Factors affecting the quality of semen of A.I. dairy bulls in South Africa

By:

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Submitted in partial fulfilment for the requirement of the Degree Magister Institunonis Agrariae (Animal Production)

PROMOTER: **Prof. E. C. Webb**

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ABSTRACT

The primary objective of this research was to study the effects of breed, age, season, and their interactions on semen morphological characteristics. The study was done on 329 bulls (271 Friesland and 58 Jersey) aged 12, 24, 36, 48, 60, 72, 84, 96 and > 96 months. The collection of semen was carried out using the artificial vagina method in all four seasons of the year. Spermatozoa were screened for the percentages normal sperm, percentage and total major defects such as knobbed acrosome, pyriform, abnormal lose head, dag defects, nuclear vacuole, degenerative heads, mid-piece reflexes, percentages and total minor defects such as normal lose heads, distal droplets, curled end-piece, lose acrosome. Statistical analyses of the data were done using the general linear model (GLM) procedure of the Statistical Analyses System (SAS, 1999).

The results of the study indicate that breed did not significantly affected the percentage normal sperm and percentage major sperm defects, but significantly affected the percentage minor defects (P = 0.01). The Least square means (LSM±SE) for the percentage normal sperm, major defects and minor defects in Friesland and Jersey bulls were 80.6 ±1.06%; versus 78.9±2.31%; 14.8±0.90% versus 15.0± 2.62%, 5.1±0.43% versus 7.6±0.94%, respectively. The results obtained show that the prevalence of sperm defects that differed significantly between breeds was higher in Jersey bulls compared to

Friesland bulls. The results of the study indicated the percentage of normal sperm to differ (P = 0.01) with season. The percentage of normal sperm during the summer, autumn, winter and spring, were 72.8±1.6%, 79.4±2.2%, 82.5±2.4% and 84.4±2.4% respectively. Season also affected the percentage of major defects (P = 0.01) and percentage of minor defects (P = 0.03). The results demonstrate that even though there was a higher variation in sperm morphology with season, better sperm morphology was recorded in spring and winter than summer and autumn. Results also indicate the percentage of normal sperm (P = 0.05) and major defects (P = 0.01) to be affected significantly by age. On the other hand, the percentage of minor defects did not differ significantly with age. Bulls of 36-48 months of age showed better semen quality than bulls older than 72 months and bulls younger than 36 months. The percentage of major defects, particularly the incidence of major defects such as knobbed acrosomes, pyriforms, dag defects and broken flagella were significantly affected by the interaction between age and breed (P = 0.05) and age and season (P = 0.05). There was an increase in the susceptibility to these sperm defects in Jersey bulls with an increase in age, while no variation was observed in Friesland bulls. With age and season combined, young bulls recorded poor semen morphology during winter, while old bulls showed poor morphology during summer.

In conclusion, the study suggested that breed, age and season and their interactions are important sources of variation in sperm morphology. For a successful AI programme, semen collection should be done at the age of 36-48 months for both breeds. It is therefore recommended that age, breed and season should be given urgent attention in any bull management system employed in South Africa in order to obtain the best semen quality.

KEY WORDS: breed, age, season, sperm morphology, percentage of normal sperm, percentage of major defects, percentage minor defects, Friesland, Jersey, artificial vagina.

I declare that the dissertation hereby submitted in partial fulfilment for the requirements of the degree **Magister Institunonis Agrariae (Animal Production)** at the University of Pretoria has not been submitted by me for any other degree at any other institution.

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DEDICATIONS

It is my greatest pleasure to dedicate this dissertation to the following people who gave their best to make my studies a success.

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LIST OF ABBREVIATIONS

i.	ABP	Androgen Binding Protein
ii.	ACTH	Adrenocorticotrophic Hormone
iii.	ADG	Average Daily Gain
iv.	AI	Artificial Insemination
v.	AT option-	At That value option
vi.	ATP	Adenosine triphosphate
vii.	DNA	Deoxyribonucleic acid
viii.	FSH	Follicle Stimulating Hormone
ix.	GLM	General Linear Model
x.	GnRH	Gonadotropin Releasing Hormone
xi.	GPC	Glycerylphosphorylcholine
xii.	LH	Luteinizing Hormone
xiii.	LSM±SE	Least Square Means ± Standard Error
xiv.	SAS	Statistic Analyses System
xv.	SC	Scrotal circumference
xvi.	THI	Temperature Humidity Index
xvii.	TSH	Thyroid Stimulating Hormone
xviii.	TVC	Testicular Vascular Cone

CHAPTER 1

INTRODUCTION AND MOTIVATION

1.1 PROJECT THEME

Reproduction management in domestic animals.

1.2 PROJECT TITLE

Factors affecting the quality of semen of AI dairy bulls in South Africa.

1.3 AIM OF THE STUDY

The aim of the study was to quantify the effects of the following factors on the quality of semen of Friesland and Jersey bulls in South Africa:

i. Age, breed and season,
ii. The interaction between age and breed,
iii. The interaction between age and season and
iv. The interaction between breed and season.

1.4 MOTIVATION

Artificial insemination (AI) is one of the most valuable management tools available to dairy cattle breeders. The successful use of AI depends on the effective harvesting of semen and collection of a relatively large number of potentially fertile spermatozoa from a genetically superior sire.

Approximately 90-95% of semen collected at AI stations in South Africa is used in dairy herds. This emphasizes the importance of studying and understanding the factors that affect the quality of semen in AI dairy bulls.

A relatively large area of South Africa is characterised as tropical and sub-tropical, where the adaptability of dairy breeds is questionable due to their sensitivity and intolerance to heat stress (McDowell, 1972). Both the Friesland and Jersey are European breeds and need better management than the indigenous breeds in order to survive and produce in these areas. This also emphasises the importance of quantifying the effects of the South African environment on these dairy breeds.

It is also postulated that the often-low fertility observed during summer in dairy cattle occurs as a result of heat stress that affects the quality of semen (Curtis, 1983). Several studies have been done on management strategies which involve the provision of shade, cooling and sprinkling with water and supplementation of adequate nutrients to enhance the reproduction potential of dairy breeds in small and commercial farmers (McDowell, 1972; Hacker, 1982; Yousif *et al.*, 1982; Curtis, 1983; Van Horn & Wilcox, 1992; King, 1993). In addition to these studies, it is of the utmost importance to all cattle farmers (small and large scale) where natural mating or AI is used, to know and understand the factors that affect semen quality so that they may effectively plan their semen collection and AI programme. Age, breed and season of semen collection are reported as some of the major factors affecting semen quality (Mathevon *et al.*, 1998; Brito *et al.*, 2002b). This study therefore aims to evaluate the effect of these factors on the semen quality of Friesland and Jersey bulls under South African conditions.

1.5 INTRODUCTION

Fertility in dairy bulls is a complex trait that is made up of several physiological processes such as the development of the reproductive system from birth to puberty, spermatogenesis, ejaculation and mating behaviour (which involves libido and copulation). For optimum semen quality, all these physiological processes should be co-ordinated.

Sperm morphology, motility, sperm concentration and volume per ejaculate are common criteria for evaluating semen quality at most AI stations (Den Daas, 1992; Colenbrander

et al., 1993; Bearden & Fuquay 1997). Sperm morphology, expressed as a percentage of normal sperm cells (Serrenson, 1979) plays an important role in fertilisation in humans (Kruger *et al.*, 1986; De Yi lui & Baker, 1982) and it also seems logical that the same will be true for domestic animals (Reinecke *et al.,* 1995). Morrow (1980) defined the relationship between spermatozoa morphology and the reproduction potential and indicated that when more than 30% of the ejaculated spermatozoa have structural defects, reduced fertility may occur in domestic animals.

Many structural abnormalities occur in the development of spermatozoa as a result of faulty spermatogenesis and also as due to semen handling both during and after collection (Salisbury *et al.,* 1978). Major factors that affect semen quality are also those related to the underdevelopment of the testis (King, 1993). All factors related to testicular degeneration, including hereditary and pathological conditions should be carefully considered as they may seriously affect semen quality via testicular development. Hoogenboezem & Swanepoel (2000) reported that testicular degeneration might be due to exposure to heat stress, nutritional deficiencies and management-related factors such as fat deposition in the scrotum and poor body condition.

The major contribution to the semen variation is environment (Curtis, 1983; Cupps, 1991; King, 1993; Bearden & Fuquay, 1997). To comprehend the impact of environment on semen quality, it is necessary to understand the biological significance of the term 'environment', which could be defined as all factors surrounding the animal (Bonsma, 1980), which include factors such as environmental temperature, nutrition, humidity, seasonal changes and management of the animal.

The frequency of abnormal sperm cells has been found to increase with factors such as extreme in temperature and heat stress (Rathore, 1970) and malnutrition (Brown, 1984), occurring mainly in the hot seasons (Anderson, 1941). This has been observed to result in a lower ejaculate volume and sperm motility, increase in the percentage of abnormal sperm and a decrease in the total live sperm (Rathore, 1970). Malnutrition includes under-feeding and over-feeding, deficiencies of a specific nutrient and included amongst

these may be the ingestion of toxic substances, which occurs largely as a consequence of poor management. Under-nutrition occurs in bulls that are on poor quality veld or as a secondary effect of heat stress. The latter depresses appetite, and hence lowers feed intake. Lower feed intake is likely to result in impaired reproductive capability of bull in terms of libido and mating behaviour and semen production and quality (McDowell, 1972).

Mathevon *et al.* (1998) reported that season significantly affected semen morphological characteristics in young bulls but did not significantly affect ejaculate volume and sperm motility in mature bulls. Significant seasonal variation occurred in the incidence of sperm head abnormalities and total sperm abnormalities. Kumi-Diaka *et al.*, (1981) reported no significant seasonal variation in sperm cell concentration, percentage of live spermatozoa and sperm cell abnormalities in the indigenous breeds, while exotic breeds showed significant seasonal fluctuation, with regard to high sperm cell abnormalities, low percentage live sperm and lower sperm cell concentration during the hot season.

The primary objectives of the study was to determine the effects of breed, age and season and their first order of interactions on the sperm morphological characteristic of Friesland and Jersey bulls in South Africa.

CHAPTER 2

LITERATURE REVIEW

2.1 DEVELOPMENT OF THE MALE REPRODUCTION SYSTEM FROM BIRTH TO PUBERTY

The production of semen by dairy bulls is dependent largely on the growth and sexual development of the bull from birth to puberty (Salisbury *et al.*, 1978). Bearden & Fuquay (1997) reported the endocrine related changes in body conformation, increase in aggressiveness and sexual desire, rapid growth of the testes and separation of the penis from the prepuce so that the extension of the penis is possible. These changes occur before the spermatozoa are present in the ejaculate, prior to puberty.

The sexual development of bulls before puberty can be divided into an infantile and juvenile stage, which are determined by luteinizing hormone (LH) and steroid hormone secretion. The infantile stage, from birth to 10 weeks of age, is characterised by the infrequent secretion of LH. The juvenile stage, from 10 -12 weeks of age, is characterised by an increase in the frequency and amplitude of LH secretion (Cupps, 1991) which stimulates testicular growth and induces spermatogenesis (Ahmad & Noakes, 1996).
Al -Gedawy & Afiefy (1984) reported that as a bull grows from birth to puberty, the rate of growth of the testicles increases rapidly until 9 months of age, slows down at 11 months of age and then accelerates again at 13 months of age. Evan *et al.*, (1995) reported that in pre-pubertal bull calves, there is a transient rise in the secretion of the gonadotrophin, LH and follicle stimulating hormone (FSH), which have been suggested to play a major role in the regulation of sexual development and semen production. Furthermore, Mamabolo (1999) reported that in the absence of these gonadotrophins, neither testicular development nor spermatogenesis may proceed. Evans *et al.* (1995) also reported that early increases in these gonadotrophins are dependent on the maturity-type of the bull. It was indicated that there is a significant increase in the rise of LH and FSH in early maturing bulls, compared to late maturing bulls at the age of 2 and 24 weeks.

Dairy calves enter the pre-puberty period at about 10-12 months of age (Bearden & Fuquay, 1997). At the onset of puberty (see Section 2.2), gonocytes migrate to the periphery of the seminiferous tubules and produce two types of cells or spermatogonia (King, 1993), namely:

(i) Stem cells or spermatogonia, which give rise to spermatogonia and succeeding spermatocytes, spermatids and spermatozoa and

(ii) Reversed stem cells, which contribute to the increase in population of stem cells between puberty and adulthood.

Hafez (1974) reported that spermatogonia and a small number of spermatocytes are present in the seminiferous tubules at 63 days of age and Leydig cells are formed at approximately three and half months of age. McMillan & Hafs (1968) reported an increase in plasma LH and hypothalamic LH releasing factor until the age of puberty, while pituitary FSH secretion declines after six (6) months. The production and release of testosterone from the testis generally increases from birth to puberty. This hormone is responsible for the maintenance of male reproductive system, initiation of spermatogenesis and male mating behaviour (Bearden & Fuquay, 1997). Foote *et al.*, (1976) reported higher seasonal variation in the secretion of testosterone in Holstein bulls, where the average level for spring of 8.0 ng/ml. Higher than the value of 5.7 ng/ml recorded in the fall. It was also indicated that testosterone levels increase with age of the bull, up to 6 to 7 years of age. Further more, Penfold *et al.* (2000) indicated the testosterone concentration to be positively correlated with the percentage of normal sperm and total number of sperm produced.

2.2 AGE AND WEIGHT AT PUBERTY

The definition of the age at puberty differs between genders. In males, it can be defined as the age at which spermatozoa are present in the ejaculate, while in females it is defined as the age at first behavioural estrus (Bearden & Fuquay, 1997).

Dairy bulls reach puberty at the age of 10-12 months, at a body weight of 160-270kg (Bearden & Fuquay, 1997). Hafez (1974) reported to be much more difficult to determine

the exact time of puberty in males because the first differentiation of spermatogenetic cells precedes the release of the first spermatozoa from the seminiferous tubules by a month or more.

Size and weight at puberty is more important than age in determining the onset of puberty. Mamabolo (1999) claimed testis weight, which is correlated with sperm production and quality, to be also positively correlated with body weight. It was also reported that approximately 80% of the variation in testicular weight to be due to the variation in body weight. Bongso *et al.* (1982) identified testicular measurements as an indicator of the reproductive potential and spermatogenic capacity of ruminants. This may explain why weight is more important than age at puberty.

Factors affecting age and weight at puberty are of major importance in the evaluation of semen quality as Day *et al.* (1984) reported puberty to be the major factor that dictates reproductive competence in bulls and heifers.

2.2.1 FACTORS AFFECTING AGE AND WEIGHT AT PUBERTY

Differences in the weight at puberty from year to year have been observed. These differences have mainly been attributed to genotype or breed variation (Bearden & Fuquay, 1997) and a difference in environmental conditions which include factors such as temperature, nutrition, photoperiod and humidity (Paterson, 1981).

The effects of breed on weight and age at puberty may be as a result of factors such as adaptability, average daily gain (ADG) and maturity type of the breed which are all related to the growth rate and hence puberty. In beef calves, growth up to the age of puberty is a measure of adaptability and weight at puberty is regarded as an important post weaning trait that can be used in the final selection of bull calves as herd sires at phase C and D at performance testing stations (Bearden & Fuquay, 1997) and on the veld (Van Zyl *et al.*, 1992; Lubout, 1987; Nauhaus, 1992).

Under tropical and sub-tropical conditions, poor nutrition accompanied by high temperatures result in stress, which reduces the feed intake and affects growth rate in the bull (McDowell, 1972), and hence lowering of the weight and delaying of the age at puberty (Lammond, 1970). If overfeeding accelerates growth rate, body weight at the time of puberty will be higher than normal and the animal reaches puberty at a younger age. On the other hand, if underfeeding retards growth rate, puberty is delayed and body weight will not reach that of a normal fed animal. Hafez (1974) reported that if nutritional status of an animal is maintained at normal levels, puberty might occur when body weight reaches approximately 45% of adult weight in cattle. In males under-nutrition results in a delay in the onset of puberty due to poor testicular development and sperm production (Bearden & Fuquay, 1997). Low energy intake and protein deficiencies alter the rate of gain and might result in a lowering of the body weight and delay the age at which puberty is attained. Vitamins and minerals are important for cellular metabolism, growth and maintenance, follicular and testicular development, all of which influence the onset of puberty in both males and females (Bearden & Fuquay, 1997). Unfavourably high ambient temperatures lead to a restriction of nutrients in the diet of the animal through the depression of appetite and hence reduced feed intake. The nutrient restriction may be enough to substantially limit somatic growth, and hence increase the age and lower the weight at puberty (McDowell, 1974).

