAR AND ACROSOME/ FLAGELLAR DEFECTS:	
Acrosomal defects:	
Total acrosomal defects	
Knobbed acrosome	
Degenerate acrosome	
Flagellar defect:	
Total flagellar defects	
Stump tail	
Double tail	
Proximal droplets	
Pseudodroplet	
Segmental aplasia of mitochondria	
Corckscrew	
Dag	
Midpiece reflex	
Other midpiece defects	
Bent midpiece	
Fractured flagellum	
Distal droplet	
Bent principle piece	
End piece defects	
Coiled principle piece	
Total acrosome and flagellar defects:	
Percentage acrosome and flagellar defects:	
OTHER ABNORMALITIES:	
Normally shaped loose heads	
Total normal, nuclear and acrosome/flagellar defects:	
Percentage morphologically normal sperm:	

The criteria used to evaluate spermatozoal morphology can be defined as follows: (LaRey, 2002; Vilakazi & Webb, 2004).

Major defects:

- Teratoid sperm: The midpiece lies over the sperm head in a bent or partially coiled form;
- Knobbed acrosome: Characterized by a localized swelling or bead on the apical ridge;
- Pyriform head: The head narrows at the post acrosomal region;
- Diadem: Appears as a dark necklace along the anterior edge of the posterior nuclear cap;
- Corkscrew: The midpiece is shaped like a corkscrew;
- Midpiece reflex: Severe bending of the midpiece;
- Dag: The tail is coiled, folded or somehow disrupted;
- Broken flagellum: Tail is broken or detached in any way;
- Proximal cytoplasmic droplet: Cytoplasmic droplet is retained in the proximal midpiece position;
- Pseudo-cytoplasmic droplet: Cytoplasmic droplet is located near the centre of the midpiece;
- Mitochondria aplasia: Characterized as a fracture in the midpiece;

Minor defects:

- Macrocephalic head: Sperm head is larger than normal;
- Abnormal loose heads: Head is detached and another abnormality is present;
- Stump tail: Characterized by a very short stump attached to the base of the nucleus;
- Normal loose heads: The sperm head is detached from the tail but there is no sign of other defects on the head;
- Degenerative or loose acrosome: This defect is considered to be very similar to the major defect degenerative head;
- Curled principle or end piece: Bending or coiling of the sperm tail;
- Distal droplet: The cytoplasmic droplet is in the distal part of the midpiece.

The 41 bulls were divided into independent breeding potential categories according to the scores they obtained for the measured reproductive traits. These categories included SC, spermatozoal morphology and motility, and overall breeding soundness. For the SC category, the 41 bulls were divided into two fertility groups with threshold measurement of: (1) SC $\geq$ 340 mm, and (2) SC < 340 mm. For the spermatozoal morphology category, the 41 bulls were divided into two fertility groups with thresholds of (1) $\geq$ 70% morphological normal sperm, and (2) < 70% morphological normal sperm. Concerning the spermatozoal motility category, the 41 bulls were also divided into two fertility groups with threshold levels of (1) $\geq$ 70% motile sperm, and (2) < 70% motile sperm. The overall breeding soundness categories (OBC) were calculated as shown in Table 9 by

combining the fertility categorisation of SC, sperm motility and sperm morphology (Parkinson, 2004; Chenoweth *et al*., 1988). The 41 bulls were categorised as being exceptional-, acceptable- and unsatisfactory-potential breeders. Potential breeders with exceptional fertility (OBC 1) achieved threshold measurements of ≥ 340 mm SC, ≥ 70% morphological normal sperm and ≥ 70% motile sperm. For the purpose of this study any bull not meeting these standards was either assigned to potential breeders with acceptable fertility (OBC 2) or unsatisfactory potential breeders (OBC 3) as shown in Table 9. Traits of the OBE have been shown to be interrelated (Chenoweth *et al*., 1988).

Table 9 Fertility groups and overall breeding soundness scores for 25 month old Bonsmara bulls

Measurement	Fertility categories		
	1	2	3
Scrotal circumference	≥ 340mm	< 340mm	< 340mm
Sperm morphology	≥ 70% normal	< 70% normal	< 70% normal
Sperm motility	≥ 70% motile	< 70% motile	< 70% motile
OBC	1 (111[1])	2 (122/221/121[2])	3 (222[3])

111[1]	Bull has an OBC 1 classification for SC, spermatozoal -morphology and -motility
122/221/121[2]	Bull has an OBC 2 classification for any two of the above mentioned measurements
222[3]	Bull has an OBC 3 classification for all three of the above mentioned measurements
OBC	Overall breeding soundness group
OBC 1	Satisfactory potential breeders with exceptional potential fertility
OBC 2	Satisfactory potential breeders with acceptable potential fertility
OBC 3	Unsatisfactory potential breeders

3.1.6. Statistical analysis

A statistical analysis of the data was done by using the general linear models (GLM) procedure of the Statistical Analyses System (SAS version 8.2 BMDP). One way analysis of variance (ANOVA) of the effect of fertility groups was done by means of the mentioned GLM procedure, and the least square means (LSM) option was used. Age was included as a continues variable. In addition, the interaction between fertility group and time was determined. All procedures were assessed at a significance level of 95% ($P \leq 0.05$) for the critical values or the *F*-statistic. Least square means procedures were used to calculate means and standard error of the means (SE). Chi- square tests (log linear analysis) of independence and trend analysis were performed on all categorical data, while a Kruskal-Wallis non- parametric test was used to do analyse the variance. Sequential hormone measurements were evaluated using ANOVA for repeated measures. The point biserial correlation was used to measure the association between the continuous variables and fertility categories.

CHAPTER 4

4.1. RESULTS

4.1.1. Growth

Palpation of the scrotum, testes and epididymis of the 41 Bonsmara bulls used during the present study, revealed no abnormalities. The average SC of the bulls after the performance test was 347.1 ± 18.9 mm and SC at the end of the trial was 370.0 ± 25.9 mm (Table 6, page 32).

4.1.1.1. Breeding potential category based on scrotal circumference

One-way ANOVA results revealed that the independent production measurements, except for SC after the performance test and at the end of the trial, did not differ between SC categories (Table 10). The production measurements include age (days), body weight (Kg), pre-weaning growth rate or 205 day growth index (Kg/day), average daily gain (g/day), Kleiber ratio, shoulder height and body length (mm) and SC. The SC measurements, both after the performance test and at the end of the trial, were numerically greater for the bulls in the high fertility category (SC ≥ 340 mm) as expected.

4.1.1.2. Breeding potential category based on spermatozoal morphology

One-way ANOVA results revealed that there were no significant differences in growth measurements of the bulls between the spermatozoal morphology categories (Table 10). The correlation (Table 11) between 205 day growth index and the percentage morphologically normal spermatozoa was $r = 0.33$ ($P<0.1$). A higher 205 day growth index was associated with a small increase in the percentage morphologically normal sperm. The correlation between body length and 205 day growth index was $r = 0.47$ ($P<0.05$) indicating that the bulls with longer body lengths had more morphologically normal sperm.

The SC measurements both at the end of the performance test and end of the trial were numerically greater for bulls with less than 70% morphologically normal sperm, but these differences were not statistically significant.

Table 10 Summary statistics of effects of the fertility categories on growth measurements for 25 month old Bonsmara bulls

Fertility category	Age ± S.D	BW ± S.D	205GI ± S.D	ADG ± S.D	KR ± S.D	SH ± S.D	BL ± S.D	ST ± S.D	SC Ts ± S.D	SC Te ± S.D
SC										
≥ 340	24.7 ± 0.7	400.8 ± 28.6	103.2 ± 6.9	104.5 ± 12.2	103.7 ± 10.4	1171.9 ± 30.0	1376.9 ± 35.9	14.1 ± 1.8	350.0 ± 18.1^{c}	375.6 ± 22.5^{a}
< 340	24.6 ± 0.6	390.4 ± 16.4	105.4 ± 10.6	111.2 ± 12.4	110.4 ± 12.6	1159 ± 20.7	1373.4 ± 27.1	14.0 ± 1.0	326.2 ± 7.9 ^{d}	330.0 ± 0.0^{b}
Morph										
≥ 70%	24.6 ± 0.5	391.4 ± 9.5	104.7 ± 9.8	103.2 ± 8.4	104.6 ± 5.7	1161.9 ± 33.8	1372.4 ± 29.7	14.1 ± 2.0	341.3 ± 20.1	367.3 ± 24.5
< 70%	24.7 ± 0.8	402.7 ± 31.4	103.0 ± 6.4	106.4 ± 13.6	104.6 ± 12.4	1173.4 ± 27.2	1378.0 ± 36.7	14.1 ± 1.6	349.0 ± 18.4	371.0 ± 26.7
Motil										
≥ 70%	24.7 ± 0.7	398.9 ± 19.3	105.1 ± 6.8	102.3 ± 8.5	102.3 ± 7.3	1170.4 ± 27.9	1380.4 ± 29.6	14.4 ± 1.9	344.0 ± 17.3	366.1 ± 23.3
< 70%	24.6 ± 0.7	400.4 ± 36.8	101.8 ± 7.8	108.8 ± 14.9	107.1 ± 13.4	1170.2 ± 31.4	1371.5 ± 40.6	13.7 ± 1.3	350.9 ± 20.6	375.0 ± 28.8
OBC										
OBC 1	24.7 ± 0.6	394.0 ± 8.9	101.0 ± 8.0^{cd}	104.3 ± 10.8	106.0 ± 7.1	1163.9 ± 38.3	1364.4 ± 29.2^{cd}	14.4 ± 2.4	339.7 ± 13.5	365.7 ± 17.2
OBC 2	24.7 ± 0.7	402.0 ± 32.7	106.8 ± 5.5^{c}	105.7 ± 11.5	103.9 ± 10.5	1176.9 ± 24.9	1388.9 ± 27.1^{c}	14.0 ± 4.6	349.0 ± 19.7	317.9 ± 27.1
OBC 3	24.6 ± 0.1	369.8 ± 21.1	96.8 ± 6.2^{d}	105.8 ± 16.3	105.6 ± 41.5	1154.5 ± 29.8	1346.5 ± 42.0^{d}	14.1 ± 1.4	347.1 ± 20.7	367.5 ± 30.1

BW	Body weights at time of test
205 GI	205 day growth index
ADG	Average daily gain
KR	Kleiber ratio
SH	Shoulder height
BL	Body length
ST	Skin thickness
SC Ts	Scrotal circumference at the end of the performance test
SC Te	Scrotal circumference at end of trial
SC	Scrotal circumference categories
Morph	Spermatozoal morphology categories
Motil	Spermatozoal motility categories
OBC	Overall breeding soundness categories
OBC 1	Satisfactory potential breeders with exceptional fertility
OBC 2	Satisfactory potential breeders with acceptable fertility
OBC 3	Unsatisfactory potential breeder
^{a,b}	Column means with the same superscript do not differ significantly (P < 0.001)
^{c,d}	Column means with the same superscript do not differ significantly (P < 0.05)
^{e,f}	Column means with the same superscript do not differ significantly (P < 0.10)

4.1.1.3. Breeding potential category based on spermatozoal motility

One-way ANOVA revealed that the production measurements of the bulls did not differ between the spermatozoal motility categories (Table 10). The correlations between spermatozoal motility and body length, and motility and the 205 day growth index were 0.36 (P<0.05) and 0.35 (P<0.05) respectively (Table 11). Higher spermatozoal motility was associated with higher values for body length and 205 day growth index.

Table 11 Correlation coefficients between spermatozoal characteristics and growth measurements for 25 month old Bonsmara bulls

	205 GI	BL	% PM	% AM	% I	% MN	Total AFD	Total D
205 GI	1	0.47**	0.36**	-0.38**	-0.31*	0.33*	-0.36**	-0.33*
BL		1	0.35**	-0.32**	-0.41**	0.06	-0.15	-0.09

205 GI	205 day growth index
BL	Body length
% PM	Percentage progressive movement
% AM	Percentage aberrant movement
% I	Percentage immotile
% MN	Percentage morphologically normal sperm
Total ND	Total nuclear defects
Total AFD	Total acrosome and flagellar defects
Total D	Total defects (normal-, nuclear-, acrosomal- and flagellar defects)
***	P < 0.001
**	P < 0.05
*	P < 0.10

4.1.1.4. Breeding potential category based on overall breeding soundness

Of the bulls examined, 17 % were classified as exceptional, and 63 % were acceptable breeders. The eight (20%) remaining bulls were classified as unsatisfactory potential breeders based on breeding soundness exanimation tests. An one-way ANOVA was used to study the effects of the production test measurements on overall breeding soundness category. Summary statistics are presented in Table 10. The results indicated that overall breeding soundness categories did not have a significant effect on the body weight, ADG and Kleiber ratio. The 205 day growth index and body length of bulls were influenced by the overall breeding soundness categories (P<0.05). Bulls in the exceptional fertility category had an average 205 day growth index of 101.0 ±8.0, bulls in the acceptable fertility category had an average 205 day growth index of 106.8 ±5.5, and bulls in the unsatisfactory category had a 205 day growth index of 96.8 ± 6.2. The 205 day growth index of the bulls in the acceptable fertility category was higher (P<0.05) than that of the unsatisfactory category. The correlation between the 205 day growth index and the fertility categories based on overall breeding soundness was r = 0.24 (P>0.1).

