The effect of dietary vitamin E supplementation on semen quality of A.I. dairy bulls

By

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Abstract

The importance of semen quality in the breeding bull is often underestimated and breeders should investigate ways to improve the semen quality in order to improve conception rates. The diet of four bulls was supplemented with a vitamin E supplement, Rovimix E, to investigate the effect on the percentage normal spermatozoa, percentage major semen defects and the percentage minor defects. The results obtained were compared to those obtained for four bulls in a control group which were fed only the concentrate diet with no vitamin E supplementation. The results indicate that abnormal loose heads and macroephalic spermatozoa were significantly ($p > 0.05$) affected, but overall vitamin E supplementation did not significantly ($p < 0.05$) influence the incidence of major and minor semen defects. It would generally seem that vitamin E supplementation could improve bull semen.

Opsomming

Die belangrikheid van bulsaadkwaliteit op die reproduksie proses word dikwels onderskat. Telers moet wyses ondersoek om die kwaliteit van bulsaad te verbeter om die kalwingspersentasie te verhoog. Ons het die dieët van vier bulle aangevul met Rovimix E, wat 'n vitamien E supplement is, om die invloed op die persentasie normale spermatozoa, persentasie major saad defekte en die persentasie minor saad defekte te ondersoek. Die resultate wat ons gekry het vir die proefondevindelike groep van bulle is vergelyk met 'n kontrole groep van vier bulle wat die normale dieët sonder vitamien E gevoer was. Daar is bevind dat van die saad defekte statisties betekensvol was soos

die abnormale loskoppe defek en die makroefaliese spermatozoa defek. Die vitamien E supplement het egter nie die persentasie major- en minor saad defekte statisties beinvloed nie. Alhoewel die effek nie statisties betekenisvol was nie, het die vitamien E supplement wel die persentasie van saad defekte verminder.

Declaration

I declare that this dissertation has not been submitted by anyone to any other tertiary institution.

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Summary

The bull is a vital part of any reproduction system, whether it be dairy or beef cattle. When analysing possible causes for reproductive inefficiency in the herd, emphasis is often placed on the female, however, the male must not be forgotten. It must also be remembered that at the beginning of any breeding season, the male must be properly tested and judged for reproductive efficiency with regards to semen quality, scrotal circumference and conformation. The bull must be tested for any diseases which may be transmitted to the female resulting in the loss of pregnancy and result in a lowered calf crop. The management of the breeding bull involves a proper balance between the environment to which he is subjected, the level of nutrition and the way in which he is treated in order to minimise stress and maximise his reproductive ability.

Examinations of bull semen must also be done as there may be genetic traits regarding the semen which can adversely affect a bull's ability to fertilise a female. Many defects can be found in the semen of breeding bulls, many of which are caused by adverse environmental conditions resulting in stress, but this can be solved and most importantly be avoided. Each semen defect has

a particular point of origin in the reproductive tract of the male and knowledge of the defects and their characteristics should help in the identification of the problem.

There are continuous investigations on aspects that could improve the quality and thus fertility of bull semen to ensure that a cow conceives and produces a healthy calf. This study investigated the effect of dietary vitamin E on the quality of semen in Friesian bulls. Eight bulls were selected which were of similar ages and were all half brothers, ensuring a similar genetic make up. They were randomly divided into two groups of four animals each. One group was fed a basic concentrate diet supplemented with a vitamin E supplement, Rovimix E, and the other group was maintained on the normal basic concentrate diet. Both group of bulls were fed *Eragrostis tef* hay *ad libitum* and had access to fresh water.

Over a three month experimental period, semen was collected from each bull in both the treated and control groups every two weeks. The semen examined for the percentage normal spermatozoa, percentage major semen defects and the percentage minor semen defects. When compared to the results obtained from the bulls in the control group, it was found that the vitamin E supplementation did not significantly affect the semen quality, either positively or negatively. The vitamin E treatment slightly decreased the occurrence of abnormal spermatozoa present in the ejaculates of the supplemented bulls.