Mabesa (1994) reported calving season to have a significant effect on weight at puberty under extensive management systems. This is in agreement with Setshwaelo *et al.* (1988) where calves that were born from November to January were associated with a higher weight at puberty, compared to those born from May to June. Similar results were recorded in the study of Holstein cattle by Hafez (1974), where calves born in spring were reported to attain puberty at 12 months of age while those born in the fall attained puberty at 16 months of age.

The endocrine mechanisms that regulate puberty are centrally regulated within the hypothalamus (Bearden & Fuquay, 1997). Alterations in hypothalamic function that may direct the cascade of the endocrinological events resulting in puberty include changes in

the populations of estradiol-receptors containing neurones that may also delay puberty. Hafez (1974) associated the onset of puberty with a decrease in the sensitivity of these steroid receptors in the brain and an increase in gonadotrophin secretion and subsequent activation of spermatogenesis. Day *et al.* (1984) reported temperature and nutrition to be the primary environmental factors that influence the timing of this change and hence influencing puberty. Bearden & Fuquay (1997) also reported that the pineal gland inhibits the onset of puberty. Sensitivity of the brain steroid receptors can be modulated by pineal gland, which is limited to counteracting the unfavourable effect of photoperiod on sexual function (which is related to the increase of pineal sensitivity). Changes in day length or photoperiod interact with the genotype of an animal in affecting the onset of puberty through its effect on sexual development of both males and females. Seasonal changes in spermatogenesis and ovarian cyclic activity are caused largely by a change in the secretion of LH and FSH, which is reduced by the exposure to the inhibitory photoperiods and elevated by exposure to stimulatory photoperiods (Straus *et al.*, 1979). Hafez (1974) also found a correlation between the attainment of puberty and the social status of the animal. It was indicated that the presence of individuals of the opposite sex hasten puberty, while the presence of individuals of the same sex delays the onset of puberty.

2.3 SPERMATOGENESIS IN BULLS

Spermatogenesis is defined as the process of formation and liberation of spermatozoa from the undifferentiated germ cells in the seminiferous tubules of the testis (King, 1993). The spermatozoa undergo maturation in the epididymis where they are stored until ejaculation takes place (Bearden & Fuquay, 1997)

2.3.1 PROCESS OF SPERMATOGENESIS

Spermatogenesis is defined as a lengthy chronological process whereby a few stem cell spermatogonia, lining the base of seminiferous tubules, divide by mitosis to maintain

their own stem cell numbers and to produce primary spermatocytes that undergo meiosis to produce spermatids which differentiate into a spermatozoa (Cupps, 1991; Figure 2.1)

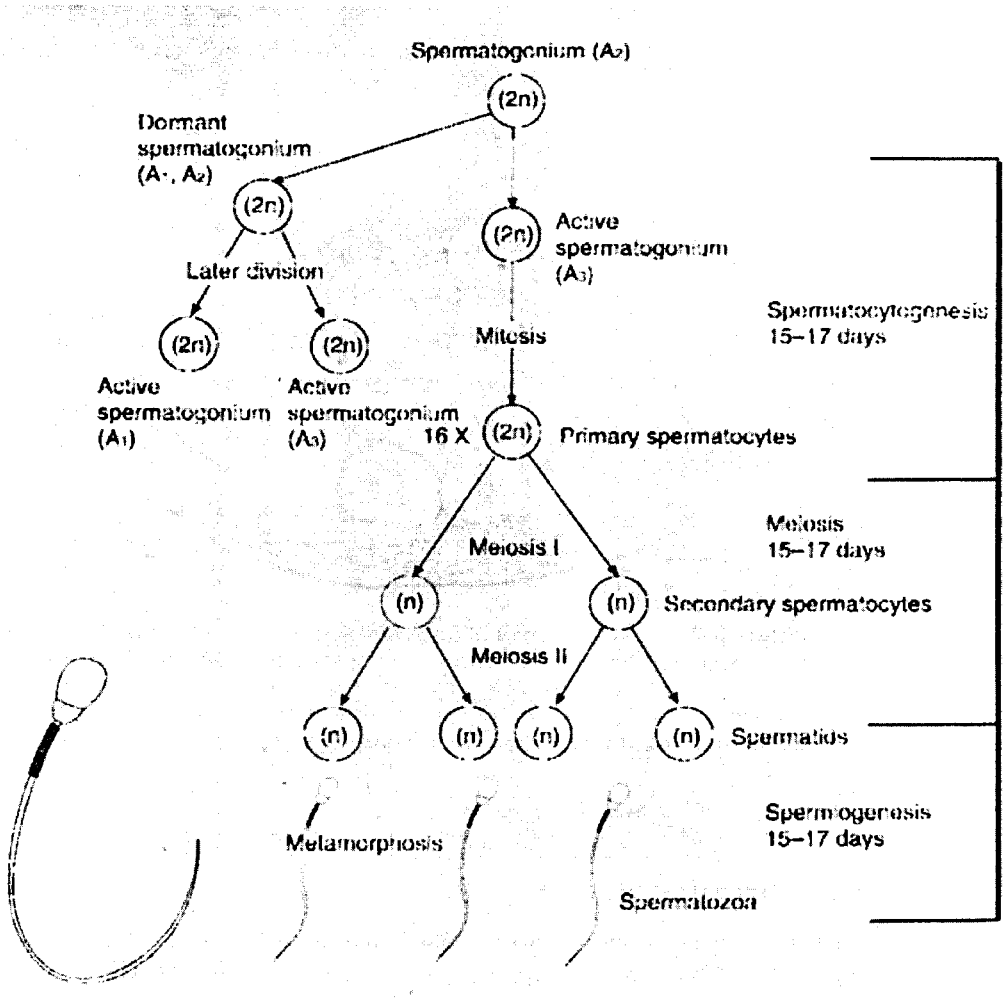

Figure 2.1 Process of spermatogenesis in domestic animal (Bearden & Fuquay, 1997)

In the bull, the process of spermatogenesis takes approximately 56-63 days and is divided into three phases namely spermatocytogenesis, meosis and spermiogenesis (Foote, 1978; Bearden &Fuquay, 1997). Spermatogenesis starts before birth when primordial germ cells of the early developing embryo migrate to the undifferentiated fetal sex cord from the yolk sac and undergo several divisions to produce gonocytes. (Cupps, 1991; King, 1993). Gonocytes persist in the male sex cord until just before puberty, after which time they divide to provide spermatogonia (King, 1993).

The produced spermatogonia undergo mitotic divisions during the process of spermatocytogenesis (first phase of spermatogenesis) to form primary spermatocytes (Figure. 2.1). Meiosis is the phase where the primary spermatocytes formed during spermatocytogenesis undergo meiotic division forming two secondary spermatocytes (Bearden & Fuquay, 1997). With this division, the chromosome complement in the nucleus is reduced to half so that the nuclei in the secondary spermatocytes contain unpaired haploid chromosomes (Cupps, 1991; King, 1993). Each secondary spermatocyte will undergo a second meiosis division, each forming two spermatids (Bearden & Fuquay, 1997;).

During spermiogenesis, the last phase of spermatogenesis, the spermatids undergo a metamorphosis, which is defined as the process by which spermatids undergo changes in morphology to form spermatozoa. During this phase, spermatids are attached to the Sertoli cells. After metamorphosis, the newly formed spermatozoa will then be released from Sertoli cells through the process called "spermiation" and be forced out through seminiferous tubules into the rete testis. From the rete testis, spermatozoa are forced to epididymis via vas efferents (Bearden & Fuquay, 1997).

During the passage through the epididymis, spermatozoa undergo maturation whereby they gain the ability to be motile, lose the cytoplasmic droplets and also become fertile (Cupps, 1991; Bearden & Fuquay, 1997; Mamabolo, 1999; Dombo, 2002). At the same time spermatozoa undergo changes in fine structures and composition involving both lipids and protein metabolism and in surface properties (Robaire & Hermo, 1988).

Dombo (2002) reported that the time required for spermatozoa to transverse the epididymis is from 4-12 days and their effective life span is 40 days. Hunter (1980) and Arthur *et al.* (1982) associated the frequent sexual activity and the small interval between semen collection with the acceleration of the movement of spermatozoa in the epididymis. This may lead to the appearance of the immature spermatozoa in the ejaculate.

2.3.2 HORMONAL CONTROL OF SPERMATOGENESIS

Bearden & Fuquay (1997) reported that the initiation of spermatogenesis is under the control of the pituitary gland. The reciprocal action of FSH, LH and testosterone is necessary for the maintenance of spermatogenesis (Salisbury *et al.*, 1978; Bearden & Fuquay, 1997).

The principal role of LH in the formation of spermatozoa appears to be in the stimulation of the release of testosterone from the Leydig cells that in turn act through the cells in seminiferous tubules to stimulate spermatogenesis. Kilgour *et al.* (1984) reported that FSH is necessary for the establishment of the normal population of Sertoli cell and the stimulation of the production of androgen-binding protein from the Sertoli cells. Androgen-binding protein binds with the testosterone making it available for its function in spermatogenesis (Bearden & Fuquay, 1997).

In sequence, the hormonal control of spermatogenesis in bulls is as follows:

- At puberty, the LH acts on Leydig cells to produce testosterone.
- Testosterone initiates the early stage of spermatogenesis in conjunction with FSH.
- The reciprocal balance of FSH, LH and the testicular androgens and estrogens maintains continued spermatogenesis.
- Testosterone through its effect on the entire male reproductive tract also aids in maintaining optimum conditions for spermiogenesis, spermatozoa transport and semen deposition near the site of fertilisation in the female.

2.3.3 FACTORS AFFECTING SPERMATOGENESIS

2.3.3.1 Environment

King (1993) reported the testes of the domestic animals to be susceptible to damage if the body temperature rises above normal, which could occur due to raised environmental temperatures. Exposure of the bull to heat stress (extreme environmental temperature)

tends to damage the primary spermatocytes, while the spermatids and spermatozoa are also sensitive to heat stress (King, 1993; Bearden & Fuquay, 1997).

Testicular temperature must be 4-5°C (Coulter, 1988; Coulter & Kastelic, 1994; Dombo, 2002) lower than the body temperature for normal spermatogenesis to occur. Casady *et al.* (1953) and McDowell (1972) reported the critical temperature for the inhibition of spermatogenesis to be 29.4 °C under conditions of continuous exposure. Higher temperature alters the scrotal thermo-regulatory mechanism (defined as the mechanism by which testes combat high and low temperature). This will increase the scrotal temperature leading to the damage of primary spermatocytes, spermatids and spermatozoa. (King, 1993; Bearden & Fuquay, 1997). Exposure of the testes to cold seems to be less damaging. Even if the testicular temperature decreases, the animal usually maintains a scrotal temperature through scrotal thermo-regulation by pulling the testes up close to the body (Setchell, 1978). Spermatogenesis is also extremely sensitive to the effect of ionising radiation and also to the humidity above 50% which destroy the dividing spermatogonia (Hafez, 1974; King, 1993).

2.3.3.2 Nutrition

The effect of nutrition on bull fertility before and after puberty is manipulated via the effect of the dietary constituents on the hypothalamic-pituitary axis, which may directly affect testicular development (Brown, 1984). Malnutrition, particularly low energy intake in males, can also impair spermatogenesis. Cupps (1991) reported circulating testosterone to often be decreased by reduced feed intake. Mann & Lutwak-Mann (1981) associated this reduction with the reduced gonadotrophin (LH and FSH) concentration, coupled with reduced sensitivity of the accessory sex gland to testosterone, which in turn may affect the efficiency of spermatogenesis.

King (1993) reported that testicular tissue is susceptible to Vitamin A and E deficiency. This deficiency act by depressing gonadotrophin secretion by the pituitary gland (Brown, 1984). Hurley & Doane (1989) reported that vitamin A and E deficiency may cause degeneration of the germinal epithelium and testicular degeneration which, may lead to

the cessation of spermatogenesis. Deficiencies in zinc and molybdenum retard testicular growth as a result of the reduced pituitary and gonadotrophin output, which affect spermatogenesis and semen outputs (Cupps, 1991). Oldham *et al.* (1978) reported that a reduction in weight of the testes is generally more marked than a reduction in body weight of domestic animals. The size of the testis is reduced if the animal is underfed. Mamabolo (1999) reported the change in testicular size, which can be achieved by change in level of roughage intake and supplementation of high quality additives, is accompanied, by a correlated change in spermatogenetic activities of the testis. This was also in line with Oldham *et al.* (1978), where a 25% increase in the testicular size was associated with 81% increase in the efficiency of spermatogenesis.

2.3.3.3 Chemical agents

Laboratory studies done by Harrison *et al.* (1997) indicated that some chemical agents in the environment, both natural and synthetic, have the potential to disrupt the endocrine system. This could at least theoretically be partly responsible for observed changes in spermatogenesis and semen quality.

A chemical such as dichlorobromopropane, which is used to eliminate soil nematodes, has been shown to cause testicular atrophy in rats (Ahmad *et al.,* 1988) and abnormal testicular biopsies (Biava *et al.,* 1987) which affect spermatogenesis. Other interesting compounds are sulphosalazine and gossypol, which are used in the treatment for ulcerative colitis in humans. Men treated with such compounds become infertile as both of them are also used as contraceptives in males (Levi *et al.*, 1982). In domestic animals, O'Morian *et al.* (1984) reported that compounds of this nature are found to affect sperm maturation rather than affecting spermatogenesis directly. Another substance, which has a very dramatic effect on the testis itself, is cadmium. Salts of this metal are reasonably toxic to the kidney and liver and also cause an extreme increase in vascular permeability in the testis of mammalian species, which leads to the stoppage of blood flow and subsequent necrosis of the testis (Setchell & Brooks, 1988).

2.3.3.4 Immunological damage

King (1993) reported that the blood-testis barrier excludes the antibodies from the seminiferous tubules. The reaction of the bull to immunology may not have an effect on the testis if the barrier is not broken. However, if the barrier is broken down (Ball & Setchell, 1983) an immunological reaction can develop and spread, leading to the loss of spermatogenic function of the testes (King, 1993).

2.4 LIBIDO AND MATING BEHAVIOUR

Libido has been identified as one of the main factors causing a variation in bull fertility. Therefore management factors that affect libido are of importance (Hoogenboezem & Swanepoel, 2000). It is important to consider the fertility of the bull via libido and mating behaviour in the dairy herd as the quality of the semen samples collected may vary according to the collection method used (Dombo, 2002). The artificial vagina method is seen as the best method for semen collection as far as the quality of sample is concerned (Bearden & Fuquay, 1997). The use of this instrument is dependent on the ability of a bull to mate and it is therefore logical to say that libido and mating behaviour determine which method is to be used.

Van Denmark & Free (1970) reported that it is obvious that the effects of season on bull fertility are complex. Its effects may be influenced by temperature, humidity and in many cases by the nutritional status of the animal. Seasonal variation in the reproduction of cattle breeds is the direct consequences of photo- periodic control of the pituitary function. Male sex drive is often less intense under hot conditions and this is evidently where libido is affected by both photoperiod and environmental temperature (Bearden & Fuquay, 1997).

Over fed and fat males may become less willing and able to mate (Cupps, 1991). Energy and protein deficiency may lead to the suppression of the endocrine system and testicular function, coupled with a diminished libido (Mann & Lutwak-Mann, 1981). Reduction in libido is associated with the nutritional deficiency of vitamin A and E (Hurley & Doane,

1989), excessive molybdenum in the diet (Brown, 1984), iodine and cobalt (Cupps, 1991).

Male sexual activities are also affected by previous sexual experience (Bearden & Fuquay, 1997). Inexperienced males are often more awkward during their first interaction with a receptive female (Cupps, 1991). Bearden & Fuquay (1997) also reported that sexual exhaustion and sexual satiety may be among the common factors that tend to lower libido when bulls are maintained exclusively for the purpose of providing semen for AI.

2.5 EJACULATION

Semen ejaculation may be defined as the ejection of semen from the body. The semen includes spermatozoa from the vas deferens and epididymis and fluid from the accessory sex gland. Ejaculation varies between the species in a number of aspects. Bearden & Fuquay (1997) indicated that the ejaculation time is less than one second in bulls, rams and bucks and more than 10-20 minutes in boars. The volume of the ejaculate is also higher in boars (200ml) compared to bulls (6ml), rams (1.5ml) and stallions (75ml). The concentration of sperm per ejaculate for bulls, rams and stallions was also reported by Bearden & Fuquay (1997) as being 1.2 billion/ml, 2.0 billion/ml and 150billion/ml respectively.