The correlation between body length and fertility category based on overall breeding soundness was r = 0.18 (P>0.1). The average body length of the bulls in exceptional fertility category was 1364.4 ± 29.2 mm, the bulls in the acceptable fertility category had a body length of 1388.9 ± 27.1 mm, and body length of the bulls in the unsatisfactory category was 1346.5 ± 42.0 mm. The bulls in the acceptable fertility category had longer body lengths (P<0.05) than the subfertile bulls.

The SC measurement after the performance test was numerically greater (P>0.1) for the acceptable and unsatisfactory fertility categories than for the exceptional fertility category. The measurements at the end of the trial were numerically greater for the exceptional fertility and unsatisfactory categories and smaller for the acceptable fertility category (P>0.1). These differences were not statistically significant but the numerical differences may be of particular importance in terms of semen production and will be discussed later in this thesis.

4.1.2. Blood hormone concentrations

Serum hormone concentrations were highly variable between the bulls, as can be seen in Table 12. Cortisol concentration before and after GnRH treatment (T_0 and T_{150}) ranged from 0 to 89.3 nmol/L and 0 to 97.2 nmol/L respectively. Cortisol concentration after sexual activity ranged form 0.3 to 78.8 nmol/L. Serum testosterone concentrations at T_0 and T_{150} were highly variable between the bulls, ranging from 2.2 to 49.8 nmol/L and 4.6 to 56.7 nmol/L respectively. Testosterone concentration after sexual stimulation ranged form 7.1 to 57.5 nmol/L.

Table 12 Summary statistics of cortisol- and testosterone concentrations (nmol/L) for 25 month old Bonsmara bulls before and after GnRH treatment, and after sexual stimulation

Variable	N	Mean ± SD	Minimum	Maximum
Cort T_0	41	33.3 ± 21.8	0	89.3
Cort T_{150}	41	36.6 ± 22.9	0	97.2
Cort T_f	41	26.5 ± 17.2	0.3	78.8
Test T_0	41	17.4 ± 12.2	2.3	49.8
Test T_{150}	41	41.5 ± 9.5	4.6	56.7
Test T_f	41	35.3 ± 12.7	7.1	57.5

Cort T_0	Cortisol concentration at starting time
Cort T_{150}	Cortisol concentration 150 minutes after GnRH treatment
Cort T_f	Cortisol concentration after sexual stimulation during the libido test
Test T_0	Testosterone concentration at starting time
Test T_{150}	Testosterone concentration 150 minutes after GnRH treatment
Test T_f	Testosterone concentration after sexual stimulation during the libido test

4.1.2.1. Breeding potential category based on scrotal circumference

An one-way ANOVA (Table 13) revealed that there were no significant differences in hormone concentrations between the SC categories.

Table 13 Summary statistics of the effect of cortisol and testosterone concentrations (nmol/L) for 25 month old Bonsmara bulls on the different scrotal circumference categories

SC mm	Cort T_0 ± SD	Cort T_{150} ± SD	Cort T_f ± SD	Test T_0 ± SD	Test T_{150} ± SD	Test T_f ± SD
≥ 340	34.1 ± 22.7	34.7 ± 22.6	26.6 ± 17.9	17.8 ± 12.3	41.3 ± 9.7	36.1 ± 12.5
< 340	27.7 ± 14.8	50.2 ± 21.8	25.3 ± 13.2	14.7 ± 12.4	43.4 ± 9.0	29.5 ± 14.6

Cort T_0	Cortisol concentration before GnRH treatment
Cort T_{150}	Cortisol concentration 150 minutes after GnRH treatment
Cort T_f	Cortisol concentration after sexual stimulation during the libido test
Test T_0	Testosterone concentration before GnRH treatment
Test T_{150}	Testosterone concentration 150 minutes after GnRH treatment
Test T_f	Testosterone concentration after sexual stimulation during the libido test

A repeated measures ANOVA was used to study the effect of the different sampling times and different fertility groups on hormone concentrations. Summary statistics are presented in Table 14 and Table 15. The results indicate that sampling time had a statistically significant effect on cortisol and testosterone concentrations for different semen morphology, motility and overall breeding soundness categories.

Table 14 Effect of sampling times and fertility category on plasma cortisol and testosterone concentrations (nmol/L) (mean (SE)) (Pooled data)

	Hormone	Sampling time		
		T_0 (SE)	T_{150} (SE)	T_f (SE)
Semen morphology	Cortisol	37.3 (5.0)^{c}	36.9 (5.6)^{c}	26.0 (5.6)^{d}
	Testosterone	19.4 (2.8)^{a}	40.9 (2.3)^{b}	35.4 (3.1)^{b}
Semen motility	Cortisol	32.5 (4.0)^{cd}	36.4 (5.1)^{c}	27.7 (3.9)^{d}
	Testosterone	17.5 (2.7)^{a}	41.6 (2.1)^{bb}	35.2 (2.9)^{b}
OBC	Cortisol	37.4 (5.9)^{c}	36.6 (7.3)^{cd}	27.7 (4.4)^{d}
	Testosterone	19.7 (3.6)^{a}	40.3 (2.9)^{b}	34.2 (4.0)^{b}

T_0	Hormone concentration before GnRH treatment
T_{150}	Hormone concentration 150 minutes after GnRH treatment
T_f	Hormone concentration after sexual stimulation
OBC	Overall breeding soundness group
^{a,b}	Column means with the same superscript do not differ significantly (P < 0.001)
^{c,d}	Row means with the same superscript do not differ significantly (P < 0.05)

Table 15 Effect of fertility category classification on cortisol and testosterone concentration at different sampling times (mean ± SE of specific categories)

Hormone	Sampling time	Fertility category	Fertility category classification		
		Semen morphology	**≥ 70%**	**< 70%**	
Cortisol	T_0		45.9 (6.2)[c]	28.7 (3.8)[d]	
	T_{150}		37.3 (7.0)	36.4 (4.2)	
	T_f		24.9 (5.3)	27.0 (3.2)	
		Semen motility	**≥ 70%**	**< 70%**	
	T_0		39.2 (4.4)[c]	25.7 (5.0)[d]	
	T_{150}		38.6 (4.8)	34.1 (5.4)	
	T_f		25.8 (3.6)	27.3 (4.1)	
		OBC	**OBC 1**	**OBC 2**	**OBC 3**
	T_0		58.0 (7.2)[a]	29.2 (3.7)[b]	25.0 (6.7)[b]
	T_{150}		35.4 (8.9)	36.7 (4.6)	37.5 (8.3)
	T_f		25.7 (6.6)	24.9 (3.4)	32.1 (6.2)
Testosterone		Semen morphology	**≥ 70%**	**< 70%**	
	T_0		23.5 (3.5)[c]	15.2 (2.1)[d]	
	T_{150}		39.4 (2.9)	42.3 (1.7)	
	T_f		35.5 (3.9)	35.2 (2.4)	
		Semen motility	**≥ 70%**	**< 70%**	
	T_0		17.0 (2.6)	17.9 (2.9)	
	T_{150}		41.1 (2.0)	42.0 (2.3)	
	T_f		36.0 (2.7)	34.4 (3.0)	
		OBC	**OBC 1**	**OBC 2**	**OBC 3**
	T_0		25.9 (4.4)[c]	14.8 (2.3)[d]	18.5 (4.2)[cd]
	T_{150}		36.2 (3.6)[e]	43.0 (1.9)[f]	41.6 (3.3)[ef]
	T_f		32.8 (4.9)	36.6 (2.5)	33.2 (4.6)

T_0	Hormone concentration before GnRH treatment
T_{150}	Hormone concentration 150 minutes after GnRH treatment
T_f	Hormone concentration after sexual stimulation
OBC	Overall breeding soundness group
OBC 1	Satisfactory potential breeders with exceptional fertility
OBC 2	Satisfactory potential breeders with acceptable fertility
OBC 3	Unsatisfactory potential breeders
[a,b]	Column means with the same superscript do not differ significantly (P < 0.001)
[c,d]	Column means with the same superscript do not differ significantly (P < 0.05)
[e,f]	Column means with the same superscript do not differ significantly (P < 0.10)

4.1.2.2. Breeding potential category based on spermatozoal morphology

The effect of sampling time on hormone concentrations is illustrated in Table 14. In the semen morphology category there was a difference (P<0.05) in cortisol concentration between the samples taken before and after GnRH treatment (37.3 (5.0) and 36.9 (5.6) nmol/L), and the sample taken after sexual stimulation (26.0 (5.6) nmol/L). There was no statistically significant difference (P>0.1) between the cortisol concentrations of samples collected before and after GnRH treatment.

Testosterone concentrations increased significantly (P<0.001) after GnRH treatment (from 19.4 (2.8) to 40.9 (2.3) nmol/L). There was also a difference (P<0.05) between the testosterone concentration before GnRH treatment (19.4 (2.8) nmol/L) and after sexual stimulation (35.4 (3.1) nmol/L), but no significant difference (P>0.1) between the concentrations of testosterone before GnRH treatment and after sexual stimulation.

A Repeated measures ANOVA was performed to test if significant differences exist in circulating cortisol and testosterone concentrations at different sampling times between the spermatozoal morphology categories (Table 15). A difference in cortisol concentration before GnRH treatment was observed between the two semen morphology categories. The bulls with ≥ 70 % morphologically normal spermatozoa had a higher (P<0.05) average cortisol concentration (45.9 (6.2) nmol/L) than bulls with < 70 % morphologically normal spermatozoa (28.7 (3.8) nmol/L). The difference in cortisol concentration after GnRH treatment between the different semen morphology categories was not statistically significant. Bulls with more morphologically normal sperm showed a decrease in cortisol concentration after GnRH treatment (45.9 (6.2) to 37.3 (7.0) nmol/L), while bulls with < 70 % normal sperm showed an increase in cortisol concentration (28.7 (3.8) to 36.4 (4.2) nmol/L). The difference in cortisol concentrations after sexual stimulation between the different semen morphology categories was not statistically significant. The bulls with ≥ 70 % morphologically normal spermatozoa had an average cortisol concentration of 24.9 (5.3) nmol/L and the bulls with < 70 % morphologically normal spermatozoa had a higher average cortisol concentration of 27.0 (3.2) nmol/L, which was similar to the concentration before GnRH treatment.

The difference in the testosterone concentration before GnRH treatment was also statistically significant between the semen morphology categories. The bulls with ≥ 70 % morphologically normal spermatozoa had higher (P<0.05) average testosterone concentration (23.5 (3.5) nmol/L) than the bulls with < 70 % morphologically normal spermatozoa (15.2 (2.1) nmol/L).

The difference in testosterone concentration after GnRH treatment between the different semen morphology categories was not statistically significant. Bulls with less morphologically normal sperm however showed a larger increase in testosterone concentration after GnRH treatment. It can be seen in Table 16 that there was a negative correlation (r = -0.21; P>0.1) between testosterone concentration before GnRH treatment and the total amount of spermatozoal defects, while the correlation between testosterone concentration before GnRH treatment and the sperm morphology categories was positive, r = 0.31 (P<0.05).

4.1.2.3. Breeding potential category based on spermatozoal motility category

The effect of sapling time on the hormone concentrations for bulls in different spermatozoal motility categories is illustrated in Table 14. Based on semen motility category, the cortisol concentration after sexual stimulation (27.7 (3.9) nmol/L) was numerically lower (P>0.1) but similar to the cortisol concentration before GnRH treatment (32.5 (4.0) nmol/L). There was a small but consistent increase (P>0.1) in cortisol concentration after GnRH treatment from 32.5 (4.0) to 36.4 (5.1) nmol/L. A significant difference (P<0.05) in cortisol concentration was noted between the samples taken after GnRH treatment and those taken after sexual stimulation.

Testosterone concentration increased significantly (P<0.001) after GnRH treatment from 17.5 (2.7) nmol/L to 41.6 (2.1) nmol/L for the samples collected after sexual stimulation. The testosterone concentration was also significantly higher (P<0.001) after sexual stimulation (35.2 (2.9) nmol/L) than before GnRH treatment (17.5 (2.7) nmol/L).

A Repeated measures ANOVA was performed to test if significant differences exist in circulating cortisol and testosterone concentration at different sampling times between the spermatozoal motility categories (Table 15). Cortisol concentrations collected before GnRH treatment differed significantly (P<0.05) for bulls in different semen motility categories. The bulls with more than 70 % motile spermatozoa had an average cortisol concentration of 39.2 (4.4) nmol/L, while bulls with less than 70 % motile spermatozoa had a much lower (P<0.05) concentration of 25.7 (5.0) nmol/L. The difference in cortisol concentration after GnRH treatment between the different semen motility categories was not statistically significant. The bulls with more than 70 % motile spermatozoa showed a small decrease in cortisol concentration after GnRH treatment of 39.2 (4.4) to 38.6 (4.8) nmol/L, while bulls with less than 70 % motile spermatozoa showed an increase after GnRH treatment from 25.7 (5.0) to 34.1 (5.4) nmol/L. This numerical difference may be of physiological significance.

Testosterone concentration before and after GnRH treatment did not differ significantly between bulls in the two semen motility categories. In addition, testosterone concentrations after sexual stimulation did not differ between bulls in the semen motility categories. The correlation (Table 16, page 53) between testosterone concentration before GnRH treatment and percentage progressively motile sperm was low (r = 0.15; P>0.1).

4.1.2.4. Breeding potential category based on overall breeding soundness category

The effect of sampling time on hormone concentrations of bulls in different OBC categories is presented in Table 14. Cortisol concentration after sexual stimulation (27.7 (4.4) nmol/L) was significantly lower ($P<0.05$) than the concentration before GnRH treatment (37.4 (5.9) nmol/L), based on OBC categories.