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Abbreviations

ABN	Abnormal Loose heads
ABAK	Abaxial implantation
A.I.	Artificial insemination
AKR	Knobbed acrosome
ANDR	Other midpiece defects
DAG	Dag defect
DEG	Degenerative heads
DLA	Degenerative loose acrosome
DPD	Distal droplet
DUBB	Double forms
C	Control group
GEBR	Broken Flagellum
KERN	Nuclear vacuole
KRTR	Corkscrew
KRUL	Curled endpiece
MAKR	Macoephalic spermatozoa
MIDS	Midpiece reflex
MIKR	Microephalic spermatozoa
MIT	Mitochondrial aplasia
NRM	Normal loose heads
PEER	Pyriform heads
PMAJOR	Percentage major defects
PMINOR	Percentage minor defects
PNORM	Percentage normal

PPD	Proximal droplet
PSD	Pseudodroplet
R	Experimental group
SD	Standard deviation
STMP	Stumptail
TERAT	Teratoid spermatozoa
VOU	Nuclear ridge or fold

TABLE OF CONTENTS

CHAPTER 1

Theme: Growth and reproduction of domesticated animals

1.1 Title

The effect of dietary vitamin E supplementation on semen quality of A.I. dairy bulls.

1.2 Aim

(i) To compile a comprehensive literature study on the factors which affect the fertility and reproductive ability of bulls

(ii) To study the effect of dietary vitamin E on the semen quality of dairy bulls at an artificial insemination centre

1.3 Motivation

An urgent need has been identified in the animal breeding industry for quantifying the effects of fat-soluble vitamins on semen quality. There is a hypothesis that the antioxidant ability of vitamin E makes a contribution towards improved semen quality. The most important function of vitamin E is as an antioxidant that protects tissue lipids from free-radical attack.

According to previous research, uncertainty with regards to about the effect of different levels of vitamin E supplementation on semen quality (Kozicki *et al.* 1981). Current research results on the effect of vitamin E on semen properties are rare and contradicting (Kozicki *et al*, 1981). It has also been found that bulls injected with synthetic vitamin E showed a decrease in

ejaculate volume and sperm numbers per ejaculate, but sperm survival time was increased while reaction time was not significantly affected. Bulls were fed additional vitamin E, in the form of maize and wheat, in a ration during a 100-day period, showed a reduction in ejaculate volume and sperm numbers per ejaculate. However sperm survival time and reaction time were increased (Stojanov *et al*, 1966).

These contrasting results may be due to the form in which the vitamin E was administered but it is clear that further investigation is needed to determine the true effects. The ability to manipulate certain semen characteristics with vitamins may hold potential for the cattle breeding industry. Thus, due to the uncertainty of the effects that dietary vitamin E may have, further investigation is needed.

1.4 Introduction

It is well known that vitamins are required for physiological processes in animals and play an essential role in metabolism. The effect of vitamin A deficiency on reproductive performance has been extensively studied (Boyazoglu, 1997; Bearden and Fuquay, 1997; Kozicki *et al*, 1981; Hafez, 1974),which suggests the possibility that other vitamins may also have an effect on reproduction and the supplementation of which may improve reproductive performance. It has been suggested that vitamin E deficiency may result in testicular degeneration, thereby reducing spermatogenesis (Cupps, 1987). According to McDowell *et al* (1996) vitamin E is essential for reproduction as well as growth, prevention of disease and the integrity of

tissues. Early research in male rats showed that a deficiency of vitamin E resulted in the degeneration of the testes, resulting in permanent sterility (Bearden and Fuquay, 1997). Kozicki *et al* (1981) found that doses of the AD_3EC vitamin complex does not affect the sperm volume, concentration, freezability, proportion of insertion abnormalities or the occurrence of proximal droplets. Evans and Bishop, as cited by Swenson *et al* (1993), recognised that the cause of failed reproduction in rats fed purified diets to be due to a vitamin E deficiency.