2.6. COLLECTION OF SEMEN

Ejaculated semen is the combination of the product of testicles and excurrent ducts and the secretion of the accessory glands (Van Denmark & Free, 1970). Collection of semen involves the proper scheduling and sexual preparation of the male as well as the proper use of the collection technique.

Cole & Cupps (1977) reported that during semen collection many sources of sperm losses occurs including losses before and during collection and during handling. It was also

indicated that the efficiency of semen collection depends mostly on the daily sperm output, effect of sexual preparation, increasing sperm output and also decreasing the sources of sperm losses. If semen collection is to be accomplished within a reasonable period of time, it should be well organised (Zemjanis, 1970).

2.6.1 METHODS OF SEMEN COLLECTION

The most common methods used are artificial vagina and the electro-ejaculation method. Irrespective of the method used, collection of semen requires appropriate facilities in order to prevent injuries to the animals and their handlers and the contamination of the ejaculate that may affect the quality of the sample collected (Salisbury *et al.*, 1978).

2.6.1.1 Artificial Vagina

The artificial vagina is designed to simulate the vaginal orifice of the female. Bearden & Fuquay (1997) reported that artificial vagina is the fastest and the most sanitary method and it provides a good imitation of the natural vagina. Maule (1962) reported that most AI stations prefer the use of short pattern artificial vagina to ensure that the semen is ejaculated directly into the cone or collecting tube and not in the liner.

King (1993) found that in the use of this technique, the length of vagina should be chosen so that the bull will ejaculate directly into the tube to avoid semen loss. It was further indicated that if more than one ejaculation is needed, more than one vagina should be used. Rao & Haranat (1984) reported the importance of the size of artificial vagina used. It was indicated that the use of smaller artificial vagina in bulls resulted in significantly higher mean seminal plasma volume and also higher sperm output.

2.6.1.2 Electro-ejaculation

Maule (1962) and Bearden & Fuquay (1997) reported that electro-ejaculation is used in older bulls, which lack the libido or ability to mount. The technique is also used for collecting semen from unco-orperative males or from bulls in which health problems limits their physical abilities (Maule, 1962; Cupps, 1991; Bearden & Fuquay, 1997). Zemjanis (1970) reported that the success rate with the use of electro-ejaculation is

highly dependent on the degree of experience of the operator with the type of the electro-ejaculator. It was further reported that this technique causes side effects such as over-extension, kyphosis, slipping and a marked pain response by the bull.

2.6.1.3 Other methods of semen collection

Semen samples may also be collected by massaging the ampullae of bulls, collecting the semen from mated females or by killing the male and stripping the cells from the reproductive tract and also by masturbation as it is done in dogs and chicken. These techniques frequently lead to contamination of semen samples with disproportionate contribution from accessory sex glands (Cole & Cupps, 1977).

2.7 PROPERTIES OF BULL SEMEN

Immediately after ejaculation samples should be viewed with naked eyes and the volume, density and colour noted. Den Daas (1992); Colenbrander *et al.* (1993) and Bearden & Fuquay (1997) reported that the percentage of normal sperm, motility, sperm concentration and volume per ejaculates are common criteria for evaluating semen quality at most AI stations.

Analysis of sperm morphology is an important part of routine spermiogramme, which gives an overall picture of the degree of semen quality (Serrenson, 1979). Phillis (1976) reported that semen evaluation should take place at the body temperature of approximately 37 °C.

2.7.1 Properties of normal semen in dairy bulls

Table 2.1 Average chemical composition of fresh bull semen (Bearden & Fuquay, 1997)

Constituent or property	Proportion [mg per ml]
Sodium	230
Potassium	140
Calcium	144
Magnesium	9
Chlorine	180
Fructose	530
Citric acid	720
Glycerylphoshorylcholine (GPC)	35×10^4
Protein [gram per ml]	6.8
Sorbitol	75

Table 2.2 Characteristics of fresh semen in dairy bulls (LSM±SE) (Serrenson, 1984)

	Friesland	Jersey
Concentration	71.2±2.7	75.6±1.3
Motility (%)	74.6±1.4	81.3±4.1
Morphological normal (%)	70-80	70-80
Abnormality (%)	13.7±1.3	10.3±1.5
Volume (ml)	5.7±0.38	4.3±0.21

2.8 MORPHOLOGY OF SPERMATOZOA

The morphology of spermatozoa is used as one of the important criteria in the evaluation of semen quality in domestic animals (Howard *et al.*, 1983). The fully formed spermatozoa are elongated cells consisting of a head containing DNA and a tail which provide the cell with motility (Mamabolo, 1999). Bull spermatozoa have an overall length of about 68-74μm. The head is about 8-10 μm. The neck, which connects the base of the head to the mid-piece and contains the proximal centriole, is about 0.3-1.5 μm long. The mid-piece is 8-10 μm and the tail is about 45-50 μm long.

Spermatozoa obtain motility from the contractile elements located in the longitudinal fibres of a tail through the process of spermatozoa metabolism. Spermatozoa metabolism is defined as a process by which spermatozoa convert nutrients into usable forms of energy (Mamabolo, 1999). The enzymes for this conversion are situated in the mitochondrial sheath. These principal energy substrates are fructose, sorbitol and GPC (Table 2.1) which are found in seminal plasma and a plasmologem (a lipid found within the spermatozoon as an energy reserve) that can be used when other substrates are limited (Bearden & Fuquay, 1997).

A detailed assessment of sperm morphology in the domestic animals can be studied as the percentage of normal sperm cells and the occurrence of major and minor sperm defects (Van Rensburg, 1957; Van Denmark & Free, 1970 Serrenson, 1979). Spermatozoa defects are classified as major and minor defects (Table 2.3). Major defects are related to impaired fertility or abnormal conditions in the testis. Minor defects should be of concern only when they exceed 10% (Van Rensburg, 1957; Van Denmark and Free, 1970; Bearden & Fuquay, 1997). Salisbury *et al.*, (1978) and Van Rensburg (1957) reported the following morphological abnormalities to be important in the reproduction of a bull (Table 2.3)

1. Abnormal acrosomes: e.g. knobbed acrosomes, which are characterised by localised swellings on the apical ridge; ruffled acrosomes, which are characterised by a wrinkled appearance.

2. Abnormal nucleus: It appears as a dark necklace along anterior edge of the posterior nuclear cap and indicates abnormal spermiogenesis.

3. Tailless sperm (Disintegrated or Decapitated sperm): This entails when the tail separates within the caput epididymis. This defect is hereditary in some of the breed such as Guernsey. The appearances of tailless sperm in the ejaculate are the early indications of testicular degeneration as a result of exposure to heat stress and severe under-nutrition.

Table 2.3 Classification of sperm morphology according to major and minor defects in bulls (Salisbury *et al.*, 1978)

Major defects	Minor defects
1. Underdeveloped	16. Narrow heads
2. Double forms	17. Small, normal heads
3. Knobbed sperm defect	18. Giants heads and short broad heads
4. Decapitated sperm	19. Free heads (normal)
5. Diadem defects	20. Detached acrosomal cap
6. Pear shaped head	21. Abaxial implantation
7. Narrow at the base	22. Distal droplets
8. Abnormal contour	23. Simply bent or coiled tail
9. Small abnormal heads	24. Terminally coiled tail
10. Free abnormal heads	
11. Corkscrew defect	
12. Other mid piece defects	
13. Proximal droplets	
14. Psuedodroplets	
15. Dag defects	

4. Abnormal mid piece: This is where the mitochondrion helix is absent and the axial fibres are frayed at the distal portion of the mid-piece. Another type of mid piece defect may be termed "psuedodroplets", which is a major sperm defect in the semen of Friesland bulls. This defect is located near the centre of the mid-piece and appears as a rounded or elongated thickening that contains dense granules surrounded by mitochondria.

5. Bent and coiled tails: This may be the most common aberration in the ejaculated semen of the bull and could be associated with reduced fertility.

6. Cytoplasmic droplets: This is usually an indication of incomplete maturation in the epididymis (Hunter, 1980).

7. Van Rensburg (1957) also reported semen abnormalities such as loose heads, acrosome abnormalities, abnormal shape of the head, mid-piece abnormalities, cytplasmic droplets, coiled tails, deformity and medusa forms

2.9 SUMMARY AND CONCLUSIONS

The production of semen by dairy bulls is dependent largely on the growth and sexual development of the bull from birth to puberty (defined as the age at which spermatozoa are present in the ejaculate). Normal reproductivity in the male is comprised of the production of semen with normal and adequate spermatozoa with the desired ability to mate. These sexual functions (sexual development, production of spermatozoa and the desired ability to mate) are under the control of gonadotrophin hormones such as testosterone, LH and FSH. These gonadotrophin hormones are influenced to a larger extent by a combination of the environmental factors such as temperature, nutrition and animal management practices.

Temperature is one of the important factors affecting reproduction. Periods of high temperature damage the spermatogenic cells and lead to testicular degeneration, reduction in the efficiency of spermatogenesis and hence poor semen quality. When bulls are fed low energy diets for a prolonged period, the onset of puberty is delayed, libido and testosterone production is affected and semen production and quality characteristics are depressed.

Scrotal circumference is highly correlated with age and sexual functions of a bull (Mamabolo, 1999). It is therefore very important to assess the relationship between

growth and development of reproductive system, scrotal circumference and semen production and quality. Knowledge of this relationship will permit the prediction of the effect of development of reproductive system on semen production and quality (Knight *et al.*, 1984).

CHAPTER 3

FACTORS AFFECTING THE QUALITY OF SEMEN

3.1 EFFECT SCROTAL CIRCUMFERENCE ON SEMEN QUALITY

Scrotal circumference (SC) is a very important trait in bull reproduction, which can be used as a measure of fertility. It is also an important component in examining bulls for breeding soundness (Hoogenboezem & Swanepoel, 2000). SC has a heritability estimate of 50% (Lasley, 1978), which means that it is influenced to a large extent by genetic rather than environmental factors. Scrotal circumference is positively correlated with the age of the animal (Brito *et al.*, 2002a). Several experiments have reported a high a positive relationship between scrotal circumference and sperm production and semen quality in growing bulls (Hanks *et al.*, 1981; Vale Filho *et al.*, 1997; Godfrey *et al.*, 1990; Gipsom *et al.*, 1985; Neville *et al.*, 1988; Palasz *at al.*, 1994; Hoogenboezem & Swanepoel 2000). This also agrees with the results in the study of Coe (1999) where scrotal circumference and age was associated with approximately 11% variation in semen quality. Hoogenboezem & Swanepoel (2000) indicated that scrotal circumference is positively correlated with the overall potential breeding efficiency and seminal characteristics (such as percentage live sperm, sperm quantity, motility and sperm concentration).

Brito *et al.* (2002a) found scrotal circumference to have a positive relationship with ejaculate volume and negative correlation with sperm motility and major sperm defects. Further more, it was also reported scrotal shape to have a negative correlation with minor sperm defects. Scrotal neck perimeter showed a negative correlation with major and total sperm defects. The temperature of a scrotum also plays a major role in determining the production and quality of semen. Coulter & Kastelic (1994) and Kastelic *et al.* (1995; 1996; 1997) reported the scrotum and testicular vascular cone (TVC) to participate in the

testicular thermo-regulation ability and therefore variation in these structures among bulls could affect the quantity and quality of semen produced. Coulter (1988); Coulter & Kastelic (1994) and Mapletoft *et al.* (1996) indicated that a pendulous scrotum may improve the testicular thermo-regulation by moving the testis away from the body and thus facilitating heat loss. Brito *et al.* (2002b) also reported that the scrotal surface temperature had a negative correlation with sperm motility and that scrotal surface temperature gradient had a positive correlation with sperm concentration.

Hoogenboezem & Swanepoel (2000) suggested that semen quality and scrotal circumference are affected by factors related to underdevelopment of the testes and testicular degeneration. These factors may be related to certain management practices such as feeding excessive energy which leads to fat deposition in the scrotum and reduced body condition of the bull (Coulter & Kozub, 1984; Cupps, 1991). Small scrotal size may also result from the lack of adequate testicular development (King, 1993) due to thermal and nutritional factors which interfere with the endocrine regulation of reproduction (Bearden & Fuquay, 1997).

3.2 EFFECT OF AGE OF THE BULL ON SEMEN QUALITY

Almquist & Amann (1976); Almquist (1982) and Everett & Bean (1982) claimed considerable attention to be paid to the evaluation of changes in semen quality due to variation in the age of bulls. Coe (1999) associated age with one of major causes in the variation of semen quality among the bulls, due to physiological changes that occur as bulls grows to sexual maturity.

Salisbury *et al.* (1978) found semen production and quality to increase beyond the first year after puberty. Salisbury *et al.* (1978) found a correlation of 0.51 between sperm production and the age at first pubertal life in Holstein bulls. Almquist (1978) reported the main factor determining the total number of sperms produced to be the size of the testis, which is correlated with age of the bull (Brito *et al*, 2002b). Testis size increases for at least 5 years after puberty (Amann & Almquist, 1976). However as maturity and

age advances, the detrimental effects of stress and disease are likely to cause that the direct relationship between the semen production and age disappear.

Serrano (1984) indicated that bulls from 2-5 years of age produce larger volumes of ejaculate compared to bulls less than 2 years of age and bulls older than 5 years of age. Several researches such as Almquist & Amann (1976); Everret & Bean (1982); Fuente *et al.* (1984); Shannon & Vishwanath (1995); Garner *et al.* (1996) and Mathevon *et al.* (1998) reported the volume of the ejaculate, sperm concentration and semen motility to improve with an advance in age of the bull.

Table 3.1 shows that quality of semen increases with advancing age of the bull. This however, contradicts Brito *et al.* (2002a) who claims no significant effect of age on sperm concentration and motility. On the other hand, Kumi-Diaka *et al.* (1981) indicated that irrespective of the breed type, either exogenous or endogenous sperm cell concentration was significantly higher in the younger matured bulls of 3-7 years compared to bulls of 7.5-10 of age.

Table 3.1 Differences on semen quality characteristic in the ejaculates of young and mature bulls (Mathevon *et al.*, 1998)

	Volume (cm^3)	Concentration (10^5 / ejaculate)	Motility (%)	Total sperm (10^4 / ejaculate)	Motile sperm (10^4 / ejaculate)
Young bull	5.48	1296	51	7090	3757
Mature bull	6.73	1380	57	9310	5339

Spermatozoa morphology is often used as one of the important criteria in the evaluation of semen quality in domestic animals (Howard *et al.*, 1983). An increase in the frequency of abnormal sperm cell has been observed with advancing age in bulls (Rao, 1971). This is also similar to the results reported by Wolfe *et al.* (2000) where it was reported that males at the age of 6 and 7 years produce fewer normal structural sperm than their younger counterparts. Even in the human Kidd *et al.* (2001) reported an increase in

semen volume of 3-22 %, sperm motility of 3-37 % and number of normal sperm of 4-18 % to be associated with an increase in age. Gustafson & Sekoni (1980) reported that old bulls (5-8 years) had higher incidence of sperm abnormalities than young bulls (1.5-2 years) and younger bulls less than 13 months had higher incidence of head abnormalities, proximal and cytoplasmic droplets than bulls of 1.5-2 years of age. This is in agreement with Salisbury *et al.* (1978) where the increase in the incidence of some defects such as abnormal mid pieces and pseudo-droplets were associated with advancing age of the bull

Dowsett & Knott (1996) reported that all semen characteristics with the exception of colour and urethral pulsation in the stallion vary significantly with age. It was further indicated that semen quality characteristic such as gel free volume, sperm concentration, total sperm number and sperm abnormalities were poorest in a stallion under the age of 3 years and over 11 years of age.

Van Denmark & Free (1970) also reported semen volume to increase with age and body weight. It was also indicated that young bulls just coming into service produce as little as 1-2 million sperm per ejaculate, while fully matured bulls produces 10-15 million sperm per ejaculate. This is also in line with the results of a study done by Mamabolo (1999) where a high correlation of 0.90 between the body size and semen production was recorded.

In dairy bulls, Bearden & Fuquay (1997) reported the characteristic of the ejaculates to differ according to the size of the breed. It was found that large dairy bulls had semen volume, motility and concentration of 7-8ml, 50-80%and 1-1.5 10^6 per ml respectively, while small breeds have 5-6ml, 50-80% and 1-1.5 10^6 per ml respectively.