Testosterone concentrations increased significantly ($P< 0.001$) after GnRH treatment (from 19.7 (3.6) to 40.3 (2.9) nmol/L). There was also a statistically significant difference ($P< 0.001$) between the testosterone concentration before GnRH treatment (19.7 (3.6) nmol/L) and after sexual stimulation (34.2 (4.0) nmol/L).

A Repeated measures ANOVA was performed to reveal if significant differences exists in circulating cortisol and testosterone concentrations at different sampling times between the overall breeding soundness categories (Table 15). Cortisol concentration before GnRH treatment differed significantly between the exceptional fertility category and the other two fertility categories ($P<0.001$) as can be seen in Figure 10. The bulls in exceptional fertility category had an average cortisol concentration before GnRH treatment of 58.0 (7.2) nmol/L while the bulls in the acceptable fertility and unsatisfactory categories had much lower concentration of cortisol ($P<0.001$) of 29.2 (3.7) and 25.0 (6.7) nmol/L respectively. The difference in cortisol concentration after GnRH treatment between the overall breeding soundness categories was not statistically significant. Bulls in the exceptional fertility category showed a large decrease in cortisol concentration after treatment (58.0 (7.2) to 35.4 (8.9) nmol/L) while the bulls in the acceptable and unsatisfactory categories showed an increase in cortisol concentration after treatment. The difference in cortisol concentration after sexual stimulation between the overall breeding soundness categories was not statistically significant. Bulls in the exceptional fertility and acceptable fertility categories had an average concentration of 25.7 (6.6) nmol/L and 24.9 (3.4) nmol/L respectively. The bulls in the unsatisfactory category had a higher cortisol concentration of 32.1 (6.2) nmol/L, and although this higher concentration was not statistically significant, it may be of physiological importance.

The testosterone concentrations before GnRH treatment differed significantly ($P<0.05$) between the exceptional fertility category (25.9 (4.4) nmol/L) and the acceptable fertility category (14.8 (2.3) nmol/L) as can be seen in Figure 11. The testosterone concentration after GnRH treatment tended to be higher ($P<0.1$) for the acceptable fertility categories (43.0 (1.9) nmol/L) than for the exceptional fertility category (36.2 (3.6) nmol/L). The bulls in the acceptable category showed a larger increase in testosterone concentration after treatment than the other two categories. The difference in testosterone concentration after sexual stimulation between the three overall breeding soundness categories was not statistically significant.

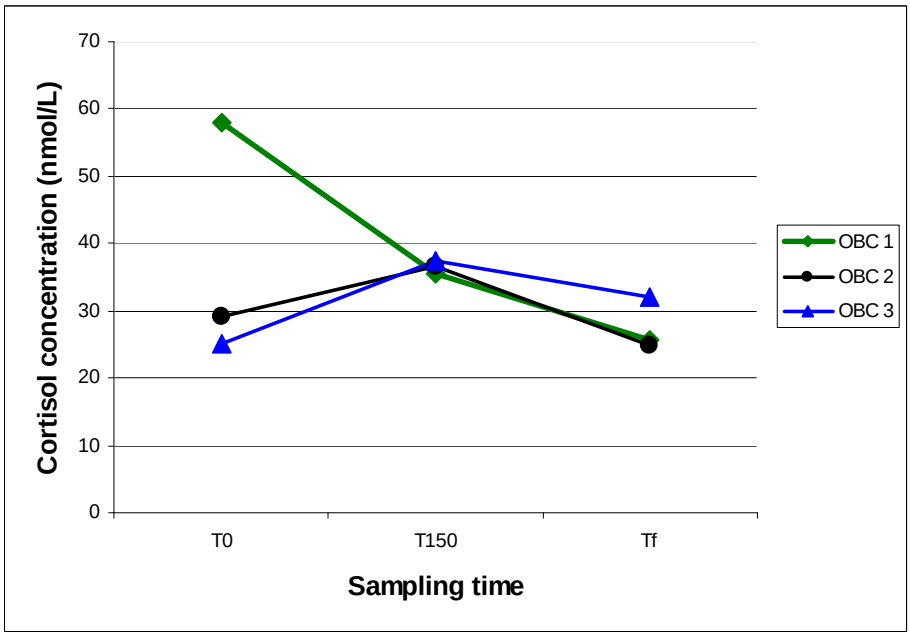

Figure 10 Cortisol concentration before- (T_0) and after- (T_{150}) GnRH treatment, and after sexual stimulation (T_f), for bulls in different Overall breeding soundness categories.

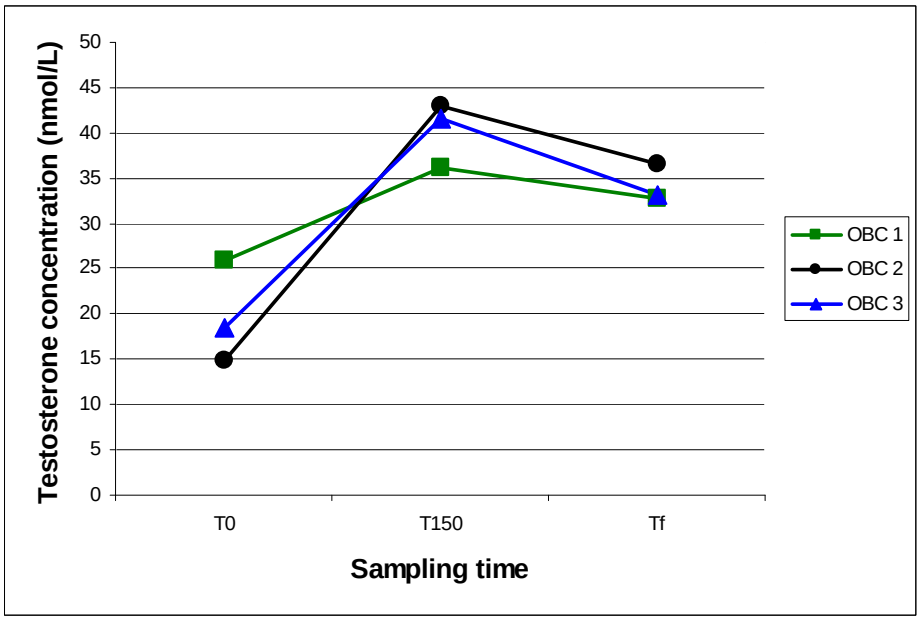

Figure 11 Testosterone concentration before- (T_0) and after- (T_{150}) GnRH treatment, and after sexual stimulation (T_f), for bulls in different Overall breeding soundness categories.

Table 16 Pearson correlation coefficients between testosterone concentrations before GnRH treatment and spermatozoal morphological defects and percentage progressive sperm movement for 25 month old Bonsmara bulls

	Test T_0
%PM	0.15
Total ND	-0.09
Total FD	-0.21
Total AFD	-0.16
Total D	-0.21
Test T_0	1

% PM	Percentage progressive movement
Total ND	Total nuclear defects
Total FD	Total flagellar defects
Total AFD	Total acrosome and flagellar defects
Total D	Total defects (normal-, nuclear-, acrosomal- and flagellar defects)
Test T_0	Testosterone concentration before GnRH treatment
***	$P < 0.001$
**	$P < 0.05$
*	$P < 0.10$

4.1.3. Libido

4.1.3.1. Libido scores

One-way ANOVA suggest that the libido score did not have a statistically significant effect on the fertility categories (Table 17). For the SC category the libido scores tended to be slightly higher (4.8 ± 1.6) for the bulls with a SC ≥ 340 mm in comparison to the bulls with a SC < 340 mm (4.2 ± 1.5). Bulls with more than 70 % morphologically normal spermatozoa had a higher overall libido score (5.3 ± 1.6) in comparison with hose with less than 70 % morphologically normal spermatozoa (4.5 ± 1.6). Libido scores were 5.1 ± 2.0 for the exceptional fertility category, 4.4 ± 4.5 for the acceptable fertility category and 5.3 ± 1.7 for the unsatisfactory category. There was no significant relationship between breeding soundness and libido for the Bonsmara bulls in this investigation.

Table 17 Summary statistics of the effect of libido score for 25 month old Bonsmara bulls on different fertility categories

Fertility category	Classification	Libido score ± SD
SCC mm	≥ 340	4.8 ± 1.6
	< 340	4.2 ± 1.5
SMPC	≥ 70%	5.3 ± 1.6
	< 70%	4.5 ± 1.6
SMTC	≥ 70%	4.7 ± 1.6
	< 70%	4.7 ± 1.7
OBC	OBC 1	5.1 ± 2.0
	OBC 2	4.4 ± 4.5
	OBC 3	5.3 ± 1.7

SCC	Scrotal circumference category
SMPC	Spermatozoal morphology category
SMTC	Spermatozoal motility category
OBC	Overall breeding soundness category
OBC 1	Satisfactory potential breeders with exceptional fertility
OBC 2	Satisfactory potential breeders with acceptable fertility
OBC 3	Unsatisfactory potential breeders

4.1.3.2. Relationship between libido and hormone concentrations

Although no statistically significant correlations were found between the libido scores and hormone concentrations, numerical relationships were observed some cases. Cortisol concentrations before and after GnRH treatment were generally higher in low libido bulls (Figures 12 and 13).

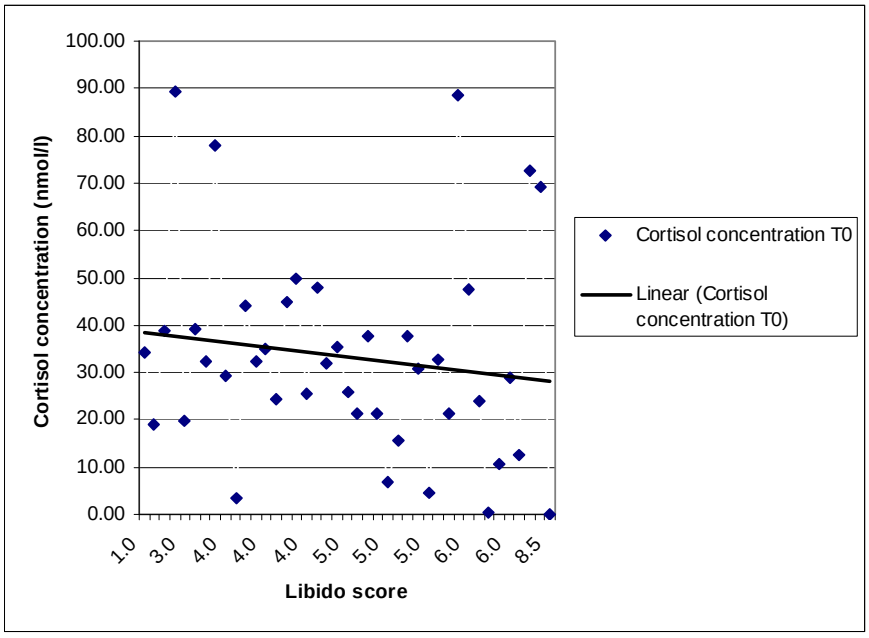

Figure 12 Relationship between libido scores and cortisol concentration before GnRH treatment for 25 month old Bonsmara bulls.

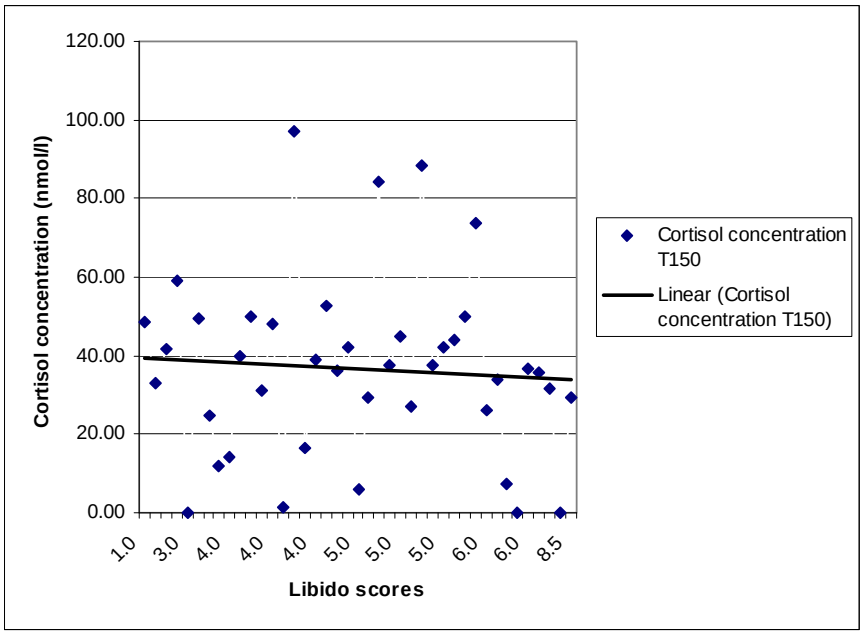

Figure 13 Relationship between libido scores and cortisol concentration after GnRH treatment for 25 month old Bonsmara bulls.

The cortisol concentration after sexual stimulation was higher in the bulls with higher libido scores (Figure 14).