The objective was to investigate the effects of dietary supplementation of vitamin E on the semen characteristics and quality in Holstein-Friesian bulls. Semen quality is determined in terms of concentration, motility and morphology. The evaluation of the morphological characteristics represents a very important part of seminal analysis, so that, after sperm motility and concentration, sperm morphology is the third essential criterion for assessment of fertility based on ejaculate analysis (Briz, Bonet, Pinart and Camps 1995)

A satisfactory semen sample will have high concentration of sperm which is indicated by an opaque milky-white colour, 40% or more progressive motility and less than 25% abnormal sperm (Bearden and Fuquay 1997). According to Chacón *et al* (1999) a bull can be classified as sound for breeding if he was clinically normal with a minimum scrotal circumference of 30 cm at 24 months or older, with no more than 15% of abnormal sperm heads (including acrosomes and midpieces), and/or a maximum of 30% total sperm

abnormalities. A bull that is classified as not being fit for breeding has more than 30% abnormal sperm heads or with a maximum of 50% total sperm abnormalities and is without clinical problems at the time of examination. Chacón *et al* (1999) also found that bulls with a long scrotum had greater percentages of abnormal heads that they were classified as unfit for breeding. The cause of these increases abnormal heads has been suggested by Riemerschmid, Setchell and Shafik (cited by Chacón *et al*, 1999) that the venous blood flow from the testicle could be affected in bulls with a pendulous scrotum. leading to blood stagnation, thus interfering with the thermo-regulation system in the testes.

A high frequency of abnormal sperm is associated with reduced fertility. The economic impact of sub-fertility or sterility on both dairy and beef operations is without doubt enormous. Both of these industries suffer losses caused by delayed calving causing a reduction in calf crop thus depleting the number of saleable livestock or breeding stock.

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CHAPTER 2

2.1 Introduction

In many cases the role of the male as the possible causes for reproductive failure in the herd is overlooked. Reproductive failure may be associated with inherited characteristics, defective semen quality, failure in mating performance, physiological and psychological causes, infectious diseases, improper management or a combination of these facts.

Bulls affect the reproductive efficiency of breeding herds, irrespective of whether they are used for natural breeding or artificial insemination. Thus impairment of bull fertility results in great economic losses, particularly in extensive cattle production systems. It is for this reason that it is essential that bulls are examined both before and during their use in breeding programmes in order to identify and remove any individuals with potential low fertility (Chacón, Pérez, Müller, Söderquist and Rodriguez-Martinez, 1999)

This chapter will outline the factors which may influence the fertilising ability of bulls, be it in an intensive or an extensive beef or dairy system using A.I. or natural mating. However most of the documented evidence deals with bulls used in an artificial insemination system but the principles are the same and can be similarly applied.

2.2 Management

This factor is discussed first, because it is the link between almost everything that the bull is exposed to in its environment. Without effective management the animal may become susceptible to factors such as disease, stress to name a few. Management is probably the most important factor in ensuring high levels of fertility and the economic success of the enterprise. The objective of feeding selected sires should be to bring them into optimum sperm-cell production early in life and to maintain this condition in their mature lives for as long as possible.

Nutrition is the one factor where most bull management systems fail, be it in overfeeding or underfeeding. It is often a difficult factor to manage and to master but it is essential for the reproductive success in the bull. Severe underfeeding or overfeeding and deficiencies of specific nutrients, especially vitamin A, are probably the most common causes of impaired reproductive capability in males (Cupps, 1987). Optimum production of healthy, normal spermatozoa depends on the health, size and condition of the testis (Salisbury *et al*, 1978), thus the bull must be fed and managed with this in mind.

Feeding adequate levels of energy, protein, minerals and vitamins are necessary to promote optimum growth and development of the young bull. Sperm-cell production is dependent on the amount of actively functioning

testicular tissue and testes size, relating directly to the size of the bull (Salisbury *et al*, 1978). The reproductive efficiency is more adversely affected by malnutrition in young males than more mature bulls and deficiencies of energy or protein can delay the onset of puberty. As the bull matures, the reproductive function becomes of a higher priority than body maintenance, therefore the effect of malnutrition are indicated by other symptoms and not in a decline in reproduction (Salisbury *et al*, 1978). Nutrition is however still important in all phases of a bull's life to ensure that the semen which is produced by that bull is of a good quality and that it will fertilise an ovum and produce offspring.