3.3 EFFECT OF THE ENVIRONMENT ON SEMEN QUALITY

The major contribution to the semen variation is via the environment (Curtis, 1983; Cupps, 1991; King, 1993; Bearden & Fuquay, 1997). To comprehend the impact of environment on semen quality, it is necessary to understand the biological significance of

this term. It could be defined as all factors surrounding the animal, which include factors such as environmental temperature, nutrition, humidity, seasonal changes and management of an animal (Bonsma, 1980).

3.3.1 Effect of temperature, humidity and pollution on semen quality

Vale Filho *et al.* (1997) reported that spermatogenesis, epididymis function, spermatic ducts and sexual ability may be altered by factors such as high temperature, humidity, deficient nutrition and high parasite infestation.

Ambient temperature is one of the most important factors affecting reproduction (King, 1993). The effect of temperature on semen quality should be treated with caution for the following reasons (McDowell, 1972):

i. Firstly, temperature often modifies feed intake and changes ascribed to temperature may really be due to altered nutrition.
ii. Secondly, temperature and photoperiod are positively correlated in many parts of the country and under natural conditions, it might be difficult to differentiate which portion of the effect is due to temperature or due to photoperiod.

Waites (1970) and Setchell (1978) reported thermo-regulation of the testis to be essential for semen production and quality. Several studies reported that temperature has an adverse effect on sperm production (Wolfe *et al.,* 1986) and semen quality (Wideus & Entwistle, 1986; Coulter, 1988; Volger *et al.,* 1993). Howard *et al.* (1965) and Burefening & Ulberg (1968) reported that besides affecting the formation of sperm, there is evidence that spermatozoa are susceptible to heat damage either while in the male or stored *in vitro* or while transported in the female reproductive tract.

Rathore (1970) and Stephan *et al.* (1971) reported an increase in the frequency of abnormal sperm cells to be associated with extreme temperatures such as heat stress.

During a period of heat stress, degenerative changes in the seminiferous tubules (Kumi-Diaka *et al.*, 1981), testicular degeneration (Mamabolo, 1999) and abnormal scrotal thermogram (Lunstra & Coulter, 1997) occur in bulls. Kumi-Diaka *et al.* (1981)

associated the degenerative changes in the seminiferous tubule to be a result of exposure to heat stress with lower semen output in terms of sperm cell concentration, percentage of live spermatozoa and percentage abnormalities. Coulter (1988); Cook *et al.* (1994) and Lunstra & Coulter (1997) reported that bulls exhibiting abnormal scrotal temperature as the result of exposure to heat stress had a lower percentage of sperm exhibiting normal head and tail morphology and a higher percentage of sperm with proximal droplets.

Skinner (1965) reported the effect of ambient temperature on semen quality to be dependent on the period of exposure to heat stress. In the trial, exposure of the bull to 40 °C for 6-8 weeks caused the reduction in sperm motility, percentage of live spermatozoa and an increase in the number of morphologically abnormal spermatozoa in the ejaculates-with little effect on semen volume, semen pH, total fructose content and total number of spermatozoa ejaculated. This is in agreement with the results reported of Curtis (1983) where a third decrease in percentage normal sperm and by a over third in of the percentage live sperm was observed in bulls exposed to heat stress for the period of two weeks.

Casady *et al.* (1953) reported the exposure of bulls to heat stress to reduce sperm motility and sperm concentration. Furthermore, Karanibus *et al.* (1997) associated these effects with the reduced sperm transport and fertilising ability in Holstein bulls. Ciereszko *et al.* (2000) reported high temperatures during spermatogenesis to cause fluctuation in acrosome activity, which may cause a disturbance in the membranes covering the entire sperm. The membranes covering the entire spermatozoa are divided into several domains (Park & Lynch, 1992). Each of these domains plays a specific role in spermatozoa such as recognition and binding of autologous zona pellucida, penetration of zona pellucida and fusion to the oocyte plasmalemma during fertilisation. These sperm membranes are structurally dynamic (Buhr *et al.,* 1993) and the rate of molecular reorganisation in these structures differ in different areas of the same contiguous membrane of the sperm and the membrane which cover the head of the sperm (Wolfe *et al.,* 1986). Buhr *et al.* (1993) reported the molecular reorganisation of these structures to be affected by the temperature to which the bull is exposed. A change in temperature during collection and storage of

semen causes changes in the composition and structures of these domains, which impair their fertilising capacity during AI or natural mating (Parks & Lynch, 1992).

Strong, progressive motility is an important index of the viability of the sperm and is usually seen as swirling, wave -like motions in highly concentrated ejaculates of the bull (Hunter, 1980). The motility of the spermatozoa varies with temperature and sperm metabolic activity (Salisbury *et al.*, 1978) The metabolic activity increases with an increase in temperature up to 37 °C, where protein denaturing occurs and thereafter sperm lose their motility and die (Van Denmark & Free 1970). Phillis (1976) found the most obvious sign of cold shock to be loss of motility and indicated that during cold shock, there is a decrease in the rate of fructose breakdown, decrease in the uptake of oxygen and fall in ATP production resulting in poor semen motility. Hafez (1974) reported semen quality and quantity to be depressed during period of high temperatures, accompanied by high humidity. This is also similar with reports by Casady *et al.* (1953) that sperm motility, sperm output and concentration decrease at a temperature humidity index (THI) of 37 °C and 70%.

Several reports have suggested that semen quality has declined throughout the world over the last two decades, as a result of environmental pollution by chemicals. Multigner *et al.* (2000) reported that chemicals in the environment, acting as endocrine disrupters, have been implicated. Chemicals with anti-androgenic properties that have been detected in the environment have significant effects on the development and functioning of the accessory sex glands and may be partly responsible for the observed decline in semen volume. Selevan *et al.* (2000) reported periods of elevated air pollution to be significantly associated with decrease in other semen quality measures, including proportionately fewer motile sperm, proportionately fewer sperm with normal morphology or normal head shape and proportionately more sperm with abnormal chromatin content. It was further indicated that this effect of air pollution is sometimes age dependent. Young men and animals may experience an alteration in sperm quality during a period of elevated air pollution without change in sperm number, compared to their older counterparts.

The interval between two collections significantly affects all semen quality traits in young and mature bulls (Mathevon *et al.*, 1998). Hunter (1980) suggested that too frequent semen collections may lead to a high frequency of sperm cells with cytoplasmic droplet, which indicates an incomplete phase of sperm maturation in the epididymis.

Schwab *et al.* (1987) indicated that for young and mature bulls, volume of ejaculate, sperm concentration and total number of sperm per ejaculate were generally highest when using an interval fewer than 4 days. These results agree with those reported by Seidel & Foote (1969); Amann & Almquist (1976); Everret *et al.* (1978) and Everret & Bean (1982) where volume of the ejaculate, sperm concentration and total number of spermatozoa per ejaculate were lower with a shorter period between collections for both young and mature bulls.

3.3.2 Effect of nutrition on semen quality

Semen quality and quantity are the major factors of concern in reproduction and can be adversely affected by malnutrition (Brown, 1984). Reduction in feed intake in animals exposed to high temperatures may lead to a deficient protein and energy intake. Severe under-nutrition or over-feeding and deficiencies of specific nutrients as the result of exposure to heat stress are the most common causes of impaired reproductive capability of the bull in terms of semen production and quality (McDowell, 1972).

Brown (1984) found reproductive function in the young bull to be more susceptible to dietary restriction of energy and protein than in adult. This was also similar to Cupps (1991) who indicated the negative effect of restricted nutrition to be more abundant in young than in old animals.

There is evidence that the effect of nutrition on semen quality is mediated via the effect of dietary constituents on the hypothalamic-pituitary axis, although there are also some indications that dietary changes affect the testis growth indirectly (Brown, 1984). A restricted diet is associated with a decrease in the release of LH and FSH, which affects semen production and quality through the effect on testicular size (King, 1993). Similar

results have been also reported by Oldham *et al.* (1978) and Martin *et al.* (1987) where the level of nutrition was associated with a reduction in testicular size-which in turn affects the production and quality of spermatozoa. A low plane of nutrition suppresses the production of gonadotrophins by the pituitary gland and the secondary sex hormones, so that atrophy of the prostrate and seminal vesicles occur thereby affecting semen quality in terms of fluid volume and concentration (Alkas *et al.*, 1982). During the periods of nutritional stress, the animal body secretes adrenocorticotropic hormone (ACTH), which in turn stimulate the secretion of glucocorticcoids, which lower the circulation or secretion of FSH and LH and hence inefficient spermatogenesis and poor semen quality (Bearden & Fuquay, 1997).

The interaction of diet and breed have been shown by researchers to affect reproductive parameters such as semen volume (Field *et al.*, 1979), epididymal sperm reserves (Buhr *et al.*, 1993) and sperm reserves (Coulter & Bailey, 1988) which may potentially affect fertility in dairy bulls. Diet has also been shown to affect the membranes associated with the protein receptors (Knazek & Lui, 1979) or enzymes in the liver (Pugh & Kate, 1980) and the salivary gland (Alam & Alam, 1986). Buhr *et al.* (1993) claimed that dietary fatty acids also exert a direct effect on these membranes. It was also indicated that dietary changes alter not only the composition but also molecular interaction within the cellular sperm membranes.

Cupps (1991) reported deficient nutrient intake for example iodine, zinc, cobalt, vitamin A, E and minerals are associated with a reduction in semen quality in terms of morphology, concentration and motility.

Scott *et al.* (1998) reported that in many species, a selenium deficiency affects the morphology and motility of the spermatozoa and may be linked to the sub-fertility in many domestic animals. The spermatozoa membranes are attacked by the increasing formation of oxygen reactive species which lower the viability and fertility of the spermatozoa (Irvine, 1996). Selenium increases the formation of anti-oxidant glutathione

peroxide activity, which decreases the reactive oxygen species and hence increase in spermatozoa viability and fertility (Bray *et al.*, 1997).

Underwood (1981) reported that zinc is responsible for larger semen production as it is involved in the nucleic acid and protein metabolism for production of the sex hormones, including testosterone and GnRH (Hambidge *et al.*, 1986). Underwood & Sommers (1969) indicated that zinc requirements for spermatogenesis are greater than that the requirements for body growth, so a deficiency may alter spermatogenesis and lead to a high proportion of abnormal spermatozoa. Saaranen *et al.* (1987) found that zinc deficiency affects the morphology and abnormalities in semen since it is associated with the attachment of the head to the tail. Bray *et al.* (1997) reported that zinc deficiency may also lead to an increase in reactive oxygen species, which affect sperm viability. Zinc also contains anti-oxidant properties that act to reduce reactive oxygen species leading to an increase in semen viability.

Prolonged dietary vitamin A deficiency impaired semen quality and semen production. Vitamin A deficiency lowered the spermatozoa concentration, semen storage capacity and also delayed sexual maturity and suppressed spermatogenesis in young bulls (Cupps, 1991). Rode *et al.* (1995) reported the proportion of spermatozoa with morphological defects to be greater in bulls with vitamin A deficiency. Vitamin B complex has a marked effect on appetite and its deficiency may cause inanition and adversely affect semen production through a reduction in testicular and body weight. Phillip & Lardy, (1940) claimed that vitamin C deficiency leads to a reduction in sex drive and semen quality in manner similar to vitamin A.

The distribution of major ions between sperm fractions and seminal plasma could provide the basis for variation in semen quality and should be considered in the interpretation of the result obtained in the evaluation of semen. Abdel-Rahman *et al.* (2000) reported that in small ruminants the percentage of motile spermatozoa decreases as the content of potassium and calcium increases and sodium, chloride, phosphorus and magnesium decreases. This is in line with Bearden & Fuquay (1997) who reported high

concentrations of potassium and calcium to be detrimental to semen metabolism of the bull spermatozoa, which determines the motility of spermatozoa On the other hand, Abdel-Rahman *et al.* (2000) also reported the percentage of live spermatozoa to be correlated positively with potassium and calcium and negatively correlated with phosphorus.

3.3.3 Effect of season on semen quality

Season can include many factors, such as temperature, photoperiod, humidity and feed quality. Differences in the quantity of feed (Siratskii, 1990) or in feed composition (Castillo *et al.*, 1987) and environmental temperature and humidity and seasonal variation (McDowell, 1972) could affect semen output.

Ahmad & Noakes (1996); Ibrahim (1997) and Mathevon *et al.* (1998) reported the month and season of the year to have a significant effect on semen quality parameters. In the area where there is marked seasonal variation in environmental temperature, bull semen quality tend to be lower during summer months (Curtis, 1983) as this results in thermal stress which causes testicular degeneration and abnormal scrotal thermo-gramm and hence lower the semen output (McDowell, 1972; Curtis, 1983).

When considering the effect of season on semen quality, ambient temperature and humidity has to be interpreted carefully. Everett & Bean (1982) associated the increase in semen output with the humidity below 50%. Curtis (1983) reported that after bulls exposed to temperature humidity index (THI) of 37 °C and 80% during specific season, the sperm concentration and total sperm output decreased markedly.

Mathevon *et al.* (1998) observed no significant variation in the volume of ejaculates and sperm motility with season in matured bulls. These results agree with Afiefy *et al.* (1984) where no variation in the volume of the ejaculate with season was observed in Friesian and Buffalo bulls. Abi Saab &d Hamadeh (1984) reported that in rams, volume of the ejaculate was affected by the season of semen collection and it was observed to be higher during August to October and lower during winter and early summer.

Regardless of the age and breed of the bull, the semen concentration and total number of cells were higher during winter and spring than summer and fall (Mathevon *et al.*, 1998). It was also stated that for young bulls, the highest percentage of motile spermatozoa was usually obtained in summer and fall. Schwab *et al.* (1987) reported that the season of collection not to affect the percentage of motile spermatozoa in mature bulls. The results found by Schwab *et al.* (1987) were contradictory to those reported by other researches such as Fuente *et al.* (1984); Rustenev (1989) and Stalhammar *et al.* (1989) who found the percentage of motile spermatozoa to be poor during spring and winter, which could be due to climate, which determines the nutritional status of the bull.

Parkinson (1985; 1987) and Soderquist *et al.* (1996) reported significant seasonal variation to occur in the incidence of sperm head abnormalities and total sperm abnormalities and the least square means for sperm abnormalities to be significantly higher during the warmer season (spring and summer) compared to the colder season (autumn and winter). On the other hand, Trudeau & Sanford (1986) reported ejaculated protein and citric acid to be higher in winter and fall and to decrease in spring (which is associated with increase in temperature).

3.4 SUMMARY AND CONCLUSIONS

The percentage of normal sperm, motility, sperm concentration and ejaculate volume are common criteria for evaluating semen quality at most AI stations. Numerous studies have shown variation to exist in semen quality of the bulls to be related to factors such as scrotal circumference (Mamabolo, 1999), age and breed (Brito *et al.*, 2002a), environmental factors such as temperature and humidity, nutrition, seasonal (McDowell, 1972) and management-related factors (Mathevon *et al.*, 1998).

Scrotal circumference is a very important trait in bull reproduction and positively correlated with age (Hoogenboezem & Swanwpoel, 2000). Several experiments have found a positive relationship between scrotal circumference and sperm production and

semen quality in growing bulls (Mamabolo, 1999; Brito *et al.*, 2002b). A decrease in scrotal circumference may result in an increase of abnormal spermatozoa. The major contribution to the semen variation is environment. Environmental temperature is one of the factors affecting reproduction. High ambient temperature lead to abnormal scrotal thermograms and testicular degeneration, which result in an increased percentage of abnormal spermatozoa and a lower percentage of normal sperm in the ejaculate. Nutritional restriction is less evident in male fertility, but nutritional deficiencies depress the production characteristic of semen such as the increase in the frequency of abnormal spermatozoa, reduction in spermatozoa motility and fertilisation capacity of the sperm. Season of semen collection affected all semen quality parameters. Where there is marked seasonal variation in temperature, humidity and nutrition, semen quality tends to be lower during summer months (Curtis, 1983). This may be due to thermal stress that reduces the appetite and hence affects feed intake leading to testicular degeneration and abnormal scrotal thermograms, resulting in poor semen quality. It is also known that for the same animal, the fertilising potential of semen does not remain the same throughout the year especially as the animal advances in age (Dombo, 2002). The determination of those factors that causes variation in semen quality and the extent of the variation is of vital importance.

CHAPTER 4

MATERIALS AND METHODS

4.1 Experimental site

The study was conducted at the Taurus Coop, Irene, (25°55 South, 28°12 East), South Africa. Taurus Coop. is situated in the highveld at an altitude of 1525m above the sea level. The long-term average rainfall for the area is 640 mm per annum with summer rainfall (October to January) and dry period from March to August. The climatic condition during the period of the study (1998) is indicated in table 4.1.