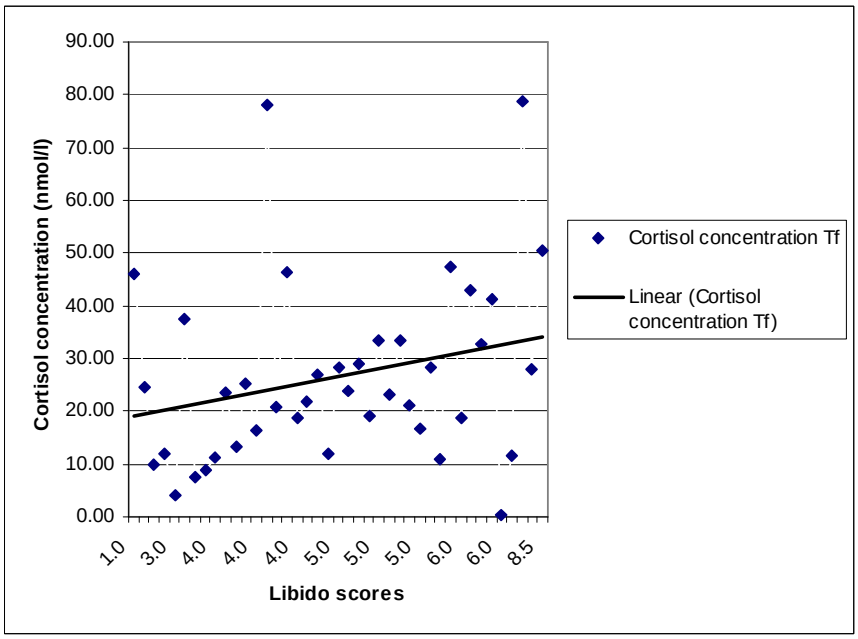

Figure 14 Relationship between libido scores and cortisol concentration after sexual stimulation for 25 month old Bonsmara bulls.

It was observed that bulls with the highest libido score also had the highest testosterone concentration before GnRH treatment (Figure 15). There was no significant relationship between libido score and testosterone concentration after GnRH treatment (Figure 16). Testosterone concentrations directly after sexual stimulation had reached higher levels in bulls with a high libido compared to bulls with lower libido (Figure 17).

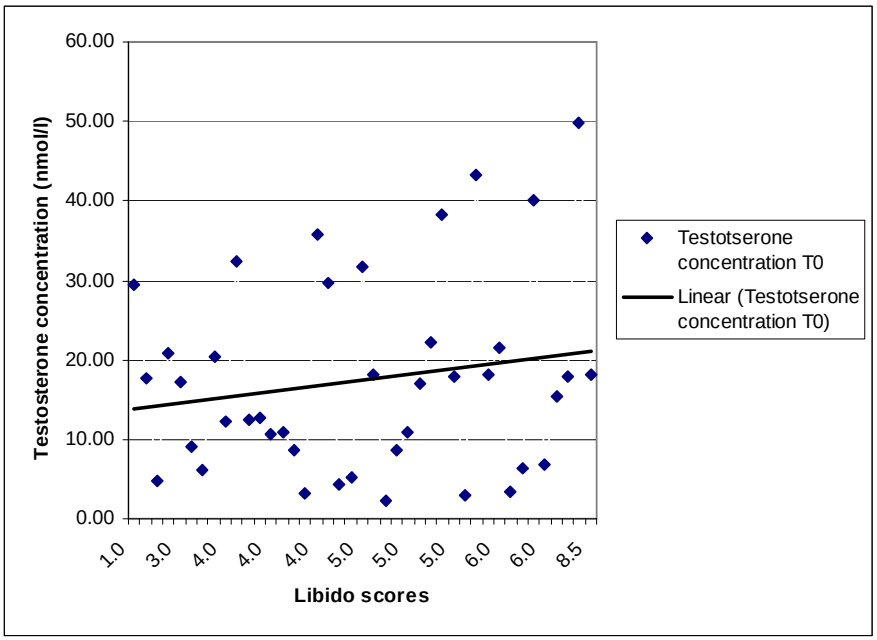

Figure 15 Relationship between libido scores and testosterone concentration before GnRH treatment for 2 month old Bonsmara bulls.

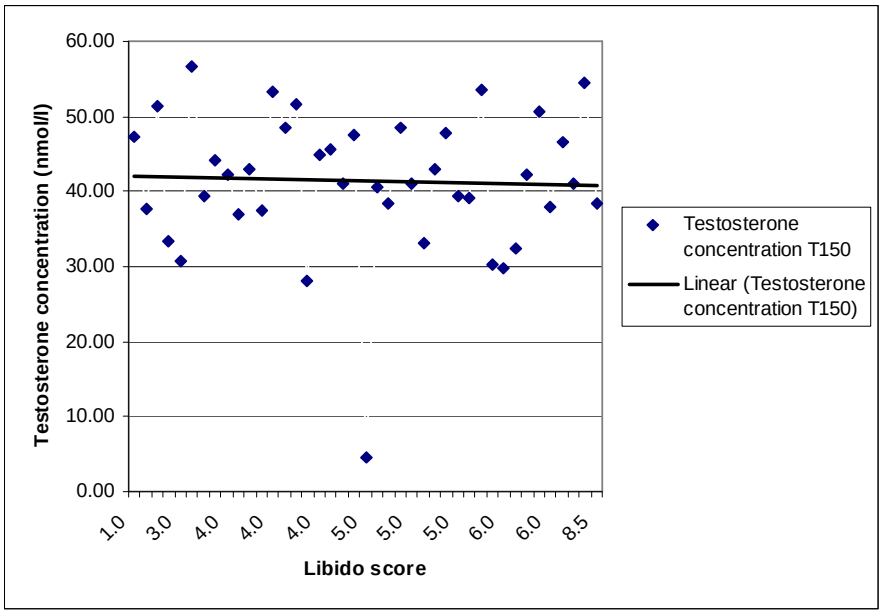

Figure 16 Relationship between libido scores and testosterone concentrations after GnRH treatment for 25 month old Bonsmara bulls.

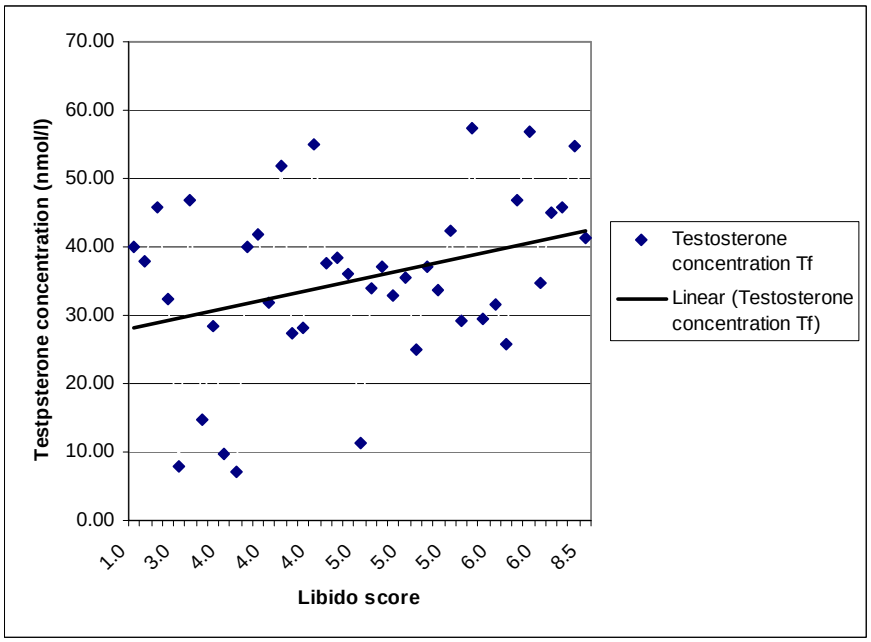

Figure 17 Relationship between libido scores and testosterone concentration after sexual stimulation for 25-month old Bonsmara bulls.

4.1.4. Spermatozoal characteristics

The results in terms of sperm morphology and motility are presented in Table 18, while Table 19 gives the summary statistics of spermatozoal defects. The large ranges and standard deviations indicate the wide variation among bulls with regard to the respective measurements.

Table 18 Summary statistics of the results in terms of spermatozoal motility for 25 month old Bonsmara bulls

Measurement	N	Mean ± SD	Minimum	Maximum
% PM	40	66.0 ± 20.6	20.0	90.0
% AM	40	16.4 ± 11.2	5.0	40.0
% I	40	17.0 ± 10.4	1.0	40.0
% MN	41	62.9 ± 14.2	20.0	85.0

PM Progressively motile sperm
AM Aberrant movement
I Immotile sperm
MN Morphologically normal sperm
N Number of bulls studied

Table 19 Summary statistics of the amount of normal, major and minor spermatozoal defects per 200 sperm cells for 25 month old Bonsmara bulls

Variable	N	Mean ± SD	Minimum	Maximum
ND: Teratoid	41	1.2 ± 1.1	0.0	4.0
ND: Macrocephalic	41	0.2 ± 0.4	0.0	1.0
ND: Pyriform	41	1.7 ± 2.2	0.0	10.0
ND: Diadem	41	7.2 ± 3.9	1.0	20.0
ND: Abnormal base	41	0.2 ± 0.4	0.0	2.0
ND: Abnormal loose heads	41	0.4 ± 0.7	0.0	3.0
ND: Double heads	41	0.1 ± 0.3	0.0	1.0
ND: Rolled/ crested heads	41	0.7 ± 0.9	0.0	4.0
ND: Tapered heads	41	0.4 ± 0.8	0.0	2.0
ND: Narrow base	41	0.1 ± 0.5	0.0	3.0
ND: Other abnormal head shape	41	0.1 ± 0.4	0.0	2.0
Total ND	41	12.0 ± 4.7	5.0	25.0
% ND	41	12.1 ± 4.7	5.0	25.0
AD: Total	41	6.8 ± 6.3	0.0	38.0
AD: Knobbed acrosome	41	1.0 ± 0.8	0.0	3.0
AD: Degenerate acrosome	41	5.9 ± 6.1	0.0	36.0
FD: Total	41	13.3 ± 8.1	0.0	42.0
FD: Stump tail	41	0.9 ± 1.0	0.0	3.0
FD: Double tail	41	0.2 ± 1.4	0.0	9.0
FD: Proximal droplets	41	3.1 ± 3.0	0.0	11.0
FD: Pseudo droplets	41	0.9 ± 1.7	0.0	9.0
FD: Aplasia of mitochondria	41	0.8 ± 1.7	0.0	9.0
FD: Corckscrew	41	0.2 ± 1.4	0.0	9.0
FD: Dag	41	2.0 ± 3.4	0.0	20.0
FD: Midpiece reflex	41	4.2 ± 3.1	0.0	14.0
FD: Other midpiece defects	41	0.2 ± 1.4	0.0	9.0
FD: Bent midpiece	41	0.1 ± 0.5	0.0	3.0
FD: Fractured flagellum	41	0.1 ± 0.3	0.0	1.0
FD: Distal droplet	41	1.7 ± 3.3	0.0	17.0
FD: Bent principle piece	41	0.2 ± 0.5	0.0	2.0
FD: End piece defects	41	0.2 ± 1.4	0.0	9.0
FD: Coiled principle piece	41	0.5 ± 0.6	0.0	2.0
Total AFD	41	23.4 ± 11.8	4.0	67.0
% AFD	41	23.4 ± 11.8	4.0	67.0
Normally shaped loose heads	41	3.5 ± 4.5	0.0	22.0
Total D	41	38.1 ± 16.1	15.0	102.0
% morphologically normal	41	62.5 ± 14.2	20.0	85.0

ND Nuclear defects
AD Acrosome defects
FD: Flagellar defects
AFD Acrosome and flagellar defects
N Number of bulls tested

4.1.4.1. Breeding potential category based on scrotal circumference

One-way ANOVA revealed that the spermatozoal characteristics had no significant effect on the SC categories (Table 20).