Liberal feeding has been shown by Davies *et al,* as cited by Salisbury *et al* (1978), to bring bulls into active sperm production and causes active secretion of the male accessory glands earlier than limited feeding does. This in turn reduces the interval between generations which is considered a genetic advantage. A nutrient deficiency is not only associated with a reduction in testicular weight and secretory output of accessory glands, but it may also decrease sperm concentration and sperm motility, thus affecting the semen quantity and quality (Cupps 1987). However, one must be careful not to overfeed bulls as this too can have its adverse effects. Scrotal circumference, epididymal sperm reserves and seminal quality can actually be reduced in bulls fed a diet containing excessive energy. Overfeeding leads to the production of excessive fat which in most cases is deposited around the testes, affecting the thermo-regulation of the testes thus increasing the temperature inside the testes. The increase in temperature leads to

degeneration of the cells lining the wall of the seminiferous tubules that affects spermatogenesis (Salisbury *et al*, 1978; Bearden and Fuquay, 1997). In an experiment where a ram's scrotum was insulated or where the testes were tied against the abdomen, sterility was the result (Bearden and Fuquay, 1997). An animal that is obese may also have less resistance to diseases and other stressors. Further more, obesity could lead to physical problems associated with the feet and legs reducing libido and thus diminishing sexual activity.

Although overfeeding has serious implications, underfeeding is more common. Reproductive functions seem to be more susceptible to dietary restrictions of energy in growing bulls than in adult bulls and severe feed restriction may result in permanent damage to gonadal tissue. Restricted feed intake in adult bulls can decrease androgen secretion and semen quality. VanDemark as cited by Salisbury *et al*(1978) found that bulls fed only 60% of their calculated needs in total digestible nutrients from eight weeks of age to forty four months of age, and tested for semen production by partial exhaustion collections every four months never caught up with control bulls in sperm-cell production. In addition to a lower semen-producing capacity, the rate of sperm-cell replenishment after partial exhaustion was slower in the underfed bulls.

Often the nutritional problem is related to energy levels in the diet. A common sign of a severe energy or even protein deficiency in males is the suppression of endocrine rather than exocrine, testicular function coupled with diminished

libido and an arrest of growth and secretary activity of accessory glands (Cupps, 1987). Patterns of luteinizing hormone secretion may be altered and the serum concentration decreased. If the luteinizing hormone levels are affected then the testosterone levels in the bull should also be affected. Since the testosterone is the hormone responsible for spermatogenesis, semen production can be adversely affected. Prolonged protein or energy deficiency can result in the cessation of spermatogenesis altogether and most species will show a decrease in semen quality, insufficient supply of water may also lead to these results (Cupps, 1987). Specific nutrient deficiencies may result in a lower physiological ability to mate. These effects are usually accompanied by a decrease in the size of the testes and accessory glands.

In addition to protein and energy deficiencies which may cause either reduced fertility or even sterility, a deficiency in the amount of vitamins and minerals in the diet may also be the cause of fertility problems in bulls. It is likely that all vitamins needed for growth and maintenance are also needed for reproduction. Vitamin A is probably the most important vitamin in reproduction. It is necessary for the integrity of the germ cells in the seminiferous tubules and a deficiency of this vitamin can reduce or even stop spermatogenesis. Vitamin A deficiency may lead to the development of pituitary cysts, loss of libido, stiffness in the joints, incoordination and a wobbly gait resulting in an inability to mount (Salisbury *et al*, 1978). The degree of these deficiency symptoms depends on the age of the bull at the onset of the deficiency and the degree of the deficiency. In later stages of the vitamin A deficiency, with the appearance of joint stiffness, the effects on

semen quality begin to become apparent. There is a decrease in sperm-cell concentration and motility and finally a marked increase in the percentage of abnormal spermatozoa. There is recovery of the spermatogenic capacity when sufficient carotene is added to the diet (Salisbury *et al*, 1978).

Vitamin E is a vitamin needed in reproduction which needs further investigation, but in earlier studies in rats vitamin E was called the "antisterility vitamin" and it was shown that a deficiency resulted in degeneration of the testes, sometimes resulting in permanent sterility (Bearden and Fuquay. 1997). Cupps(1987) stated that a vitamin E deficiency might result in testicular degeneration thereby reducing spermatogenesis.

Minerals are also of great importance in the diet of breeding bulls and appear to affect the libido of bulls. Zinc appears to be of primary importance for the reproductive function in males (Cupps, 1987). A zinc deficiency may result in retarded testicular growth, atrophy of tubular epithelium, decrease in pituitary gonadotropin output and decrease androgen production. A deficiency may also influence gonadotropin activity by influencing the gonadotropin receptor complex (Hurley and Doane, 1989).