Table 4.1 Monthly climatic variation in terms of temperature, rainfall and relative humidity at Taurus Coop. Irene, during the period of January to December 1998

Months	Rainfall (mm)	Min. temperature (°C)	Max. temperature (°C)	Rel. humidity (%)
January	137.2	15.9	26.8	67
February	44.8	16	27.9	60
March	72.7	15.6	27.8	59
April	13.7	12.6	26.4	50
May	0	6.7	22.8	34
June	0	5.9	22.1	26
July	0	5.8	19.0	40
August	0	6.4	21.4	38
September	49.6	11.5	24.9	46
October	63.1	13.6	24.8	57
November	162.4	14.4	25.3	66
December	96.2	14.7	24.9	69

4.2 Experimental animals

In this study, 329 dairy bulls from two breeds namely Friesland and Jersey were used. Friesland bulls were dominant in the study (n =271 Friesland bulls, which comprise 82% of the experimental animals), while only 58 Jersey bulls were available. Each bull was

used for more than one observation on the effect of age, breed, season and their interactions. One set of observations was selected randomly from each bull. The age of the bulls ranged between 12 to 156 months. All bulls younger than 12 months and older than 150 months were discarded from the study. Eight classes were derived from this age range as indicated in Table 4.2. The two breeds tested (Friesland and Jersey) differed in scrotal circumference in relation to age and body weight as indicated in Table 4.3.

Table 4.2 Number of bulls and classification of age groups during the semen collection

CLASS	NUMBER OF BULLS	PERCENTAGE	AGE GROUP
1	01	0.30	12 to 23 months
2	81	24.62	24 to 35 months
3	49	14.89	36 to 47 months
4	58	17.63	58 to 69 months
5	62	18.84	60 to 71 months
6	54	16.41	74 to 83 months
7	10	3.04	84 to 95 months
8	14	4.26	≥ 96 months
	Total 329	**100**	

Table 4.3 Mean scrotal circumference (mm), body size (kg) and age (months) of bulls sampled

AGE CLASS (m)	BREED			
	FRIESLAND		JERSEY	
	Weight (kg)	Scrotal Circumference (cm)	Weight (kg)	Scrotal Circumference (cm)
24 months	530	30.5	325	27.9
24-60 months	680	35.8	450	33.1
60-72 months	720	42.5	590	39.6
72 months and above	770	37.3	635	34.4

4.3 Animal management

4.3.1 Feeding of animals

Bulls were fed 16kg Eragrostis hay and 4 kg 17 % protein Epol feed per 700-kg bull per day. Water was provided *ad libitum.*

4.3.2 Preparation of bulls and sexual stimulation

During the study, no artificial hormones were used to sexually stimulate the bulls. Sexual excitements prior to semen collection was induced by parading of the dummy or teaser bulls around the bull. At least three false mounts were allowed to enhance the degree of sexual excitement.

4.4 Semen collection

4.4.1 Collection interval

Semen collection was done twice a week (Monday and Thursday or Tuesday and Friday). One ejaculate was collected from each bull during each collection session. Collection was always performed in the morning between 7 and 9 am.

4.4.2 Semen collection schedule

The collection of semen was scheduled during all four seasons of the year, namely summer, autumn, winter and spring. The number of observations obtained in each season varied according to the number of bulls used in each season. For each season, the total number of bulls used per breed varied as indicated in Table 4.4.

Table 4.4 Distribution of the number of bulls by season and breed

SEASON	TOTAL BULLS (n)	FRIESLAND BULLS (n)	JERSEY BULLS (n)
Autumn	62	44	18
Summer	140	109	31
Spring	63	58	5
Winter	64	60	4

4.4.3 Preparation of the Artificial Vagina

A standard bovine artificial vagina with rough latex liners, at a temperature of 42° C was used for all bulls. During the collection process, the same semen extender (Triladyl) was used as a lubricant. Lubrication was used to provide a comfortable passage for the penis during mounting and careful attention was paid to prevent the excessive lubrication in order to prevent contamination of the ejaculate.

4.5 Laboratory evaluation of semen samples

The morphology of spermatozoa morphology was assessed after staining with Bloms eosin-nagrosin stain and examining 100 sperm cell at 100x. The following defects were evaluated

The percentage normal sperm, percentage major sperm defects, percentage minor sperm defects, teratoid, knobbed acrosomes, pyriform, nuclear vacuole, nuclear ridge, macrocephalic, microcephalic, abnormal lose head, double forms, degenerated or lose heads, corck screw, stump tail, mid-piece reflexes, other mid-piece, dag defects, broken flagellum, proximal droplets, pseudo-droplets, normal lose head, degenerative or lose acrosome, abaxial implantation, mitochondrial plasia, curled end-piece, distal droplets

4.6 Statistical analyses of the data

Statistical analyses of the data were done by using the general linear model (GLM) procedure of the Statistical Analyses System (SAS, 1999).

Analyses of variance on the effect of breed, season and the interaction between breed and season were done with the use of mentioned GLM procedure together with least square means (LSM) option where age was used as a continuous variable with an average of 56 months.

Effect of age on the different age groups was analysed using the (AT) value option of the general linear model (GLM) procedure, which made it possible to select realistic and logical co-variates we may consider interesting. In the study, this procedure was used to

get the LSM (least square means) for the effect at different values of age namely, 12, 24, 36, 48, 60, 72, 84 and 96 months instead of the average of 56 months, which was used as a continuous variable for the effect of breed and season. Similar procedures were also used on the analyses of variance for the effect of the interactions between age and breed and the interaction between age and season.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 EFFECTS OF BREED ON SPERM MORPHOLOGY

A. Effect of breed on percentage of normal sperm

The results of the study indicate that breed has no significant effect on the percentage normal sperm (Figure 5.1). The two breeds tested, Friesland and Jersey, both had a high percentage of normal sperm (80.6±1.07% and 78.9±2.31%, respectively) (Table 5.1). These results are in line with those reported by Coe (1999) who considered more than 70 –80 % normal sperm to be acceptable for optimal sperm morphology. On the other hand, the results of this study differ from that reported by Coe (1999), in that no significant breed differences were observed in the present study. These results obtained are similar to those in a study on the effect of breed on semen quality of Hereford and Simmental bulls by Buhr *et al.* (1993), where no significant effect of breed was recorded in semen characteristics.

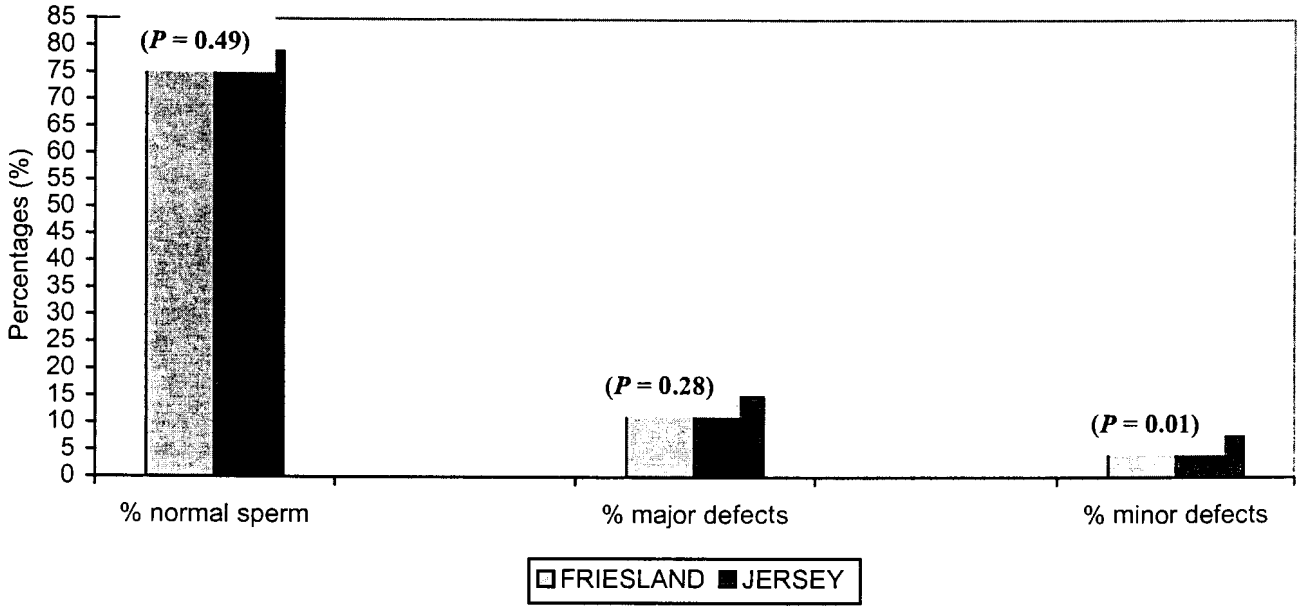

Figure 5.1 Graphical presentation of the effect of breed on the percentage normal sperm, percentage major and minor sperm defects

B. Effect of breed on major sperm defects

The study demonstrated that breed has no significant effect on the percentage major sperm defects. The two breeds tested (Friesland and Jersey) recorded 14.8±0.90% and 15.0±1.97% major sperm defects respectively (Figure 5.1). Based on the observations by Van Rensburg (1957); Salisbury *et al.* (1978) and Bearden & Fuquay, (1997), the effect of breed on the percentage major sperm defects was insignificant in this study as they fall below the critical value of 20%. The results obtained in this study are similar to those of Brito *et al.* (2002b) who found no differences between *B. indicus* and *B. taurus* bulls regarding the percentage major defects. Even though there was no significant difference between breed regarding percentage sperm defects (Table 5.1), major sperm defects such as abnormal lose head ($P = 0.05$) and nuclear vacuole ($P = 0.02$) differed significantly between breeds. This is similar to the results obtained by Soderquist *et al.* (1996), where abnormal lose heads and detached heads were observed to vary significantly between breed. On the other hand, major defects such as degenerative heads and double forms did not differ significantly between the breeds studied in the study.

Table 5.1 Effect of breed on sperm morphology (LSM±SE)

Semen defects	Friesland	Jersey	*P* value
% Normal sperm	80.6±1.07	78.9±2.31	0.49
Major defects			
% Major defects	14.8±0.90	15.0±1.97	0.89
Nuclear vacuole	2.4±0.43	0.8±0.96	0.02
Abnormal head loose	0.3±0.07	0.01±0.16	0.05
Double forms	0.1±0.03	0.2±0.06	0.27
Degenerative heads	0.3±0.06	0.1±0.13	0.16
Minor defects			
% Minor defects	5.1±0.43	7.6±0.94	0.01
Distal droplets	2.5±0.37	5.2±0.80	0.01
Loose acrosome	1.32±0.17	0.89±0.37	0.27
Normal loose head	0.6±0.08	0.8±0.10	0.01

C. Effect of breed on minor sperm defects

The study showed that breed affected the percentage minor sperm defects significantly (P = 0.01) (Figure. 5.1). Friesland and Jersey recorded 5.1±0.43% and 7.6±0.94% minor defects respectively. According to Van Rensburg (1957), the effect of breed on minor defects was also insignificant in this study as these did not exceed the critical value of 10%, but the Jersey were more susceptible. Minor defects such as distal droplets (P = 0.01) and normal head lose (P= 0.01) also varied significantly with breed while degenerative or lose acrosome did not differ significantly with breed (Table. 5.1). The results of this study indicated that even though there is a variation regarding the appearance of semen defects between the two breeds, Friesland bulls showed better semen characteristics as they numerically had a higher percentage normal sperm with a lower percentage of major defects and significantly less minor defects, compared to Jersey bulls (Figure.5.1).

The effect of breed on sperm morphological characteristics could probably be due to the difference in breeds in terms of adaptability phenomena (Bonsma, 1980) and scrotal circumference (Coulter and Keller, 1982; Lattimer *et al.*, 1982; Mamabolo, 1999). Even though the adaptability of the two breeds was not assessed in the study, from the results of this study one may conclude that Friesland bulls are better adapted to the conditions where the study was conducted than the Jersey bulls.

The effect of breed on scrotal circumference has been well documented (Coulter & Keller, 1982; Lattimer *et al.*, 1982). Scrotal circumference is a good indicator of testis size and hence semen quality (Mammabolo, 1999) and degree of sexual development among breeds (Swanepoel, 1986). Brito *et al.* (2002a) reported a negative correlation between scrotal circumference and the prevalence of sperm defects. In the current study, the data in Table 4.3 indicates that Friesland bulls have a higher scrotal circumference than Jersey bulls. As scrotal circumference is positively correlated with semen quality, this may be the reason why Friesland bulls showed a higher percentage normal sperm and abnormalities than Jersey bulls.

5.2 THE EFFECT OF SEASON ON SPERM MORPHOLOGY

A. Effect of season on percentage normal sperm

The results of the study indicated that season significantly affected (P = 0.05) the percentage normal sperm (Table. 5.2 and Figure. 5.2). For summer, autumn, winter and spring, percentage normal sperm was 72.8±1.58%, 79.4±2.2%, 82.4±2.37% and 84.4±2.36% respectively. Even though percentage of normal sperm for all the seasons ranged above 70-80% which is considered to be acceptable (Coe, 1999), there was a significant (P = 0.01) variation in the percentage normal sperm between summer, autumn, winter and spring.

Table 5.2 Effect of season on sperm morphology in bulls (LSM ± SE)

Semen defects.	Summer	Autumn	Winter	Spring	*P* value
% normal sperm	72.8 ± 1.58^{A}	79.4±2.20^{B}	82.5 ± 2.37^{B}	84.4 ± 2.36^{B}	0.01
Major defects					
%Major. Defects	20.3± 1.34^{B}	15.3±1.88^{A}	12.5 ±2.0^{A}	11.5 ±2.0^{A}	0.01
Knob. Acrosome	1.2 ±0.19	1.0 ±0.27	1.4 ±0.29	0.8 ±0.29	0.37
Pyriform	8.2 ±0.71^{B}	6.6±0.10^{AB}	3.7±1.07^{AC}	5.0±1.07^{A}	0.01
Mid-piece. Reflex	3.1±0.43	2.6±0.60	4.1±0.65	3.4±0.64	0.36
Proximal droplets	4.4±0.52^{C}	2.3±0.74^{BC}	1.7±0.79^{B}	1.1±0.79^{A}	0.03
Minor defects					
% minor defects	7.9 ±0.64^{B}	6.2 ±0.89^{A}	6.3 ±0.96^{A}	5.0 ±0.96^{A}	0.03
Distal droplets	4.6 ±0.82	4.2 ±0.76	3.7 ±0.89	2.6 ±0.82	0.16
Normal lose head	1.06±0.13^{B}	0.55±0.18^{A}	0.77±0.19^{A}	0.53±0.19^{A}	0.02

A-C Means in the same row with different superscripts differed significantly (P < 0.05)

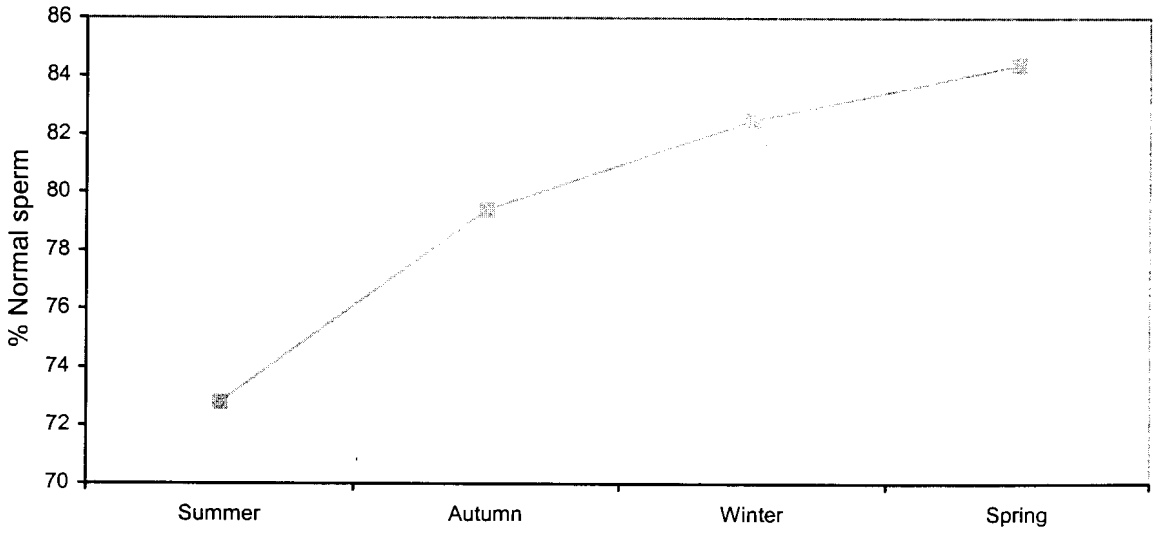

Figure 5.2 Graphical presentation of the effect of season on the percentage normal sperm in bulls

The results in Table 5.2 and Figure 5.2 indicate that the percentage of normal sperm was significantly lower during summer and tended toward a value of 70% which is considered critical according to Coe (1999). These results are also in line with those reported by Corcuera *et al.* (2002) where a higher variation and lower semen quality was observed in boars during the summer, compared to the other seasons. A lower percentage normal sperm during summer may be due to the higher temperature-humidity index (THI) which occurs at maximal level during summer (McDowell, 1972; Curtis, 1993). This is also in line with Everret & Bean (1982) and Mamabolo (1999) where periods of high temperature and humidity were associated with testicular degeneration and a reduction in the percentage normal sperm.