Table 20 Summary statistics of effect of the fertility categories on spermatozoal characteristics for 25 month old Bonsmara bulls

Fertility category	%PM ± SD	%AM ± SD	%I ± SD	%MN ± SD	Total ND ± SD	Total FD ± SD	Total AF ± SD	Total D ± SD
SC								
≥ 340	67.3 ± 19.2	15.6 ± 10.5	16.5 ± 9.7	61.7 ± 14.6	12.4 ± 4.5	13.7 ± 8.3	23.6 ± 12.2	38.8 ± 16.7
< 340	57.0 ± 30.0	22.0 ± 15.3	21.0 ± 15.2	67.6 ± 10.8	10.4 ± 6.0	12.0 ± 6.4	22.2 ± 8.8	32.4 ± 10.8
Morph								
≥ 70%	77.5 ± 11.6^{c}	10.5 ± 6.4^{e}	12.0 ± 5.4^{e}	76.5 ± 6.0^{a}	9.6 ± 2.2^{c}	7.1 ± 5.0^{a}	13.0 ± 5.4^{a}	23.6 ± 6.0^{a}
< 70%	62.2 ± 21.6^{d}	18.4 ± 12.0^{f}	18.7 ±11.2^{f}	57.4 ± 12.8^{b}	13.0 ± 5.0^{d}	15.6 ± 7.8^{b}	27.3 ± 11.2^{b}	43.4 ± 15.4^{b}
Motil								
≥ 70%	79.8 ± 7.6^{a}	9.6 ± 5.0^{a}	10.3 ± 4.3^{a}	66.4 ± 9.8	11.3 ± 4.3	11.9 ± 6.1	20.5 ± 7.8	33.6 ± 9.8
< 70%	47.4 ± 17.7^{b}	25.7 ± 10.6^{b}	26.2 ± 9.1^{b}	57.5 ± 17.3	13.2 ± 5.0	15.1 ± 9.9	27.2 ± 14.9	43.7 ± 20.6
OBC								
OBC 1	81.1 ± 8.5^{c}	8.6 ± 4.8^{c}	10.0 ± 4.1^{c}	75.6 ± 6.2^{c}	9.4 ± 2.2	8.3 ± 5.4	13.9 ± 6.3^{c}	24.4 ± 6.2^{c}
OBC 2	71.9 ± 11.4^{c}	13.4 ± 7.3^{c}	14.3 ± 6.8^{c}	61.9 ± 11.7^{d}	13.3 ± 5.3	13.1 ± 6.6	22.9 ± 8.0^{d}	38.1 ± 11.7^{d}
OBC 3	28.6 ± 9.0^{d}	35.7 ± 4.5^{d}	34.3 ± 7.3^{d}	52.9 ± 18.7^{d}	10.8 ± 2.4	18.4 ± 11.8	33.8 ± 17.8^{d}	49.9 ± 24.8^{d}

% PM	Percentage progressive movement
% AM	Percentage aberrant movement
% I	Percentage immotile
% MN	Percentage morphologically normal sperm
Total ND	Total nuclear defects
Total FD	Total flagellar defects
Total AFD	Total acrosome and flagellar defects
Total D	Total defects (normal-, nuclear-, acrosomal- and flagellar defects)
SC	Scrotal circumference categories
Morph	Spermatozoal morphology categories
Motil	Spermatozoal motility categories
OBC	Overall breeding soundness categories
OBC 1	Satisfactory potential breeders with exceptional fertility
OBC 2	Satisfactory potential breeders with acceptable fertility
OBC 3	Unsatisfactory potential breeders
^{a,b}	Column means with the same superscript do not differ significantly (P < 0.001)
^{c,d}	Column means with the same superscript do not differ significantly (P < 0.05)
^{e,f}	Column means with the same superscript do not differ significantly (P < 0.10)

4.1.4.2. Breeding potential category based on spermatozoal morphology

Seventy two point seven percent of the bulls with more than 70% morphologically normal spermatozoa had an ivory semen colour and 9.1% had white semen colour. There was, however, an equal distribution of bulls with less than 70 % morphologically normal spermatozoa with a white (33.3%), grey (33.3%) and ivory (33.3%) semen colour (P<0.1). The relationship between semen morphology category and semen marbling was not statistically significant (Figure 18).

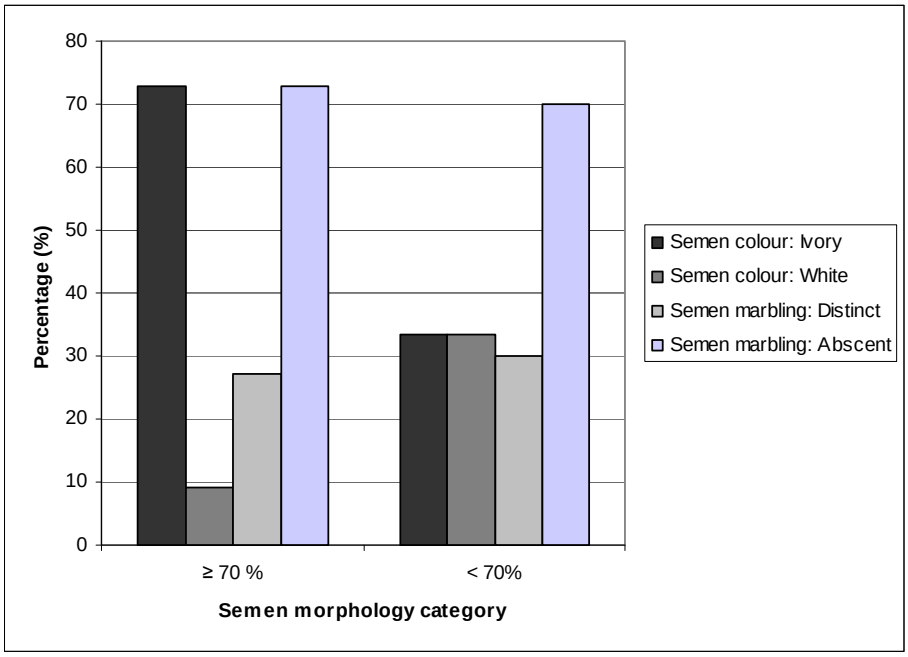

Figure 18 Macroscopic semen characteristics for the two spermatozoal morphology categories.

Table 20 gives the summary statistics of spermatozoal characteristics of bulls in the different spermatozoal morphology categories. The partial correlation coefficients between spermatozoal morphology categories and separate spermatozoal characteristics are given in Table 21.

Spermatozoal morphology categories significantly influenced semen characteristics of experimental bulls. The correlation between progressive motility and fertility category based on sperm morphology was 0.33 (P<0.05). The bulls with ≥ 70% morphologically normal spermatozoa had an average percentage of 77.5 ± 11.6 % progressively moving spermatozoa while the bulls with < 70% morphologically normal spermatozoa had a lower percentage (P<0.05) of 62.2 ± 21.6%. Bulls with ≥ 70% morphologically normal spermatozoa had an average percentage aberrant movement of 10.5 ±6.4 %, and the bulls with < 70% morphologically normal spermatozoa tended to have greater sperm movement (18.4 ± 12.0 %; P<0.1). There tended to be a difference ((P<0.1) in terms of percentage immotile spermatozoa between the two spermatozoal morphology categories.

Bulls with more than 70% morphologically normal spermatozoa had an average percentage of 12.0 ± 5.4 % and the bulls with less than 70% morphologically normal spermatozoa had an average of 18.7 ± 11.2 % of immotile sperm.

Table 21 Correlation coefficients between the spermatozoal morphology category and individual spermatozoal characteristics for 25 month old Bonsmara bulls

	% PM	% MN	Total ND	Total FD	Total AFD	Total D
Spermatozoal morph	0.33**	0.60***	-0.34**	-0.47**	-0.54***	-0.55***

% PM	Percentage progressive movement
% MN	Percentage morphologically normal sperm
Total ND	Total nuclear defects
Total FD	Total flagellar defects
Total AFD	Total acrosome and flagellar defects
Total D	Total defects (normal-, nuclear-, acrosomal- and flagellar defects)
***	P < 0.001
**	P < 0.05
*	P < 0.10

The correlation between the two spermatozoal parameters, percentage morphologically normal sperm and percentage progressively moving sperm, was 0.50 (P<0.001), while the correlations between percentage morphologically normal sperm and aberrant and immotile sperm was 0.48 (P<0.05) for both variables. Thus, as the percentage morphologically normal sperm decrease the sperm motility also decreased.

The correlation between percentage morphologically normal sperm and the fertility categories based on sperm morphology was 0.6 (P<0.001). The difference in fertility classification was highly significant (P<0.001) for percentage morphologically normal sperm as was expected. Bulls in the high fertility classification had an average percentage of 76.5 (± 6.0) % morphologically normal sperm while bulls in the subfertile classification had a significantly lower average (P<0.001) of 57.4 (± 12.8) %. There was a negative correlation between fertility category based on sperm morphology and the total amount of defects (Table 21) (r =-0.55; P<0.001.) while the negative correlation between the two spermatozoal characteristics, percentage progressively moving sperm and total spermatozoal defects, was r = -0.52 (P<0.001). Bulls with ≥ 70% morphologically normal spermatozoa had and average of 23.6 (± 6.0) % total spermatozoal defects while bulls with < 70% morphologically normal spermatozoa had a significantly higher (P<0.001) average of 43.4 ± 15.4 % spermatozoal defects.

Most of the total defects were acrosomal and flagellar defects, rather than nuclear defects. The amount of total defects was highly correlated (P>0.001) with the flagellar and acrosomal defects (r = 0.72 and r = 0.93 respectively) and lowly correlated to the total amount of nuclear defects (r = 0.32; P<0.05). The nuclear defects did not correlate significantly with the total acrosome and flagellar defects (Table 22).

Table 22 Pearson correlation coefficients between spermatozoal morphological defects and percentage progressive sperm movement for 25 month old Bonsmara bulls

	%PM	Total ND	Total FD	Total AFD	Total D
%PM	1	-0.06	-0.35*	-0.53***	-0.52***
Total ND		1	0.02	0.03	0.32**
Total FD			1	0.78***	0.72***
Total AFD				1	0.93***
Total D					1

% PM	Percentage progressive movement
Total ND	Total nuclear defects
Total FD	Total flagellar defects
Total AFD	Total acrosome and flagellar defects
Total D	Total defects (normal-, nuclear-, acrosomal- and flagellar defects)
***	P < 0.001
**	P < 0.05
*	P < 0.10

4.1.4.3. Breeding potential category based on spermatozoal motility

Thirteen of the bulls (56.5%) with more than 70% motile spermatozoa had an ivory semen colour while 26.1% had a white semen colour. Of the bulls having less than 70% motile spermatozoa, 27.8% had an ivory colour and 27.8% had a white semen colour. This difference in semen colour between the two spermatozoal motility categories (Figure 19) was not statistically significant. The difference in semen marbling between the two categories was not statistically significant. The majority of the semen samples showed no semen marbling.

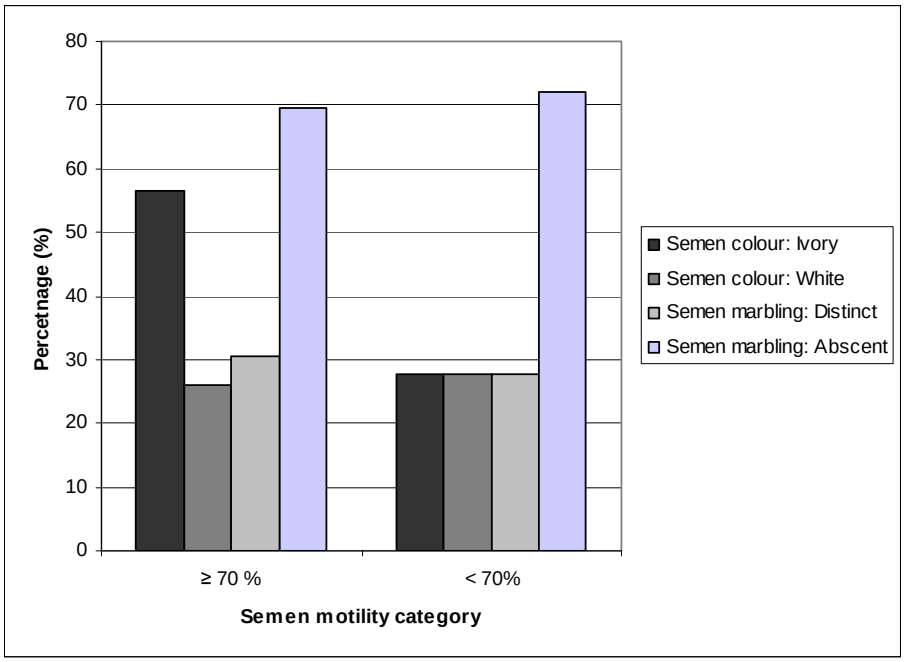

Figure 19 Macroscopic semen characteristics for the two spermatozoal motility categories.

The summary statistics of the spermatozoal characteristics of bulls in different spermatozoal motility categories are given in Table 20. Results suggests that spermatozoal motility categories did not influence spermatozoal characteristics

As was expected, the correlation ($P<0.001$) between the fertility category based on semen motility and percentage progressively moving sperm was high ($r = 0.79$). Another logical and significant difference ($P<0.001$) was observed for percentage aberrant sperm movement between the two categories. A negative correlation ($P<0.001$) was recorded between semen motility category and percentage aberrant sperm movement ($r = -0.72$) while the correlation (Table 23) between the two spermatozoal characteristics, progressive movement and the percentage aberrant sperm movement, was -0.96 ($P<0.001$). There was a negative correlation ($r = -0.77$, $P<0.001$) between semen motility category and percentage immotile sperm, and the correlation between the two spermatozoal characteristics, progressive movement and percentage of immotile sperm, was -0.94 ($P<0.001$). The partial correlation coefficient between different semen motility characteristics can be seen in Table 23. The correlation between aberrant sperm movement and immotile sperm was very high, 0.83 ($P<0.001$).

Table 23 Correlation coefficients between different semen motility characteristics for 25 month old Bonsmara bulls

	% PM	% AM	% I
% PM	1	-0.96***	-0.94***
% AM		1	0.83***
% I			1

% PM	Percentage progressive movement
% AM	Percentage aberrant movement
% I	Percentage immotile
***	P < 0.001
**	P < 0.05
*	P < 0.10

4.1.4.4. Breeding potential category based on overall breeding soundness

Seventy one point four (71.4) % of the bulls with exceptional fertility had an ivory colored semen while 14.3% had a white semen colour. Of the bulls in the acceptable fertility category, 42.3%, 26.9% and 30.8% had an ivory, grey and white semen colour respectively. In the unsatisfactory classification, 25% of the bulls had ivory semen colour, 50% had grey and 25% had a white semen colour. The difference in semen colour was not statistically significant (Figure 20). The relationship between the fertility category and semen marbling was not statistically significant. The majority of the semen samples in all fertility categories showed no marbling.