Animals which are fed diets deficient in zinc grow more slowly than normal animals. If the deficiency occurs when the bull is young, age of puberty is delayed. Puberty is usually reached when the young bull is at a certain weight and proper spermatogenesis can only occur when the testicular size has a diameter of not less than 30cm.

An iron deficiency has been associated with depressed libido and deterioration of semen quality, while a deficiency of cobalt has led to anaemia and a lowered libido. Bulls mainly require calcium and phosphorus for skeletal development and unlike producing cows, bulls do not secrete any product containing these minerals. This is the reason for a separate diet being offered to breeding bulls. If bulls are fed the same diet as producing cows they will suffer from excess calcium intake. An excess of calcium in the diet of young bulls was found to result in the deposition of exceedingly dense bone which may cause the normal bone marrow to disappear (Salisbury *et al*, 1978). The excess bone forms in and around the backbone interferes with normal activity of the bull causing lameness and it suppresses the willingness and the ability of the bulls to mount (Salisbury *et al*, 1978). A deficiency of phosphorus is presumed to affect reproduction by reducing the appetite and thus reducing the intake of all nutrients, but it would seem that the affect of a phosphorus deficiency is not as detrimental to bulls as it is to cows.

Some minerals which are needed in the diet of bulls for normal body functions and reproduction may have a toxic effect. The cattle producer should be aware of these toxic minerals and ensure that they are not present in the diet that is being fed to his animals. One such toxic mineral is molybdenum. Diets high in molybdenum show the usual symptoms of copper deficiency such as infertility, anaemia, poor growth and bone disorders (McDonald *et al*, 1998) together with a lack of libido and the prevention of spermatogenesis (Salisbury *et al*, 1978). Cadmium is another mineral that is highly toxic when fed in excess and causes testicular necrosis.

Many nutritional problems, if recognised, can be corrected and in most cases normal semen production and reproductive activity will result. However, it must be remembered that the cumulative effects of nutritional deficiency together with any infectious diseases or injuries can affect the bull's reproductive effectiveness, thus the old saying of "prevention is better than cure" rings true.

Management of reproduction also includes the effect of the age and size of the bull when relating to semen production. Puberty is the time when fertile spermatozoa are observed in the ejaculate and this is usually between the ages if 10 to 12 months in bulls (Bearden and Fuquay, 1997). Puberty is however not an indication of the sexual maturity of the bull. Testes size and total production of spermatozoa increase until about 18 months of age and have been shown to continue increasing until 3 years of age. Many studies have shown a high correlation between testes size and body weight (Salisbury *et al*, 1978 and Bearden and Fuquay, 1997). There is also a correlation of 0.80 between testes size and sperm output (Salisbury *et al*, 1978 and Bearden and Fuquay, 1997). The above mentioned correlations have been shown to decrease as the bull increases in age, thus the period between puberty and sexual maturity are of great importance from a management perspective to ensure that the young bull is at the expected weight or size for his age if adequate or above average fertility is to be expected. Due to the presence of the correlation testes size and body weight and between testes size and sperm output, selection can be done at a

relatively young age, for example a bull can be eliminated from a breeding programme if the scrotal circumference is lower than the minimum standards of the breed, at a specific age and weight.

The way in which a bull is handled in the breeding system can also have an effect on his reproductive ability. Boyd as cited by Salisbury *et al* (1978), found that bulls given extra attention such as brushing and clearing, showed a higher sexual interest than those that received no extra attention. The frequency that a bull is used can have an adverse effect on his reproductive performance. In an artificial insemination centre environment, if semen is collected from the same bull every day, the volume and total sperm collected have shown to decrease in some bulls to a point where it is not commercially viable to process the semen. If semen from a certain bull is desired then it is suggested that semen is collected every second day in order to obtain ejaculates of relatively high sperm concentration. If the bull is in an extensive farming system, it is advised that he be used with the correct number of females which he can breed with successfully to prevent exhaustion during the mating season. A mature bull can be used at a ratio of 1:35 cows to achieve acceptable conception levels. If a young bull is used then the ratio to female animals must be less. The exhaustion level of bulls is measured by the level of his libido, as a bull will continue to mate with as many females as his libido will allow. This is similar for artificial insemination centres where semen is collected. The semen quality has not been proven to decrease with over use but the libido declines and with it the ability and willingness to mount (Bearden and Fuquay, 1997).