The results of this study indicated that a higher percentage normal sperm occurred during spring and winter. These results agree with those reported by Mathevon *et al.* (1998) where spring and winter were also associated with a higher percentage of normal sperm, regardless of the age and breed of the bull.

In this study there was a significant (P = 0.01) increase of 10.2% in normal sperm percentage from summer to winter and a significant decrease (P = 0.01) of 11.6% from spring to summer. The decrease in the percentage normal sperm from spring to summer

may be attributed to the increment in heat stress from spring to summer that causes testicular degeneration (Mamabolo, 1999) and an abnormal scrotal thermogram, which in turn have a negative impact on the quality of the semen (King, 1993).

In the area where the study was conducted, summer is characterised by a rapid increase in temperature and humidity (Table 4.1), which is negatively correlated with fertility in domestic animal (King, 1993; Curtis, 1983). The decrease in percentage normal sperm in this regard agrees with that of Casady *et al.* (1953) where the increase in THI above 37°C and 70% is associated with a decline in semen quality. Furthermore, this is also in line with the reporting by Curtis (1983), where the decline in percentage normal sperm by a third or more was observed when bulls were exposed to the increased THI during summer.

The results also indicate a quantitative change of 3.2% in percentage normal sperm from autumn to winter and 2.1% changes from winter to spring. Both changes were not statistically significant.

B. Effect of season on major sperm defect

The percentage major sperm defects for summer, autumn, winter and spring were 20.3±1.34%, 15.3±1.88%, 12.5±2.0% and 11.5±2.0%, respectively (Figure 5.3).

This indicates that percentage major sperm defects mostly fall below 20% for all seasons, which is considered to be acceptable according to Bearden & Fuquay (1997) and Coe (1999). Even though the percentage major sperm defects were below 20% for all seasons, there was a significantly difference ($P = 0.01$) in the percentage major sperm defects between summer, autumn, winter and spring. The major defects that were significantly affected by season were proximal droplets ($P = 0.03$) and pyriform ($P = 0.01$) while others such as knobbed acrosome and mid-piece reflexes were not significantly different between the seasons (Table 5.2).

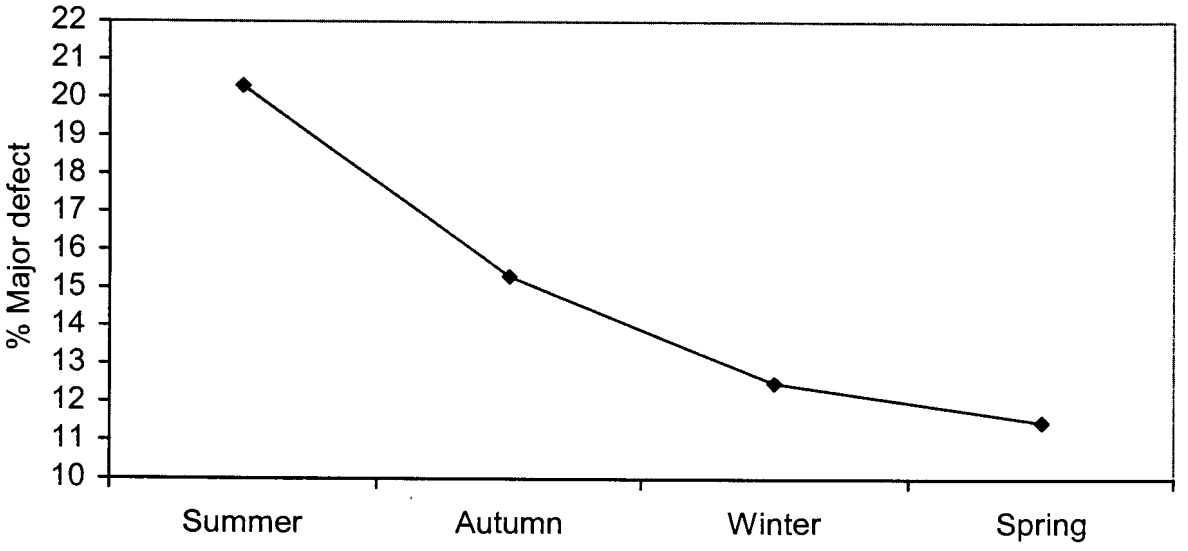

Figure 5.3 Graphical presentation of the effect of season on the percentage major sperm defects in bulls

Figure 5.3 indicates that the percentage major defects were higher and tended toward a critical value of 20% during summer. These results agree with Rathore (1970) where an increase in the frequency of abnormal sperm cells was associated with the hot season. The higher percentage of major defects in summer may also be a result of a higher THI during summer, which causes heat stress and results in abnormal scrotal thermograms and testicular degeneration. According to Cook *et al.* (1994) bulls with abnormal scrotal thermograms produce semen that has a lower percentage of sperm exhibiting normal head and tail morphology and a higher percentage of sperm with proximal droplets.

The result of the study also indicated that the percentage major defects were significantly lower during spring than the other seasons. Figure.5.3 indicated a significant (P = 0.01) increase of 8.8% in the occurrence of major defects from spring to summer. In turn, there was a significant decrease (P = 0.02) of 5% in the occurrence of these defects from summer to autumn. These changes may also be attributed to the changes in THI. From spring to summer, THI increases leading to the reduction in scrotal circumference

(Downey *et al.*, 1984) and an increase in major sperm defects (Brito *et al.*, 2002a). As THI drops from summer to autumn, the effects are reversed.

There were no significant changes in percentages major sperm defects observed from autumn to winter and winter to spring. From the climatic analyses of the area where the study was conducted (Table.4.1), this results may be attributed to the minimal changes in THI between these seasons.

C. Effect of season on minor defects

The results of the study indicate that season has a significant effect (P = 0.03) on the percentage minor defects. For summer, autumn, winter and spring, percentage minor sperm defects was 7.9±0.6%, 6.2±0.9%, 6.3±1.0% and 5±1.0%, respectively (Figure. 5.4). Based on the reports by Van Rensburg (1957) and Salisbury *et al.* (1978), the effect of season on minor defects in this study had an insignificant effect on the semen quality, as these defects were less than 10% for all seasons.

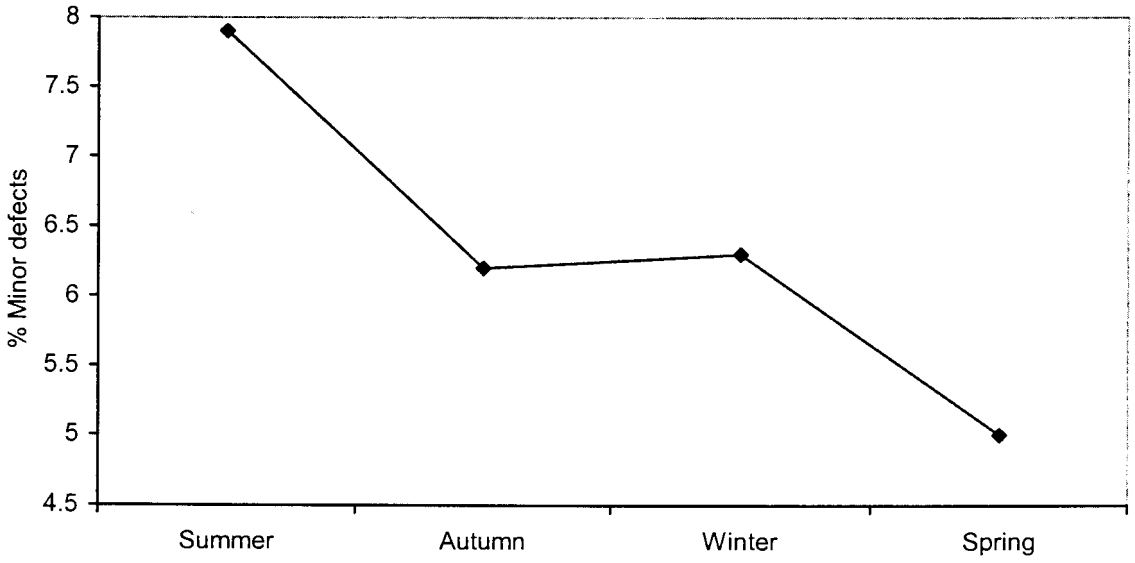

Figure 5.4 Graphical presentation of the effects of season on the percentage minor sperm defects in bulls

Even though the proportion of minor defects was insignificant, Figure 5.4 indicates that summer tended towards the critical value of 10% for minor defects, more than the other three seasons. This may also be attributed to the higher THI in summer than during the other seasons. This has an impact on semen quality. The results obtained in the study also indicate that minor defects such as normal lose heads differed significantly (P = 0.02) with season while there was a tendency for distal droplets to vary significantly with season. Overall the results of the study demonstrated sperm morphological traits to differed significantly (P = 0.05) with season. In general, a better morphology was observed during spring and winter, compared to summer and autumn. These results are similar to those reported by Anderson (1941); Ibrahim (1997) and Mathevon *et al.* (1998), where season was also found to affect sperm morphology significantly with higher semen quality being observed during the spring compared to the summer season.

5.3 EFFECT OF AGE ON SEMEN MORPHOLOGY

A. Effect of age on percentage normal sperm

In Table 5.3 and Figure 5.5 the percentage normal sperm for bulls younger than 36 months, matured bulls of 36-48, 60 months and 72 months old were 78.1%, 81.5% 78.0%and 77.1% respectively are set out.

Table 5.3 Effect of age (months) on sperm morphology in bulls

Semen defects (%)	12	24	36	48	60	72	84	96	*P* value
% normal sperm	77.0	78.1	81.5	81.5	78.0	77.1	73.2	73.0	0.05
%major defects	22.3	17.2	13.8	12.2	12.5	14.4	18.2	23.8	0.01
Knobbed acrosomes	2.8	1.8	1.1	0.7	0.6	0.72	1.2	1.9	0.02
Pyriforms	7.8	5.8	4.6	4.2	4.5	5.5	7.3	9.9	0.03
Mid-piece reflexes	5.0	3.4	1.9	1.4	1.5	2.2	4.2	6.8	0.01
Dag defects	2.9	2.16	1.5	1.0	0.74	0.6	0.7	1.02	0.02
Broken flagellum	0.5	0.3	0.24	0.2	0.24	0.3	0.5	0.7	0.16
% minor defects	6.7	6.8	7.1	7.3	7.7	8.2	8.7	9.3	0.97
Normal lose heads	2.14	1.6	1.2	0.8	0.64	0.5	0.6	0.8	0.03

Even though percentages normal sperm for all age groups were above 70% (which is acceptable according to Coe (1999)), it also differed significantly (P = 0.05) with age. Figure 5.5 indicates that the percentage normal sperm was higher in bulls aged 36-48 months and lower in bulls younger than 36 months and older than 72 months of age.

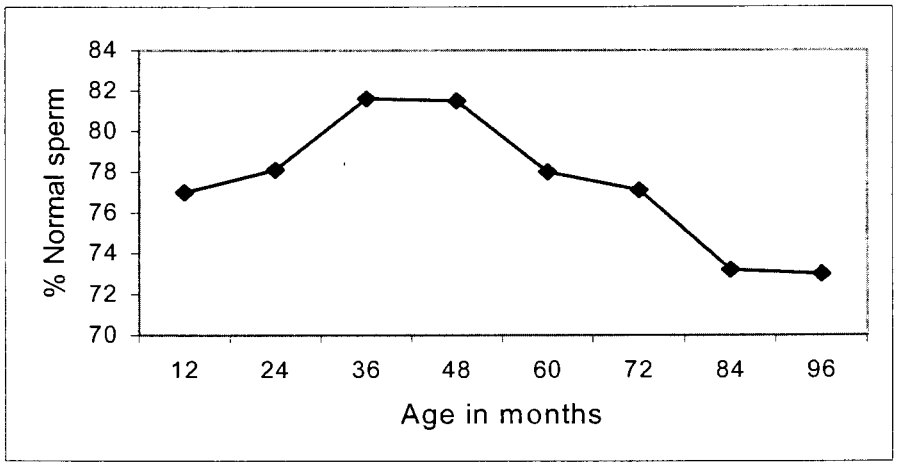

Figure 5.5 Graphical presentation of the effect of age on the percentage normal sperm in bulls

The results obtained in the study are in agreement with those reported by Kumi-Diaka *et al.* (1981) and Dowsett & Knott (1996) where higher semen quality in terms of normality and concentration was observed in bulls of 3-7 years of age, compared to bulls younger than 3 years and older than 7 years. Figure 5.5 indicates a numerical increase of 4.5% in the percentage normal sperm from 12 months to 36 months and also a numerical decrease of 8.5% in normal sperm percentage from the age of 36-48 months to 96 months and above.

B. Effect of age on major sperm defects

The results indicated that age has a significant (P = 0.01) effect on the percentage major sperm defects. In Table 5.3 and Figure. 5.6 the percentage major sperm defects were higher in bulls younger than 36 months and older than 72 months, compared to bull aged 36-60 months. These results are contrary to those reported by Kumi-Diaka *et al.* (1981)

where no significant differences were observed regarding the major and total sperm defect with an increase in the age of the bull.

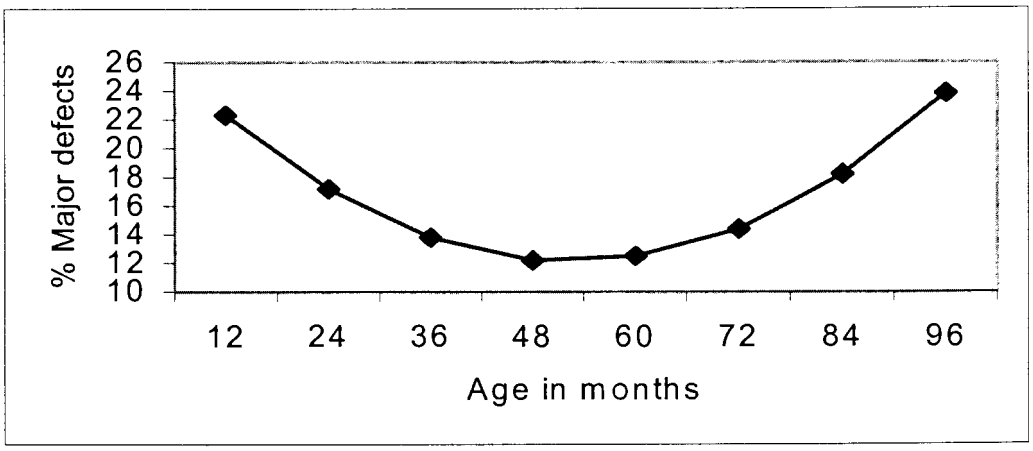

Figure 5.6 Graphical presentation of the effect of age on the percentage major sperm defects in bulls

The results in Figure 5.6 indicate that in bulls younger than 24 months and older than 84 months the percentage major defects were above 20% (which is critical according to Bearden & Fuquay, 1997). Table 5.3 indicate that major sperm defects which were affected by age are knobbed acrosomes ($P = 0.01$), dag defects ($P = 0.01$) and pyriforms ($P = 0.03$). These results agree with those reported by Rao (1971); Dowsett & Knott (1996) and Soderquist *et al.* (1996) where a variation in total abnormalities and the increased frequency of abnormal sperm cells was observed with advancing age.

C. Effect of age on minor sperm defects

The results of the study indicate that the percentage minor defects did not differ significantly with age (Table 5.3 and Figure. 5.7).