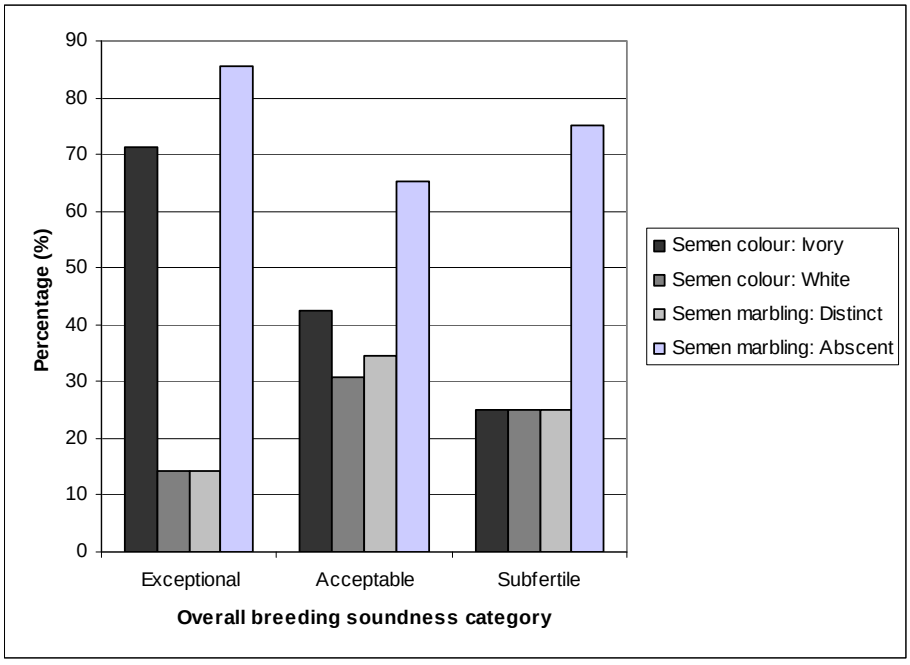

Figure 20 Macroscopic semen characteristics for the overall breeding soundness categories considered in this study.

Table 20 gives the summary statistics of spermatozoal characteristics of bulls in different overall breeding soundness categories. Partial correlation coefficients between the overall breeding soundness categories and spermatozoal characteristics are given in Table 24.

Table 24 Correlation coefficients between the overall breeding soundness categories and spermatozoal characteristics for 25 month old Bonsmara bulls

	% PM	% AM	% I	% MN	Total AFD	Total D
OBC	0.77***	-0.73***	-0.70***	0.45**	-0.51***	-0.48**

% PM	Percentage progressive movement
% AM	Percentage aberrant movement
% I	Percentage immotile
% MN	Percentage morphologically normal sperm
Total AFD	Total acrosome and flagellar defects
Total D	Total defects (normal-, nuclear-, acrosomal- and flagellar defects)
***	P < 0.001
**	P < 0.05
*	P < 0.10
	P > 0.1

Overall breeding soundness category influenced the progressive sperm movement measurements significantly (P<0.001). Bulls in the exceptional fertility category had 81.1 ± 8.5 % progressively moving sperm and the bulls in the acceptable category had 71.9 ± 11.4 % (P>0.1). The bulls in the unsatisfactory category had a much lower percentage morphologically normal spermatozoa (28.6 ± 9.0; P<0.05) than the other two categories. The correlation between progressive sperm movement and fertility category based on overall breeding soundness was high (0.77; P<0.001).

There was a significant difference between the overall breeding soundness categories in terms of the percentage aberrant sperm movement (P<0.001). The correlation between the overall breeding soundness category and percentage aberrant sperm movement was -0.73 (P<0.001). Bulls in the exceptional fertility category had 8.6 ± 4.8 % aberrant movement, bulls in the acceptable category had 13.4 ± 7.3 % and those in unsatisfactory category had a much higher percentage (P<0.05) of aberrant sperm movement of 35.7 ± 4.5. Another significant difference (P<0.001) was that between the three categories for percentage immotile sperm. The bulls in the unsatisfactory category tended to have a higher (P<0.05) percentage (34.3 ± 7.3) immotile spermatozoa than the other two categories. The correlation between fertility category based on overall breeding soundness and percentage immotile sperm was -0.70 (P<0.001).

The percentage morphologically normal spermatozoa in the ejaculate was significantly effected (P<0.001) by the overall breeding soundness classification. The correlation between fertility category based on overall

breeding soundness and the percentage morphologically normal sperm was r = 0.45 (P<0.05). Bulls in the exceptional fertility classification had a higher (P<0.05) percentage morphologically normal spermatozoa (75.6 ± 6.2) than those in the acceptable and unsatisfactory classifications (61.9 ± 11.7 and 52.9 ± 18.7 respectively). There were negative correlations between fertility category based on overall breeding soundness and the total amount of defects (r = -0.48) and acrosomal and flagellar defects (r = -0.51). The overall breeding soundness classification had an effect (P<0.05) on the total, acrosomal and flagellar defects. Bulls in the exceptional fertility classification had a lower percentage (P<0.05) total, acrosomal- and flagellar defects than bulls in the other classifications.

4.2. DISCUSSION

4.2.1 Growth

Reproduction rate is at least five times more important economically than growth performance and at least ten times more important than product quality for the average cow-calf producer (Trenkel & Willham, 1977). The overriding objective of a cow-calf production system should be to maximize the number of calves born or weaned for a given number of cows in a herd under prevailing environmental and managerial conditions (Rust & Groeneveldt, 2001; Burrow & Prayaga, 2004).

The average SC after the performance test was 347.1 ± 18.9 mm and SC at the end of the trial was 370.0 ± 25.9 (Table 6, page 32). Chenoweth *et al*. (1984) reported a similar SC of 371.0 mm for two year old Angus and Hereford bulls while Coulter and Kozub (1989) reported a SC of 355.0 mm for crossbred European breeds.

4.2.1.1. Breeding potential category based on scrotal circumference

Chenoweth *et al*. (1984) did a study to investigate the effect of age on reproductive parameters in bulls and found that the average SC of yearling bulls was lower (P<0.01) than that of older bulls. Therefore the SC of bulls must be adjusted for by age when animals of different ages are compared.

Data are lacking on the relationship between performance test results and reproductive parameters but according to Ologun *et al*. (1981) an unfavorable relationship exists. The usefulness of the SC measurement as an indicator of fertility was not significant based on the results of the present study. However, bulls with a smaller SC tended to have better performance test results (P>0.1).

4.2.1.2. Breeding potential category based on spermatozoal morphology

Knights *et al*. (1984) reported that semen traits are lowly correlated phenotypically with growth traits (r = -0.08 to r = 0.08). Very low genetic correlations were observed between the semen traits of Angus bulls and their SC, while the corresponding phenotypic correlations were moderate.

Weaning weight is an important production trait because it reflects the milk producing ability of the dam, and is a measure of the bull's potential genetics for early growth. The adjusted weaning weight, usually listed as 205 day weight, is the bull's actual weight at weaning corrected to a standard age of 205 days and adjusted to other known variables (eg. dam age). In the present study the correlation between the 205 day growth index and the percentage morphologically normal spermatozoa was positive (r = 0.33) and tended towards significance (P<0.1). Higher 205 day growth indices were associated with a small increase in the percentage morphologically normal sperm. These results differ from that reported by Makarechian & Farid (1985) of a negative correlation (r = -0.47; P<0.05) between fertility and pre-weaning average daily gain.

There was also a positive correlation (r = 0.47; P<0.05) between body length and the 205 day growth index, indicating that bulls with a longer body length had more morphologically normal sperm.

The SC measurements at both the beginning and end of the trial were numerically greater for the bulls with < 70% morphologically normal sperm, but these differences were not statistically significant. These results are in agreement with results reported by Smith *et al.* (1981). They found that there was no statistically significant relationship between SC and the percentage of normal spermatozoa but numerically, their data show that bulls with a higher SC tended to have a lower percentage of normal sperm cells.

The finding that bulls with greater SC had less morphologically normal sperm, disagree with the findings of Moser *et al*. (1996). Moser *et al*. (1996) found that bulls with a large SC tended to have more morphologically normal spermatozoa (P<0.1) than bulls with smaller circumferences. During that study the total abnormalities tended to be lower for bulls with smaller testicular circumferences (P<0.1).

The relationship between scrotal infrared temperature patterns and the natural mating fertility in yearling beef bulls was studied by Lunstra & Coulter (1997). The testes in the scrotum must be maintained at a temperature of 2 to 5 ºC below body temperature to maintain normal spermatogenesis. They reported a negative relationship between testis size (SC r= -0.29, testis volume r = -0.34; P<0.1) and pregnancy rates. These results suggests that larger testes may be associated with lower fertility, even though it is well known that larger testes in bulls are associated with better semen quality (Lunstra *et al*., 1978). Their data indicated that bulls that had larger scrotal sizes (350 mm) exhibited higher scrotal temperatures when compared to bulls with smaller SC (340 mm), which may partially explain the less efficient testicular thermoregulation and lower semen quality.

4.2.1.3. Breeding potential category based on spermatozoal motility

The results from the present study suggest that relatively high growth rates before weaning may have a positive effect on fertility under normal extensive feeding conditions. The 205 day growth index and body length measurements of bulls were numerically greater in the fertility category with high spermatozoal motility. There were positive correlation between spermatozoal motility and the 205 day growth index ($r = 0.35$), and spermatozoal motility and body length ($r = 0.36$; $P<0.05$). Higher spermatozoal motility was associated with higher values for body length and 205 day growth index. By contrast, numeric differences in growth after weaning suggest that high growth rates after weaning may have a negative effect on fertility.

4.2.1.4. Breeding potential category based on overall breeding soundness

The overall breeding soundness categories was influenced ($P<0.05$) by the 205 day growth index and body length of bulls. The correlation between the 205 day growth index and the fertility categories based on overall breeding soundness was $r = 0.24$ ($P>0.1$). The subfertile bulls had a lower 205 day growth index ($P<0.05$) than the bulls with acceptable fertility. The correlation between body length and fertility category based on overall breeding soundness was $r = 0.18$ ($P>0.1$). Smith *et al.* (1981) disagree with these finding. They reported that neither body lengths, SC, nor any of the other growth traits showed any correlations with overall breeding soundness scores. Chenoweth *et al.* (1988) also found that bulls in different fertility categories did not differ ($P<0.05$) in terms of scrotal circumference measurements.

Bulls in the acceptable fertility category had the largest ($P>0.1$) SC measurement after the performance test and the smallest ($P>0.1$) SC at the end of the trial. The differences in SC between the categories were not statistically significant but the numerical differences may be of importance in terms of semen production. Moser *et al*. (1996) reported that bulls with larger SC obtained a better overall breeding soundness score than bulls with smaller SC ($P<0.1$).

4.2.2 Blood hormone concentrations

Physical, anatomical and behavioral characteristics of bulls are maintained by androgens in the blood plasma. Other male characteristics that are also androgen depended include sexual performance, the initiation and maintenance of spermatogenesis, normal functioning of the accessory sex glands and social interaction with other males (Swenson & Reece, 1993). It has also been shown that androgens play a role in the regulation of body growth (Sitarz *et al*., 1976). Testosterone is secreted in an episodic fashion that makes it difficult to assess normalcy of testosterone production and thus Leydig cell function. Testosterone production can be declared normal when the particular testosterone value obtaining is at least equal to values observed at the lowest point of

the episodic cycle of about 3.5 nmol/L of plasma. Testosterone value can be as high as 34.67 nmol/L of plasma (Swenson & Reece, 1993). Three hours after an increase in the LH concentration in the blood there is a pulsatile release of testosterone. These pulses occur three to four times daily at 6 to 8 hour intervals. Only after the testosterone concentration has decreased to basal levels will there be another increase in LH level, leading to the next testosterone release. Individual bulls show random sequential changes in plasma testosterone and there is no evidence of diurnal discrepancy (Nancy *et al*., 1977).

During the present study sampling time (before and after GnRH treatment and after sexual stimulation) had a statistically significant effect on cortisol and testosterone concentrations for the different fertility categories based on semen morphology, motility and overall breeding soundness.

Testosterone concentration increased significantly ($P<0.001$) after GnRH treatment. The observations of the present study agree with those of Gabor *et al.* (1998) who reported that plasma testosterone concentration increased substantially after GnRH treatment, even though the concentrations before GnRH treatment were more repeatable than those after GnRH treatment ($P<0.01$).

During the present study there was also a significant increase ($P<0.001$) in the testosterone concentrations of bulls after sexual stimulation when compared to the basal concentrations. Malak & Thibier (1985) reported that the testosterone concentration of dairy bulls does not show an elevation after ejaculation in the absence of a female animal. Those ejaculations were stimulated by unnatural mating stimuli, such as a teaser bull and artificial vagina. A study was done by Lunstra *et al*. (1989) to determine the effect of natural mating stimuli on serum testosterone concentrations. They also investigated the effect of nasogenital investigation (the Flehmen response) on hormonal responses in the bull. Bulls are provided with a variety of sexual stimulations from the estrus female, including auditory, visual and olfactory cues. Yearling beef bulls were bled four hours before and five hours after a 10 minute exposure to an estrus female. During exposure the number of Flehmen responses was recorded. The serum testosterone concentration of bulls that achieved a service did not change ($P>0.1$) relative to their pre exposure concentrations, while that of bulls that mounted without achieving a service increased ($P<0.1$). In bulls that showed no interest in cows the testosterone concentration tended to be lower ($P>0.1$) after exposure. The post exposure testosterone response was highly correlated ($r^2 = 0.4 - 0.66$; $P<0.1$ to 0.001) with the number of Flehmen responses achieved, regardless of the number of mounts and or services. Their data showed that repeated nasogenital investigation (Flehmen response) of cow by the bull stimulates an elevation in testosterone concentrations in the bull. The Flehmen response may be a mechanism used to rapidly increase serum androgen levels and to arouse and sustain sexual interest in the beef bull. Lunstra *et al*. (1989) also studied the effect of the mating stimuli on testosterone concentration. Blood samples were taken from bulls pre- and post-exposure to an estrus female. The post exposure testosterone concentration did not change for those bulls that achieved a service ($P>0.1$) but it increased dramatically for bulls that achieved a mount with no

service (P<0.01). They concluded that the Flehmen response elicits the elevation in serum testosterone concentrations in a bull.

Elevated blood concentrations of ACTH results in increased adrenocorticol activity, leading to increased secretion of cortisol and resultant decreased adrenal cholesterol due to the increased cholesterol conversion to pregnenolone, which is the precursor for cortisol synthesis (Swenson & Reece, 1993). For the production of testosterone, cholesterol is converted to pregnenolone and then the biosynthetic pathway involves either Δ5-intermediates (pregnenolone, 17α-hydroxypregnenolone, dehydroepiandrosterone and 5-androstenediol) or Δ4-intermediates (progesterone, 17α-hydroxyprogesterone and 4-androstenedione) to the production of testosterone. Moberg (1991) stated that increases in circulating concentrations of the adrenal glucocorticoids are used as indicators of stress because of the responsiveness of the adrenal axis to physiological stress. Grandin (1997) argued that short term stress from handling can be assessed by cortisol concentrations and three stress categories were proposed based on cortisol concentrations. Baseline levels indicate procedures that were not stressful and the concentrations range between 8 - 17 nmol/L for *Bos taurus* cattle. Normal levels of cortisol that occur during restraint in a head gate ranges between 99 and 174 nmol/L. Mean values larger than 256 nmol/L indicate that animals experienced extreme stress.

The results of the present study showed that for all of the fertility categories, GnRH treatment did not affect cortisol secretion because the pooled cortisol concentration did not differ between the samples taken before and after GnRH treatment. The cortisol concentrations were also higher before (37.3 (5.0) nmol/L) and after GnRH treatment (36.9 (5.6) nmol/L), in comparison to the concentration after sexual stimulation (26.0 (5.6 nmol/L)). This may indicate that the bulls were stressed more before and after GnRH treatment, compared to that after sexual stimulation. The handling of bulls could have had a more significant effect on cortisol concentrations than the different fertility categories. By contrast, the testosterone concentration was lower (P<0.001) before GnRH treatment for all the fertility categories, compared to the concentrations after sexual stimulation.

A reciprocal relationship may exist between plasma cortisol and testosterone. Nancy *et al.* (1977) pointed out that when evaluating normal temporal variation in testosterone concentration, sequential collection of blood from bulls unfamiliar to restraint and capture cannot be used due to the negative effect that stress has on blood testosterone and LH levels. The release of corticoids during stress can directly or indirectly cause a decrease in testosterone concentration in the blood. This problem can be overcome by exogenous GnRH providing exogenous stimulation to the reproductive endocrine axis (Post *et al.*, 1987a). Hendricks *et al.* (1984) reported that when plasma cortisol concentrations are high, the coincident plasma testosterone concentrations are low. The adrenal axis is known to regulate the gonadal axis and this implies that the adrenal cortex modulates

reproduction. Excess blood cortisol concentrations have been shown to inhibit LH release and to enhance the ability of the sex steroids (testosterone) to suppress gonadotropin secretion through negative feedback (Swenson & Reece, 1993). Daley *et al*. (1999) also reported that stress induced concentrations of cortisol secretion enhances the negative feedback potency of estradiol in cows, and reduces estrogen-dependant accumulation of GnRH receptors in pituitary tissue, leading to a decrease in LH pulse frequency of cows. Glucocorticoids can act directly on the GnRH- secreting cells of the hypothalamus because they can readily penetrate the blood-brain barrier. Breen & Karsch (2004) reported that cortisol inhibits the pulsatile release of LH by suppressing the pituitary responsiveness to GnRH rather than inhibiting hypothalamic GnRH release. Their findings demonstrated that a stress induced increment in cortisol inhibits pituitary responsiveness to GnRH pulses. Management related stressors have been shown to increase glucocorticoid concentrations and at the same time decrease the pituitary's responsiveness to exogenous GnRH (Breen & Karsch, 2004). It can be concluded that cortisol is a reasonable indicator of stress and affects the interpretation of testosterone as indicator of fertility. If an antagonism exists between these testosterone and cortisol, as demonstrated by Johnson *et al.* (1982) as cited by Hendricks *et al.* (1984), cortisol would inhibit protein anabolism via a fall in plasma testosterone. Regardless of this response of the testis to the adrenal axis, there is no evidence that male fertility is severely affected (Moberg, 1991).

4.2.2.1. Breeding potential category based on scrotal circumference

Thompson *et al.* (1994) did a study on 13 month old crossbred bulls to examine the relationship between testosterone concentration and testicular function. They hypothesized that the efficiency of testosterone production will be lower in bulls with testicular immaturity. The bulls were divided into two groups according to SC (group 1 having SC < 28 cm and group 2 SC > 28cm) and were injected with exogenous GnRH. Blood samples were collected immediately prior to injection (t = 0), 30 minutes after injection (t = 30) and 2 to 3 hours after injection (t= 150). At 30 minutes after GnRH injection the testosterone concentration increased ($P<0.0001$) and continued to increase ($P<0.0001$) to t = 150 but did not differ ($P<0.1$) between the two SC groups. It was concluded that testosterone production was not affected by small SC.

The results of the present study are in agreement with a study done by Post *et al.* (1987b), with four, 29 month old, Hereford – Shorthorn bulls selected for different rankings for testosterone response to GnRH treatment. The measurements of liveweight, weight gains and scrotal circumference showed no relationship with testosterone concentrations 2 to 3 hours after GnRH treatment.

4.2.2.2. Breeding potential category based on spermatozoal morphology

A statistically significant difference ($P<0.05$) in cortisol concentration before GnRH treatment was revealed between the two semen morphology categories. The bulls with ≥ 70 % morphologically normal spermatozoa had a cortisol concentration of 45.9 (6.2) nmol/L in comparison with the lower ($P<0.05$) concentration of 28.7 (3.8) nmol/L for the bulls with less normal spermatozoa.

The results from the present study suggest that high basal testosterone concentrations can be indicative that there are less spermatozoal morphological defects. The difference in testosterone concentrations before GnRH treatment was statistically significant between the semen morphology categories. Bulls with more than 70 % morphologically normal spermatozoa had a higher ($P<0.05$) average testosterone concentration before GnRH treatment than bulls with < 70 % morphologically normal spermatozoa. Testosterone concentrations after GnRH treatment were numerically higher ($P>0.1$) for bulls with < 70% morphologically normal sperm. Those bulls with less morphologically normal sperm however showed a large increase in testosterone concentration after GnRH treatment while bulls with ≥ 70 % morphologically normal spermatozoa showed a smaller increase in testosterone concentration after GnRH treatment. This smaller increase in testosterone concentration may be explained by the sensitive negative feedback–system that operates between LH and testosterone secretion. Luteinizing hormone, secreted from the adenohypophysis, is under the control of episodically released GnRH and is released four to five times per 24 hours. Testosterone is produced and secreted by the Leydig cells under control of LH. A critical minimum frequency of LH releases is required for the secretion of testosterone. Increased levels of testosterone can be observed 30 to 60 minutes after increased LH secretion, and the elevated testosterone lasts for one to several hours. A bulls that is accustomed to capture and breeding has an average testosterone value of 14 to 24 nmol/L (Thibier, 1976). Levels of testosterone barely above physiological amounts can inhibit LH release and inhibit further testosterone synthesis. The negative feedback inhibition of LH secretion by testosterone is followed by a resultant decline in testosterone secretion (Swenson & Reece, 1993).

There was a negative correlation ($r = - 0.21$; $P>0.1$) between testosterone concentration (T_0) and the total amount of spermatozoal defects, while the correlation between testosterone concentration (T_0) and sperm morphology categories was $r = 0.31$ ($P<0.05$). Malak & Thibier (1985) found no difference between the testosterone concentrations of good and poor semen producing bulls. These conflicting results could be caused by differences in sampling time after GnRH treatment, age and breed of the bulls as well as stress susceptibility.

4.2.2.3. Breeding potential category based on spermatozoal motility category

Cortisol concentrations before GnRH treatment differed ($P<0.05$) between the semen motility categories. Bulls with less than 70 % motile spermatozoa had much lower ($P<0.05$) average cortisol concentrations than bulls with more than 70 % motile spermatozoa.

The results of the present study agree with those of Post *et al.* (1987b) in that there is no relationship between testosterone response to GnRH treatment and spermatozoal movement. Post *et al.* (1987b) did a study on four, 29 month old, Hereford – Shorthorn bulls selected for their different rankings for testosterone response to GnRH treatment. The measurements of percentage viable sperm showed no relationship with ranking for testosterone 2 to 3 hours after GnRH treatment. In the present study the correlation between testosterone concentration and percentage progressively moving sperm was $r = 0.15$ ($P>0.1$). There was a negligible difference in testosterone concentrations between the bulls with ≥ 70% and < 70% motile sperm for each of the sampling times.

4.2.2.4. Breeding potential category based on overall breeding soundness category

The cortisol concentration before GnRH treatment differed significantly between the exceptional fertility category and the other two fertility categories ($P<0.001$). Bulls in the exceptional fertility category showed a large decrease in cortisol concentration after treatment, while those in the acceptable and unsatisfactory categories had an increase in cortisol concentration after treatment.

The relationship between testosterone concentration and overall bull fertility is not clear form the result of this study. The testosterone concentrations before GnRH treatment was significantly higher ($P<0.05$) for the exceptional fertility category than for the acceptable fertility category. The testosterone concentration after GnRH treatment tended to be higher ($P<0.1$) for the acceptable fertility and subfertile categories than for the exceptional fertility category. Bulls in the acceptable and subfertile categories showed a larger increase in testosterone concentration after treatment than the exceptional fertility categories. Before GnRH treatment the bulls with exceptional fertility had the highest testosterone concentrations ($P<0.05$), but after GnRH treatment they had the lowest plasma testosterone concentrations ($P<0.1$). This observation may be explained by the negative feedback–system that operates between LH and testosterone secretion as mentioned above.

4.2.3 Libido

Even though the intensity of libido is extremely difficult to measure,it is important for cattle enterprises to have and indication whether or not a certain bull is likely to mate. It must be pointed out that the measure of libido obtained in a test situation may not reflect sexual activity in the mating paddock. Tests for libido assessment in males determine the sexual responsiveness of bulls to females according to different criteria

(Petherick, 2005). During the present study a score was assigned to each bull according to a combination of those criteria.

Many inconsistencies exist among libido testing and makes the interpretations of results very difficult (Petherick, 2004). When differentiating between bulls of high, medium and low libido, the bulls must be given the opportunity to express their maximum libido (Blockey, 1981a). There are many precautions to be taken when testing bulls for libido and mating ability (Chenoweth, 1999). The bulls should not be tested during periods of adverse weather conditions such as extreme heat, cold or wind (Chenoweth, 1999). Extremes of thermal environments and climates can operate to reduce the expression of libido (Petherick, 2004; Quirino *et al*., 2004) and must be considered when interpreting libido score results (Landaeta-Hernandez *et al.*, 2001). Existing methods used to determine the sexual behavior of bulls may also be impeded due to the fact that they involve the use of restrained, non-estrus females that does not represent natural mating stimuli (Bailey *et al.*, 2005; Quirino *et al*., 2004). It has been shown that a group of unrestrained, sexually receptive females induce greater sexual responsiveness in bulls than sequentially pairing them with individual females. Other practices such as using restrained, non-estrus females or even restrained male cattle in serving capacity tests seem questionable (Bailey *et al.*, 2005). Testing of bulls immediately following other procedures such as electroejaculation, vaccination and parasite control measures should also be avoided (Chenoweth, 1999).

Petherick (2004) pointed out that cattle in groups interact and develop relationships with each other, one form of relationship being a dominance hierarchy or social order. It has been reported by López *et al*. (1999) that the sexual behavior of other males can be inhibited by the presence of a more dominant male. The length of time that the bull has spent in the herd tends to determine the dominance of that bull and this is closely associated with the bull's age. Bulls that have been reared together and are of a similar age are less likely to fight (Ologun *et al.,* 1981) and libido testing should therefore be done in groups of similar ages to reduce the effect of social dominance on expression of libido (Chenoweth, 1999). Dominance is of little importance when yearling or two year old bulls are used (Petherick, 2004).

Landaeta- Hernandez *et al.* (2001) reported that the repeatability of the libido scores of yearling *Bos taurus* bulls is in the moderate range of r = 0.64 which indicates large environmental influences that can lead to difficulties in interpretation of a libido score. The variance of a libido test can be reduced to 69 % when repeated eight times, suggesting that environmental influences, deficiency in test design and misinterpretation of behavioral patterns influence the expression of libido. Libido testing in this study was only done on one occasion because of time constraints. The low repeatability of test procedures and the necessity to perform a number of repetitions to reduce the environmental variance reduces the practicality of libido tests for routine application.

4.2.3.1. Libido scores

The results obtained during the present study indicate that the SC and spermatozoal morphology fertility categories may be more accurate predictors of libido than the spermatozoal motility and overall breeding soundness categories.