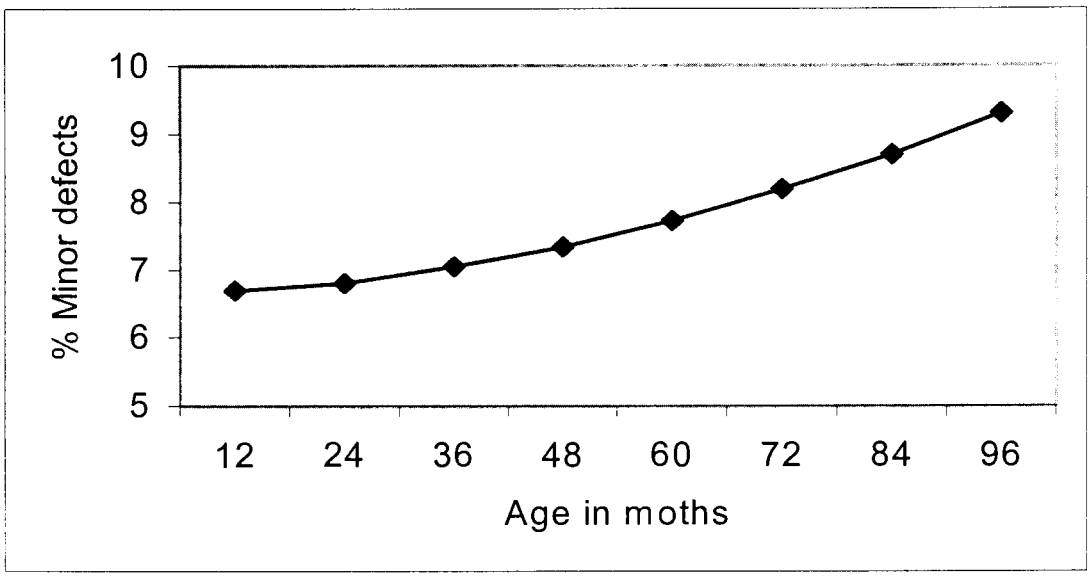

Figure 5.7 Graphical presentation of the effect of age on the percentage minor sperm defects in bulls

These results obtained did not agree with those reported by Brito *et al.* (2002a) where the increase in age of the bulls was associated with a significant increase in the percentage minor sperm defects. The results in Table 5.3 indicate the minor defect such as normal lose heads to differ significantly ($P = 0.01$) with age. According to Van Rensburg (1957), the effect of age on the percentage minor sperm defects obtained in the study are insignificant, being less than 10%. Bulls older than 96 months tended towards the critical value.

Overall results on the effects of age on semen morphological traits showed that sperm morphology is better in bulls aged 36-48 months than bulls of 72 months and older and bulls younger than 36 months. According to researchers, these results may probably be attributed to the factors such as scrotal circumference (Mamabolo, 1999; Coe, 1999; Brito *et al.*, 2002b), the regulating balance mechanism (McDowell, 1972), fat deposition and brain and reproductive tissues (Salisbury *et al*, 1978; King, 1993) which affect semen production and quality.

Scrotal circumference as an indicator of testis size (Mamabolo, 1999), was reported to change as bulls progress in age (Brito *et al.*, 2002a). Table 4.3 indicates scrotal circumference to increase with age, similar to Coulter & Foote (1977) who found scrotal circumference to increase rapidly in younger bulls, gradually in mature bulls and even decrease in older bulls. This may explain why mature bulls of 36-60 months have a higher semen quality than bull younger than 36 months and older than 72 months in this study.

Dairy bulls reach puberty at the age of 12 months (Bearden & Fuquay, 1997) and maturity at the age of at 3-4 years (Almquist, 1982). The results of the study indicate that bulls prior to maturity recorded higher sperm defects. This may probably be due to the fact that in younger bulls, the testicles are still developing and hence the semen in the ejaculate is of low quality (Coulter & Foote, 1979). Younger bulls also have an underdeveloped thermo-regulatory mechanism and hence scrotal thermo-regulatory mechanism which has a negative impact on semen quality (McDowell, 19720. Lower semen outputs in older bulls may be associated with the degenerative changes in seminiferous tubule (Coe, 1999), fat deposition which may take place in scrotum (Salisbury *et al*, 1978; King, 1993) and the break down of body tissues particularly testicular tissues (King, 1993) with advancement in age. At an older age, testicular tissues, are broken down faster than they are replaced, which may cause testicular degeneration resulting in poor semen output (Salisbury *et al*, 19781). Fat deposition as a bull progress in age may take place around the scrotum. This may affect semen quality by reducing the heat radiation capacity from the scrotal neck (Brito *et al.*, 2002a), thereby increasing the temperature of the testis (Coulter *et al*, 1997). As a result, abnormal scrotal thermogramm and testicular degeneration may occur and hence higher semen defects and a lower percentage of normal sperm (Cook *et al.*, 1994). The results of the study also indicate that semen quality starts to deteriorate in the bulls older than 72 months. This deterioration may be associated with the accumulative hazards of life including non-specific infections, nutritional stress, diseases and accidents, which all combine to cause the direct relationship between semen quality and age to disappear (Salisbury *et al*, 1978).

5.4 THE INTERACTION BETWEEN AGE AND BREED ON SPERM MORPHOLOGY

The results of the study indicated no significant interaction between age and breed in the effect on the percentage normal sperm and minor sperm defects. There was, however a significant interaction between age and breed ($P = 0.01$) in their effect on the percentage major sperm defects (Table 5.4).

Table 5.4 The interaction between age and breed on sperm morphology (LSM±SE)

Semen defects	Age in months								*P* value
	12	24	36	48	60	72	84	96	
% Major defect.	**15.7 ±3.3**	**14.9 ±2.1**	**14.3 ±1.26**	**14.0 ± 1.0**	**13.9±1.1**	**14.0±1.3**	**14.4±1.5**	**15.1±1.9**	0.01
	28.9 ±7.9	19.4 ±4.9	13.3 ±3.19	10.5± 2.8	11.0±2.9	14.8±3.1	22.0±3.8	32.5±5.6	
	P = 0.11	*P = 0.38*	*P = 0.75*	*P = 0.24*	*P = 0.35*	*P = 0.80*	*P = 0.06*	*P = 0.004*	
Knobbed Acrosomes	**2.5 ± 0.5**	**2.0 ±0.3**	**1.5± 0.18**	**1.2 ± 0.1**	**0.9±0.2**	**0.8±0.2**	**0.7±0.2**	**0.6±0.3**	0.03
	3.1 ± 1.1	1.7±0.7	0.7 ± 0.5	0.2 ± 0.4	0.2±0.4	0.7±0.4	1.6±0.5	3.1±0.8	
	P =0.55	*P = 0.70*	*P = 0.08*	*P = 0.02*	*P = 0.09*	*P = 0.83*	*P = 0.08*	*P = 0.004*	
Pyriforms	**7.2 ±1.8**	**6.5±1.1**	**5.8 ± 0.7**	**5.4 ± 0.5**	**5.0±0.6**	**4.7±0.7**	**4.5±0.8**	**4.5±1.0**	0.11
	8.4 ± 4.3	5.2±2.6	3.3 ± 1.7	2.9 ± 1.5	3.9±1.6	6.3±1.7	10.2±2.0	15.4±3.0	
	P = 0.78	*P = 0.63*	*P = 0.17*	*P = 0.13*	*P = 0.50*	*P = 0.36*	*P = 0.01*	*P = 0.001*	
Mid-piece Reflex	**3.0 ± 1.0**	**2.5±0.6**	**2.0± 0.4**	**1.8± 0.3**	**1.7±0.3**	**1.8±0.4**	**2.1±0.5**	**2.5±0.6**	0.01
	7.9 ± 2.5	4.2±1.51	1.8± 1.0	0.9± 0.8	1.4±0.9	3.2±1.0	6.4±1.2	11.0±1.8	
	P = 0.06	*P = 0.26*	*P = 0.88*	*P = 0.35*	*P = 0.71*	*P = 0.17*	*P = 0.001*	*P = 0.001*	
Dag defects	**0.85± 0.4**	**0.7±0.2**	**0.7± 0.1**	**0.7 ± 0.1**	**0.6±0.1**	**0.6±0.1**	**0.6±0.2**	**0.6±0.2**	0.01
	5.1 ± 0.9	3.5±0.5	2.2± 0.4	1.4 ±0.3	0.9±0.3	0.7±0.3	0.8±0.4	1.4±0.6	
	P = 0.001	*P = 0.001*	*P=0.001*	*P = 0.03*	*P = 0.50*	*P = 0.85*	*P = 0.59*	*P = 0.27*	

LEGEND: **BOLD** ⇒**FRIESLAND**

NORMAL ⇒JERSEY

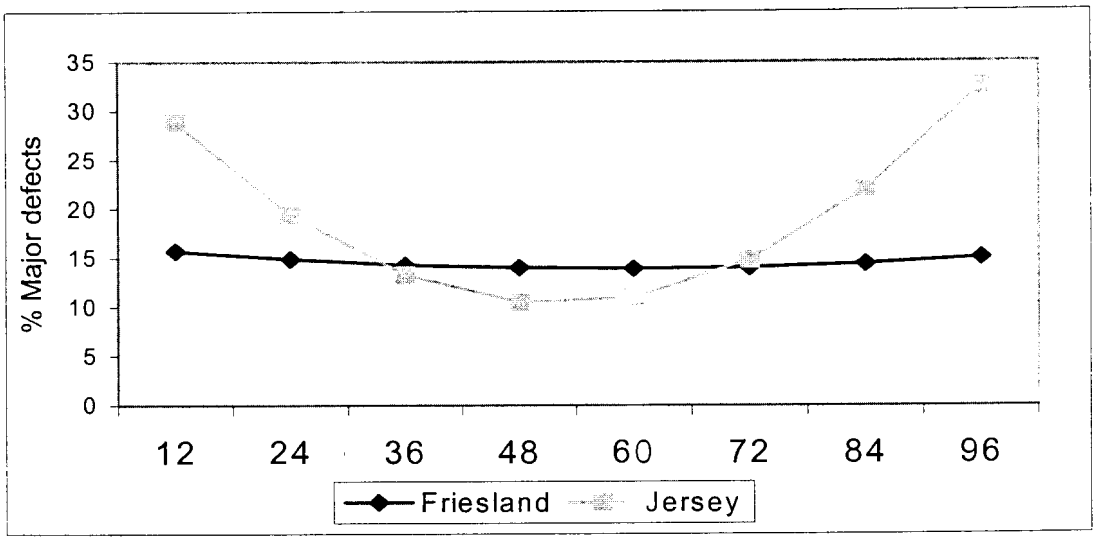

Figure 5.8 Graphical presentation of the interaction between age and breed on the percentage major sperm defects in Friesland and Jersey bulls

Figure 5.8 indicates that young and old Jersey bulls recorded major sperm defects above the critical value of 20% when compared to Friesland bulls of the same age. In both breeds between 36-60 months of age, the percentage major sperm defects were below the critical value. Friesland bulls were more prevalent when compered to Jersey. These results differ with those reported by Serrano (1984) where the interaction between age and breed was found to have no affected on semen quality. Major sperm defects such as knobbed acrosomes ($P = 0.03$) dag defects ($P = 0.01$), broken flagellum ($P = 0.02$) and mid-piece reflexes ($P = 0.003$) are affected significantly by the interaction between age and breed (Table 5.4).

For all sperm defects that were affected significantly by the interaction between age and breed, these could probably be attributed to the differences in scrotal circumference with age between the breeds and the maturity-type of bull (as related to fat deposition). Almqiust (1982); Swanepoel (1986) and Mamabolo (1999) reported scrotal circumference as an indicator for testis size and a reliable parameter for sexual development among different breeds at different ages. The variation in scrotal circumference with breed and age may explain the interaction between age and breed on sperm morphology. This also agrees with the results reported by Coe (1999) where the

sperm morphology. This also agrees with the results reported by Coe (1999) where the interaction of scrotal circumference and age was associated with 11% of the variation in semen quality. Curtis & Amann (1981) and Mamabolo (1999) also reported that breeds with larger scrotal circumference have greater sperm production and higher semen quality. Table. 4.3 demonstrate that Frieland bulls have a larger scrotal circumferences at all ages when compared to Jersey. This may explain why Friesland have a lower percentage major defects sperm and is less prevalent to major sperm defects indicated on (Table 5.4), compared to Jersey.

The Jersey breed is an early maturing types of animal which, deposits fat in the body at an earlier chronological stage, compared to Friesland (Casey & Maree, 1993). The fat deposition in the body increases with age and may also take place in the scrotum (King, 1993). This fat deposition in the scrotum may decrease scrotal thermo-regulation by reducing the heat radiating capacity of the scrotal neck (Brito *et al.*, 2002a). This may result in an abnormal scrotal thermogram and testicular degeneration, which has an inverse effect on semen quality (Coulter, 1988; Mamabolo, 1999). This also explains why younger growing Jersey bulls are more susceptible to sperm defects compared to Friesland of the same age. Due to the early fat deposition in Jersey bulls

5.5 THE INTERACTION BETWEEN AGE AND SEASON ON SPERM MORPHOLOGY

The results of the study indicate that the interaction between age and season significantly ($P = 0.04$) affect the percentage of major sperm defects (Figure 5.9) and the incidence of major defect such as knobbed acrosomes ($P = 0.05$) (Figure 5.10) and dag defects ($P = 0.05$). These results are contrary to that reported by Curtis & Amann (1981) where no significant interaction between age and season on any parameters of semen quality was observed.

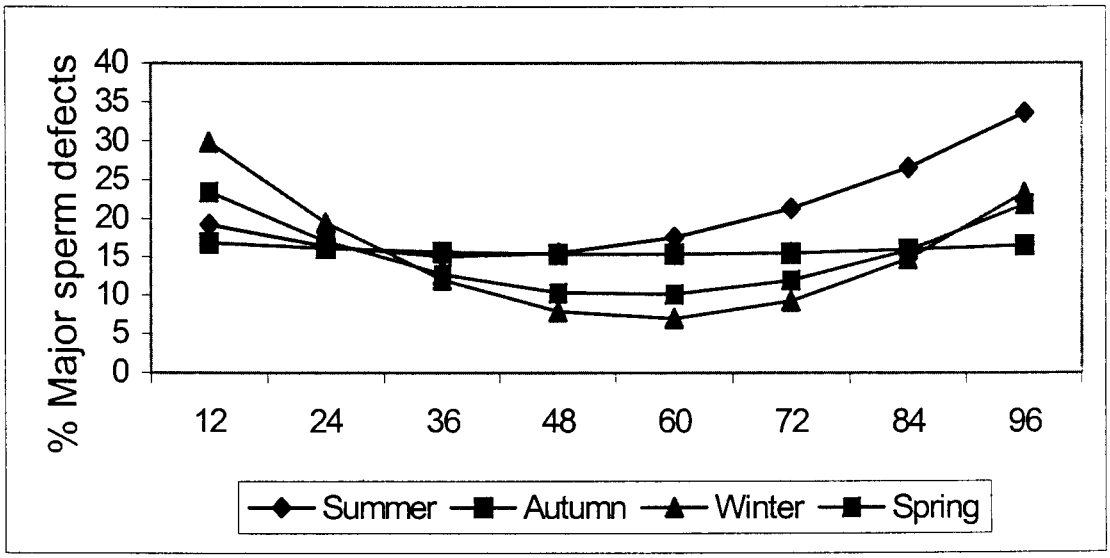

Figure 5.9 Graphical presentation of the interaction between age and season on the percentage major sperm defects in bulls