In the present study, the libido scores of the bulls in the SC ≥ 340 mm category tended to be numerically slightly higher (4.8 ± 1.6) than bulls with an SC < 340 mm (P>0.1). Ologun *et al*. (1981) reported different results. These authors compared the average daily gain and weight of yearling bulls after a 140-day performance test, with the libido/ serving capacity scores they obtained in a libido test where restrained non-estrus females were used. There was a negative correlation (P<0.01) between both the ADG and final weight with serving capacity score. The genetic correlation between selection for high growth rate and SC is 0.5 indicating that selection for growth will lead to a significant increase in scrotal size, which can have a positive effect of sperm production and thus male fertility (Rust & Groeneveldt, 2001; Burrow & Prayaga, 2004). The results obtained by Ologun *et al*. (1981) indicate a non-favorable relationship between production traits and libido. Another study was done by Quirino *et al*. (2004) to access the relationship between libido and seminal traits in two to five year old zebu bulls. They reported a libido score of 2.4 for the two year old zebu bulls. There was a negative correlation between libido and scrotal circumference (r= - 0.2; P<0.05) meaning that the bulls with the highest libido had smaller scrotal circumferences.

Bulls with more than 70 % morphologically normal spermatozoa had a numerically higher (P>0.1) overall libido score.

The overall breeding soundness classification did not affect the libido of bulls in this study. The bulls in the unsatisfactory overall breeding soundness category had the largest libido score while the acceptable fertility category had the lowest score. Chenoweth *et al.* (1988) also found no differences (P<0.05) in sex drive between bulls with different breeding soundness classifications. Thus optimal bull reproductive evaluation should include the assessment of both breeding soundness and libido.

The genetic correlation between libido and spermatozoa defects was r = -0.43, indicating that a bull that obtains the best libido score would have the lowest amount of spermatic defects (Quirino *et al.*, 2004).

4.2.3.2. Relationship between libido and hormone concentrations

The summary statistics of the overall libido scores obtained from the 41 bulls in the present study (average weight 399.5 ± 27.4 Kg) was 4.7 ± 1.6. In serving capacity studies done on different breeds of bulls by Bertram *et al*. (2002) the following libido scores were reported for two year old bulls. Santa Gertruidis and Brahman bulls, with an average weight of 586 ± 102 Kg and 385 ± 53 Kg respectively, had average libido scores of 6.5 ± 2.9 and 3.4 ± 3.0 respectively.

Although no statistically significant correlations were found between the libido scores and hormone concentrations, a numerical relationship was observed in some cases. Cortisol concentrations before and after GnRH treatment were higher in the low libido bulls. The cortisol concentration after sexual stimulation was higher in the high libido bulls. The relationship between cortisol concentrations and libido score in the present study agree with those of other researchers. Byerley *et al.* (1990) reported that in yearling bulls the cortisol concentration after GnRH treatment is higher in the low libido bulls. The design of the present study differ's form the study of Byerley *et al.* (1990) because they only compared the highest and lowest libido bulls from their experimental population and bulls were tested for libido at a bull to female ratio of 4:3. The female animals that they used were non- estrus, restrained cows.

Bulls that obtained the highest libido score also had the highest testosterone concentration before GnRH treatment. There was no relationship between libido score and testosterone concentration after GnRH treatment. Similar results were obtained by Byerley *et al*. (1990) during a study on GnRH induced testosterone response in yearling bulls. The four bulls with highest and lowest libido were selected from 84 yearling bulls tested for libido. They found that the testosterone concentration was elevated ($P<0.05$) in high libido bulls in the two hours prior to GnRH injection as opposed to the low libido bulls. There was no difference ($P<0.1$) between high and low libido bulls in the testosterone concentration following GnRH injection.

In the present study the testosterone concentration directly after sexual stimulation reached higher levels in the bulls with high libido than the bulls with lower libido.

Post *et al.* (1987b) did a study on 29 month old Hereford – Shorthorn bulls to assess the efficiency of using testosterone response, 2 to 3 hours after GnRH treatment, to predict the reproductive performance of bulls. They defined libido as the amount of estrus females mounted during a five day period. Their results showed a relationship between ranking for testosterone and number of estrus cows mounted. Bulls with the lowest 'libido' scores had the lowest testosterone concentration and those with high 'libido' scores had higher testosterone concentrations. They proposed that testosterone status can be a valuable test used to select herd sires.

Petherick (2005) pointed out that libido scores generally increased with bull age. In serving capacity studies done in different breeds of bulls by Bertram *et al*. (2002), it was reported that the percentage of bulls displaying services tended to increase with age ($P<0.05$). Eighty-two, 83 and 86 % of Belmont Red bulls and 50, 50 and 66 % Santa Gertruidis bulls that are two-, three-, and more than four years of age completed one or more services.

4.2.4 Spermatozoal characteristics

Chenoweth *et al*. (1988) determined the relationship between OBE and the sex drive of bulls. They found that the factors exerting the principal effects on OBE classification were qualitative seminal characteristics. Spermatozoal motility and SC did not influence the classification of the bulls in that study but a study by Tomky *et a*l. (1979) showed that SC affected the OBE score. Chenoweth *et al*. (1988) found that there is no significant relationship (r = -0.16 to 0.24) between sex drive and breeding soundness traits in beef bulls.

4.2.4.1. Breeding potential category based on scrotal circumference

The results suggest that the SC categories considered in this study does not affect the spermatozoal characteristics of Bonsmara bulls. This may be due to the fact that all of the bulls studied had sufficiently well developed testes.

4.2.4.2. Breeding potential category based on spermatozoal morphology

Semen ejaculates may show as few as 5 % abnormal sperm, while others may approach 100 %. Fertility is usually not affected until the level of abnormal sperm exceeds 20 to 2 5% (Bearden *et al*., 2004).

The result of the present study also suggests that as the percentage morphologically normal sperm decreased the spermatozoal motility also decreased. The correlation between progressive sperm motility and fertility category based on sperm morphology was 0.33 ($P<0.05$). Bulls with ≥ 70% morphologically normal spermatozoa had more progressively motile spermatozoa ($P<0.05$), tended to have less aberrant sperm movement ($P<0.1$) and a lower percentage immotile spermatozoa ($P<0.1$). The correlation between the two spermatozoal characteristics, percentage morphologically normal sperm and percentage progressively moving sperm, was 0.50 ($P<0.001$), while the correlations between percentage morphologically normal sperm and aberrant and immotile sperm was 0.48 ($P<0.05$) for both variables.

As was expected, the correlation between percentage morphologically normal sperm and the fertility categories based on sperm morphology was high ($r = 0.6$; $P<0.001$).

There was a negative correlation of $r = - 0.55$ ($P<0.001$) between fertility category based on sperm morphology and the total amount of defects (while the negative correlation between the two spermatozoal characteristics, percentage progressively moving sperm and total spermatozoal defects, was $r = - 0.52$ ($P<0.001$). Bulls with ≥ 70 % morphologically normal sperm had a significantly lower percentage ($P<0.001$) of total spermatozoal defects.

Total defects was influenced to a greater extend, by acrosomal and flagellar defects ($P<0.001$) than nuclear defects ($P<0.05$). The amount of total defects was highly correlated ($P<0.001$) with the flagellar and acrosomal defects ($r = 0.72$ and $r = 0.93$ respectively) and lowly correlated to the total amount of nuclear defects

(r = 0.32; P<0.05). The nuclear defects did not correlate significantly with the total acrosome and flagellar defects. Acrosomal defects occur if the spermatogenesis cycle has been disturbed and this abnormality leads to reduced motility of the sperm. Flagellar defects usually involve the coiling or bending of the principle piece. Motility is impaired and this causes a negative effect on potential fertility because the sperm can not move in a foreword direction due to the bending of the tail, and is unable to penetrate the zona pelucida (Barth and Oko, 1989). The occurrence of acrosomal and flagellar defects can also be due to semen collection and handling after fact and due to lengthy storage in the epididymis before and ejaculation (senescent sperm). During the present study the bulls were given the opportunity to service a cow and thus ejaculate (during the libido test) prior to the semen evaluation and it can therefore be assumed that these results can not be ascribed to lengthy storage of sperm in the epididymis.

From the results of the present study it seems that the semen morphology category is a better indicator of semen quality than the SC and semen motility categories.

4.2.4.3. Breeding potential category based on spermatozoal motility

Sperm motility is an indication of the percentage live spermatozoa in an ejaculate and can range from 0 to 80 %. Samples of less than 40 % would lead to decreased pregnancy rates depending on the type of breeding system employed. Progressively motile sperm is moving from one place to another in a straight line. Circular and reverse movements are associated with tail abnormalities and vibrating or rocking movement is associated with aging. Abnormal sperm do not show progressive motility so as the percentage of abnormal sperm decrease the progressive motility percentage increases (Bearden *et al*., 2004).

4.2.4.4. Breeding potential category based on overall breeding soundness

Smith *et al.* (1981) studied the relationship among fertility, SC, seminal quality and libido in two year old Santa Gertruidis bulls. The correlation they found between individual semen measurements and the overall breeding soundness score were low. Their data emphasized the difficulty of accurately predicting the fertility of an individual bull in natural service on the basis of a single semen evaluation.

During the present study there was a significant difference (P<0.001) in percentage progressive sperm movement between the overall breeding soundness categories. The correlations (P<0.001) between fertility category based on overall breeding soundness and progressive sperm movement was r = 0.77, the correlation with the percentage aberrant sperm movement was r = - 0.73 and the correlation with percentage immotile sperm was r = - 0.70. These results suggest that the overall breeding soundness classifications of bulls are largely influenced by spermatozoal motility. Contradictory results were reported by Chenoweth *et al.* (1988) where the

percentage spermatozoal motility between bulls classified as highly fertile (72.6 ± 1.9) and subfertile (64.2 ± 4.6) did not differ ($P<0.05$).

During the present study the correlation between fertility category based on overall breeding soundness and the percentage morphologically normal sperm was $r = 0.45$ ($P<0.05$). These results suggest that the overall breeding soundness classifications of bulls were significantly influenced by spermatozoal morphology. The bulls in the exceptional fertility classification had a higher ($P<0.05$) percentage morphologically normal spermatozoa than the acceptable and unsatisfactory classifications. There were negative correlations between fertility category based on overall breeding soundness and the total amount of defects ($r = - 0.48$) and acrosomal and flagellar defects ($r = - 0.51$). The highly fertile bulls had a lower percentage ($P<0.05$) spermatozoal defects. Similar results were reported by Chenoweth *et al.* (1988) that bulls in the exceptional fertility- breeding soundness category had fewer ($P<0.01$) total spermatozoal abnormalities than bulls of lower fertility.

CHAPTER 5

5.1. CONCLUSION

The results of this study indicate that production data is less useful than semen characteristics as predictor of the breeding potential of young bulls. The negative correlations between 205 day growth index and total acrosomal and flagellar defects, and the positive correlation between the 205 day growth index and spermatozoal motility suggest that relatively high growth rates before weaning may have a positive effect on fertility under normal extensive feeding conditions.

Sampling time had a statistically significant effect on cortisol and testosterone concentrations for all of the breeding potential categories. Testosterone concentration increased significantly ($P<0.001$) after GnRH treatment. The results of the present study showed that when plasma cortisol concentrations were high, the coincident plasma testosterone concentrations were low, implying that cortisol affects the interpretation of testosterone as an index of fertility. The results from the present study suggest that high testosterone concentrations may be indicative that there might be less spermatozoal morphological defects in the ejaculate of a 25 month old Bonsmara bull. The bulls with exceptional fertility had a much higher ($P<0.001$) cortisol concentration before GnRH treatment when compared to the less fertile bulls. Before GnRH treatment the bulls with exceptional fertility had the highest testosterone concentrations, but after GnRH treatment they had the lowest plasma testosterone concentrations. This observation may be explained by the negative feedback system that operates between LH and testosterone secretion.

None of the reproductive and production measurements had a significant effect on the libido scores. The results indicated that the SC- and spermatozoal morphology categories may be more accurate predictors of libido than the spermatozoal motility and overall breeding soundness categories. Although no statistically significant correlations were found between the libido scores and any of the circulating hormone concentrations, a numerical relationship was observed in some cases. The cortisol concentrations before and after GnRH treatment were higher in the low libido bulls. Bulls that obtained the highest libido score had the highest testosterone concentration before GnRH treatment, but there was no difference between high and low libido bulls in terms of the testosterone concentration following GnRH treatment. These results imply that, as none of the measured traits showed a significant correlation with libido scores, optimal bull reproductive evaluation should include the assessment of both breeding soundness and libido.

From the results of the present study it seems that the semen morphology category is a better indicator of semen quality than the SC and semen motility categories. The percentage spermatozoal defects were influenced to a greater extent by morphological abnormalities leading to reduced motility of the sperm than by any other abnormalities. The result revealed that, as the percentage morphologically normal sperm decreased, the spermatozoal motility decreased. Overall breeding soundness classifications of bulls were largely influenced by spermatozoal motility and to a lesser extend by spermatozoal morphology and SC.

5.2. RECOMMENDATIONS

The results obtained during the present study may have been influenced by the relative small number of animals used. The results of one animal have a significant effect on the average and standard deviations obtained. A trial that used more animals would have reduced the standard deviations.

The experiment was only conducted during one season (summer). It would have been interesting to see result obtained during winter months. Perry *et al.* (1991) reported that GnRH induced testosterone responses were lower in early winter and higher during spring months in Australia. The bulls used during that study was just post pubertal at the start of winter and their age in the spring was 912d (age may play a role in testosterone concentration and season may not really have such a significant effect).

5.3. CRITICAL EVALUATION OF THE PROJECT

The project has been successfully conducted and the primary objectives were achieved. During the course of the project suggestion on libido- and fertility testing of 25 month old Bonsmara bulls were made which can be of practical importance in the South African beef cattle farming industry.

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