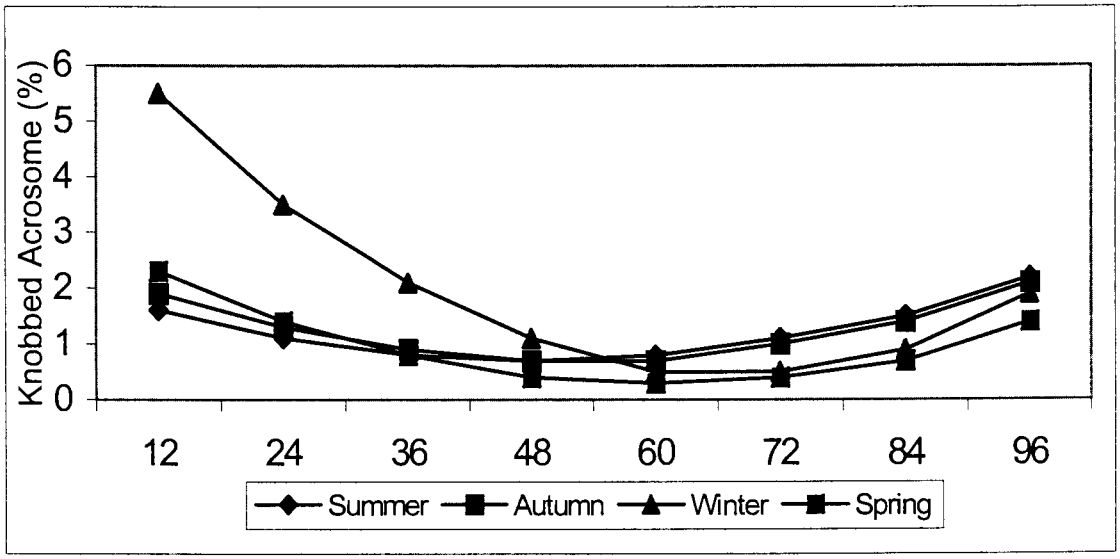

Figure 5.10 Graphical representation of the effect of the interaction between age and season on the incidence of knobbed acrosomes in bulls

The percentage of major defects and the incidence of knobbed acrosomes and dag defects tended to vary with age more in summer, winter and spring and remain relatively

unaffected in autumn. Winter was critical for bulls younger than 36 months as the percentage of major defects during this season were above 20% (which is the critical according Bearden & Fuquay, 1997). The effect of winter on younger bulls declined drastically up to 60 months of age and then increased but to a lesser degree between 60 and 96 months of age. At an age below 36 months, winter differed significantly to summer ($P = 0.04$) and non-significantly with spring. These results are contrary to those reported by Salisbury *et al.* (1978) where even though there is a variation in semen quality of bulls younger than 36 months of age with season, a higher performance was observed during winter and it declined during the subsequent warmer seasons. Summer was less of a concern in bulls younger 36 months of age but had an increasing influence than all the other seasons as animal progress in age. Results also show no quantitative variation on sperm morphology of bulls between 36-48 months of age with season. As bulls advance in age however, they became more susceptible to higher percentage of major sperm defects and the incidence of knobbed acrosomes and dag defects during summer than during the other seasons. At the age of 72 months, 84 months and 96 months, summer differed significantly from spring and winter. Summer differed significantly from spring and winter as follows, at 72 months ($P = 0.02$) and ($P = 0.01$), at 84 months ($P = 0.02$) and ($P = 0.02$) and at 96 months ($P = 0.02$) and ($P = 0.05$) respectively.

The interaction between age and season may probably be attributed to factors such as heat regulating mechanisms, fat deposition and the fate of the body tissues among bulls of different ages at different seasons. Salisbury *et al.* (1978) reported fat deposition in cattle to increase with an advancement of age. As a bull ages, this fat may be deposited in the scrotum. Coulter *et al.* (1997) and Brito *et al.* (2002a) reported that fat deposition in the scrotum affects testicular thermo-regulation through a reduction in the heat that can be radiated from the scrotal neck. As the results, the efficiency of the scrotal thermo-regulation may be reduced in old bulls during summer, which may cause testicular degeneration and abnormal scrotal thermograms and hence poor semen quality. As bulls age, body tissues are broken down faster than they are replaced and testicular tissues are far more sensitive tissues of the body to factors such as heat and nutritional stress than

other tissues of the body (King, 1993). Reduction on feed intake as the results of thermal stress during summer could also lower the amount of glucose available to tissues for metabolism causing the break down of this tissue (McDowell, 1972). As a result, testicular degeneration may occur in old bulls and hence poor semen quality. Younger bulls are considered to have higher nutritional requirements for growth, maintenance and reproduction than older bulls (Salisbury *et al.*, 1978). During winter, poor pasture quality and quantity may lower the availability of nutrients required to maintain the requirements for growth and reproduction for example, spermatogenesis and hence semen quality (Hacker, 1982). This agrees with Brito *et al.* (2002b) where the excessive body loss or reduced feed quality and quantity was associated a reduction in semen quality during winter in young growing males.

5.6 THE INTERACTION OF BREED AND SEASON ON SPERM MORPHOLOGY

The results of the study indicate no significant interaction between breed and season in the effect on percentage major sperm defects but significantly (P = 0.01) affected the incidence of dag defects (Table 5.5 and Figure 5.12).

Table 5.5 The interaction between breed and season on sperm morphology

	% Major defects (LSM± SE).		Dag defects (LSM± SE).	
	Friesland	**Jersey**	**Friesland**	**Jersey**
Summer	20.88±1.3	18.83±2.5	0.86±0.9	1.07±0.3
Autumn	13.47±2.1	17.88±3.4	0.78 ± 0.2^{A}	1.55 ± 0.4^{B}
Winter	11.93±1.8	13.85±7.1	0.46±0.2	0.11±0.8
Spring	11.16±1.8	16.52±6.3	0.56 ± 0.2^{A}	3.8 ± 0.7^{B}
P value	P = 0.52		P = 0.001	

A-B Means in the same row with different superscripts within a season, differed significantly (P < 0.05)

The results in Table 5.5 and Figure 5.12 indicate that for the two breeds tested, (Friesland and Jersey), the incidence of dag defects was 0.86% versus 1.07%, 0.78% versus 1.55%, 0.46% versus 0.11% and 0.56% versus 3.8% for summer, autumn, winter and spring respectively. This indicates that Jersey bulls are more susceptible to the incidence of this defect during summer, autumn and spring compared to the Friesland, which is only susceptible during winter when compared to the Jersey.

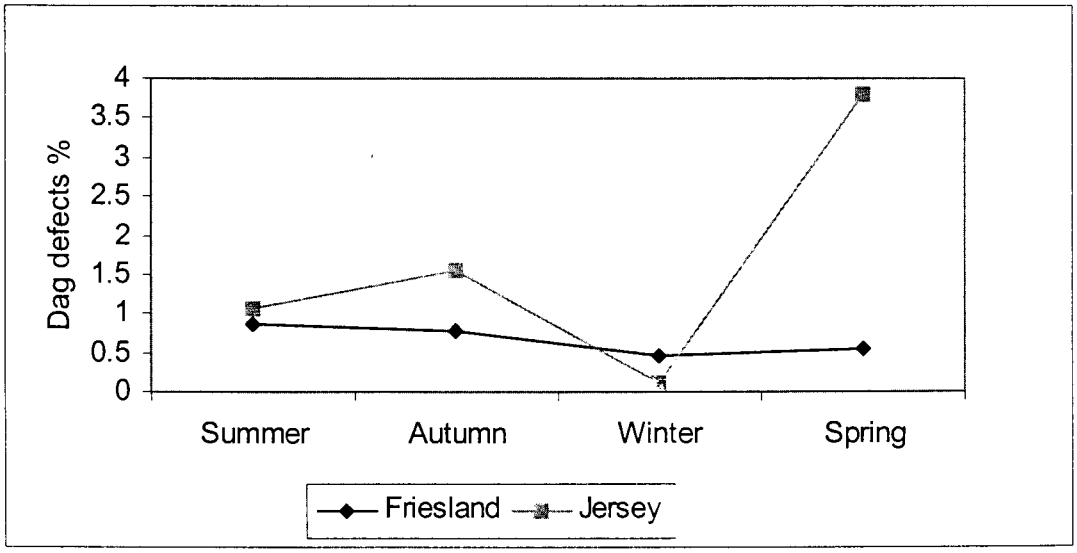

Figure 5.12 Graphical presentation of the interaction between breed and season on the incidence of dag defects in Friesland and Jersey bulls

Table 5.5 and Figure 5.12 show a significant (P = 0.01) variation in the -prevalence of dag defects between Friesland and Jersey bulls during spring. During spring, the Jersey bulls were approximately 85% more susceptible to the incidence of dag defects than Friesland bulls.

The results of the study also indicated that the interaction between breed and season did not significantly affect the occurrence of minor defects On the other hand, Figure 5.13 illustrate a tendency for the incidence of normal lose heads (a minor defect) to vary with the interaction between breed and season.

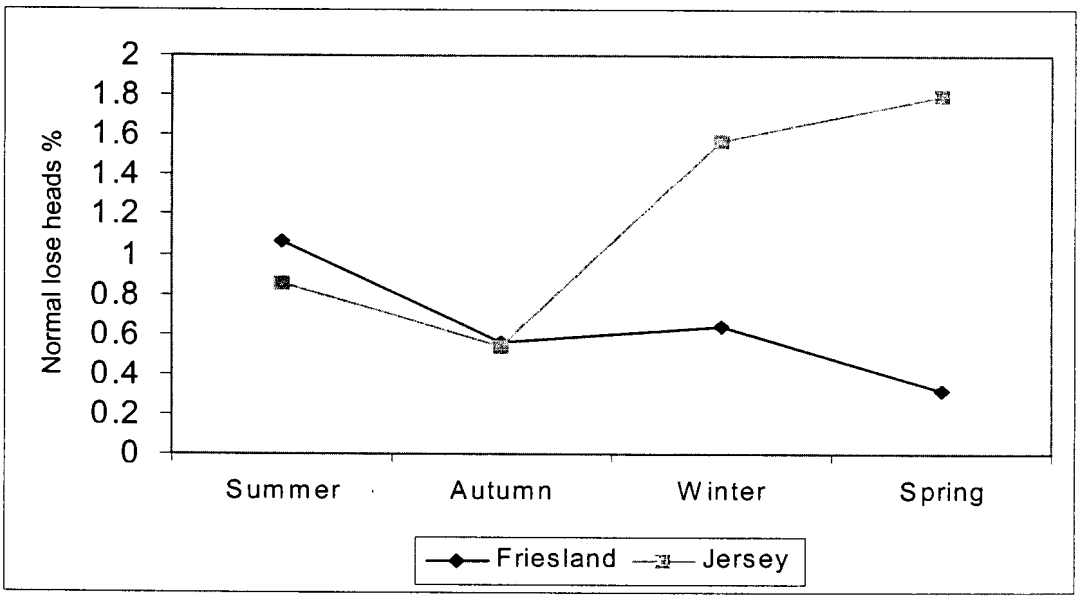

Figure 5.13 Graphical presentation of the interaction between breed and season on the incidence of normal lose head in Friesland and Jersey bulls

Overall, the results indicate that Jersey bulls are more susceptible to the incidence of dag defects during the hotter seasons (spring and summer) than in winter, compared to Friesland. Friesland bulls were more susceptible to the incidence of normal lose heads during the hotter season (summer) than in other seasons, compared to the Jersey bulls.

5.7 SUMMARY OF THE RESULTS AND DISCUSSION

The study indicated the detailed assessment of semen morphology as percentage of normal sperm cell (Seronson, 1979) and major and minor sperm defect (Van Rensburg, 1957; Van Denmark and Free, 1970) to be the useful bio-markers of the effect breed, age, season and their interaction on the quality of semen of AI dairy bulls.

For both breeds that were tested, all season and different age groups, minor defects did not have significant effects on the quality of semen as a result obtained indicate that they never exceeded the critical value of 10%. For the two breeds tested, (Friesland and Jersey), the results of the study indicated that Friesland had better sperm morphology

than Jersey. This was due to the higher percentage normal sperm and lower incidence of abnormal sperm that was observed in Friesland bulls compared to Jersey bulls.

Optimal reproductive performance requires optimally co-ordinated function of tissues and endocrinological regulatory system, which is affected to a large extent by the extremes of temperatures and nutritional deficiencies, which are season dependent (Hurley and Doane, 1989). The study demonstrated seasonal variation on semen morphology. Least square means for semen defects (major and minor) were significantly higher during summer and autumn compared to spring and winter. Least square means for percentage normal sperm was significantly higher during spring and winter compared to summer and autumn. The results obtained shows a clear suppression in sperm morphology of AI bulls during summer period of South Africa as a result of THI, which has inverse effect on semen quality.

Higher percentage of normal sperm and less incidence of sperm defects was observed in bulls aged 36-48 months of age, compared to the bulls younger than 36 months and older 72 months of age. Younger bulls (less than 36 months) had better semen morphology than their older counterparts (older than72 months). This was attributed the rapid increase of the scrotal circumference in younger bulls than in older bulls (Coulter & Foote, 1977) as scrotum circumference is positively correlated with semen quality in bulls (Mamabolo, 1999)

The percentage major defects, particularly the incidence of knobbed acrosomes, dag defects, pyriforms, mid-piece reflexes, normal lose heads and broken flagellum differed significantly with the interaction between age and breed and the interaction between age and season.

There was a distinct variation in the susceptibility to these sperm defects in Jersey bulls as age progresses, while the variation in the incidence of these defects with age was almost constant in Friesland bulls. Results also demonstrate that young and old Friesland bulls are less susceptible to the incidence of these sperm defects compared to Jersey bulls

of same age groups. Irrespective of the breed, young bulls have poor sperm morphology during winter while old bulls showed poor morphology during summer.

CHAPTER 6

CONCLUSIONSAND RECOMMENDATIONS

6.1 CONCLUSIONS

The results of the study indicate that breed, age season and their interactions are important sources of variation in the sperm morphology of AI dairy bulls in South Africa. Between the two breeds tested, Friesland bulls have higher semen quality than Jersey bulls as they recorded a higher percentage of normal sperm and the incidence of abnormal sperm remained below the critical value of 20%. Jersey bulls younger than 24 months and older than 72 months recorded an incidence of abnormal sperm above the critical value of 20% when compared to Friesland bulls of the same age. For a successful dairy production system and AI programme, semen collection or the introduction of bulls to heifers and cows, bulls should be between age of 36-48 months of age in both breeds during spring and winter. With breeds combined, summer is not an ideal season for semen collection from bulls that are 72 months or older, while winter is not an ideal for bulls younger than 36 months of age.

6.2 RECOMMENDATIONS

Manipulation of temperature in dairy cattle through the provision of management practices to reduce the effect of heat stress on bull reproduction is recommended as a primary tool in optimising the quality of semen harvested in AI dairy bulls in South Africa. It is also recommended that the relationship between the nutrition, reproduction physiology and management should be given a higher priority in dairy production systems.

Further researchers and considerable attention should be directed in determining the primary effects of breed variation (scrotal circumference), age, season and environment

(temperature and nutrition) and their interactions on the fertility and quality of semen of AI dairy bulls in South Africa.

CHAPTER 7

CRITICAL EVALUATION OF THE PROJECT

A. General Evaluation of the project

- The project has been successfully conducted as all the primary objectives were achieved.
- During the course of the project, imperative aspects (recommendations), which should be taken in to account in dairy reproduction management, were identified.
- There was excellent corporation and participation among people involved in the project. That is the supervisor; staff members and the Head of Department, Dept. of Animal and Wildlife Science UP; Dept of Statistics, UP; Technical Officers from Taurus Irene (ARC).
- The particular study of this nature is among the primary aspects in agricultural industry since 90-95% of semen from AI Stations in South Africa goes to the dairy industry where AI is practised. The fact that a large number of commercial dairy farmers use AI in which its success is highly dependant up on the collection of maximum number of viable sperm also make this study a highly recommended aspect in livestock production in South Africa.

B. Positive aspect of the project

- The results of the project may be used by both the public and private sector and also by both commercial and communal farmers in dairy production system to develop management programs that will result in the increased fertility of bull in terms of semen quality to enhance productivity.
- The supervisor of the project was fully dedicated to the project from the onset to the end of the study.

C. Negative aspects of the project

- Mr. Anderson who helped in collection and analysis of the sample had to leave before the study was concluded. This was detrimental to both the student and the Dept. of Statistics and it also resulted in the prolonged statistical analysis of the data.

D. What should be done

- The supervisor and the head of the department have to sign a contract that will bind all the parties involved in the project ensuring that there will be no withdrawal until the project is complete.

E. Personal evaluation and contribution of the project to me as a student

- Postgraduate studies should increase the student' s scientific knowledge, practical skills to the student characters. The particular nature of this study has contributed to my knowledge and added outstandingly to a practical experience, which I will be able to apply with confidence in practice of any nature in agricultural industry.
- From this, project I have personally benefited the basic and the practical knowledge about the semen collection, analysis and evaluation protocols which I think are amongst the most important required skill in dairy production industry due to the higher demand of high quality semen that are used in AI programs
- The study also thaught me how to plan and organise research project and resulted in improved experienced in office work and computer skills as I did the typing, editing and formatting myself which required a knowledge of computer MS word, Ms Excel and Ms Power point.

CHAPTER 8

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