Exercise was once thought to be essential in maintaining semen quality and fertility levels in bulls, but when an experiment was conducted where bulls were exercised for 30 minutes a day and another group of bulls were confined, there was no differences in semen quality (Bearden and Fuquay, 1997). Exercise is important from a physical aspect, such as maintaining the strength of the bull's legs, reducing lameness and preventing excess growth of hooves. These factors are all important in maintaining the male in an optimum breeding condition.

When transporting bulls, care should be taken to ensure that the conditions in which the bulls are transported are comfortable and the chance of injury is prevented. Transportation of bulls does not seem to have any negative effects on semen production and quality but care should be taken when transporting bulls to minimise stress(Salisbury *et al*, 1978; Bearden and Fuquay, 1997).

2.3 Inherited Causes of Reproductive Failure

Rollinson (1955), estimated that approximately 10% of all sterility is hereditary (Salisbury *et al*, 1978). Certain anatomical defects are in most cases hereditary and can reduce or even eliminate reproductive performance. Inbreeding should thus be avoided as far as possible because this can give rise to the phenotypic expression of mutant genes and defects. Heterosis has been shown by many authors to improve fertility (Hafez, 1974). In a study

performed by McNitt *et al* (1966), it was found that in a herd of half-sib bulls, with physical defects of the genitalia were highly heritable which, shows that selection against these defects will be successful and can thus be done to eliminate the appearance of them in a breeding herd (Hafez, 1974).

One such heritable defect of the genitalia is cryptorchidism. It is a genetic defect that occurs only in males where either one or both testes do not pass into the scrotum. A bull with bilateral cryptorchidism results in sterility because spermatogenesis cannot occur at body temperature due to both the testes being retained in the body cavity. The condition can be corrected by surgery if the bull has the genetic ability to produce excellent offspring, but this solution is not recommended due to this defect being hereditary. It would be better to destroy these animals or rear them purely for meat but to breed with them is not advisable. A bull with unilateral cryptorchidism can still produce normal semen but the testis that is still in the body cavity is not functional because of complete degeneration of the spermatogonia. *Impotentia Coeundi* is another heritable defect that is considered to be caused by an autosomal recessive gene which only affects males. This condition is defined by the inability of the retractor penis muscle to relax. This condition can also be corrected by surgery and the ability to copulate will return but this is also not advisable for genetic reasons (Salisbury *et al*, 1978). It is suggested that environmental factors may be involved in causing this condition (Salisbury *et al*, 1978).

Certain aspects of the spermatozoa morphology is also hereditary, and increased heterosis has been shown to decrease primary morphologic abnormalities of the spermatozoa. All seminal traits appear to be moderately heritable except for secondary morphologic abnormalities which can be selected, however progress might be slow (Hafez, 1974). It is for this reason that the sperm morphology of breeding bulls must be checked regularly for any abnormalities, as it could be a possible reason for lower reproductive efficiency and the failure of cows mated with or inseminated with the semen of a particular bull. Donald and Hancock (1953), found a specific gametic sterility in England and Holland, which is regulated by a recessive autosomal gene. Bulls homozygous for this gene produced large numbers of abnormal spermatozoa and are completely sterile (Hafez (1974) and supported by Donald and Hancock (1953)). A congenital anomaly such as testicular hypoplasia is another heritable defect where the potential for complete development of the spermatogenic epithelium is lacking in one or more epithelial components (Hafez, 1974).

Fertility itself can be considered as lowly heritable, approximately 5% (Bearden and Fuquay, 1997), however the influence of managerial practices and the effect of the environment greatly impacts on an animal's reproductive performance. Because fertility can be roughly measured according to the bull's libido and ability to serve a female, the heritability of these two traits should be taken into consideration. Johnson and Rendel (cited in Salisbury *et al,* 1978) reviewed data which indicated that libido and the ability to mate has heritability values of between 0,30 and 0,40 (Salisbury *et al,* 1978). Thus the

fertility of the breeding bulls in a herd cannot be solely due to the genotypic makeup of the bull and this is why management of breeding bulls is so important.

The indicators of fertility, however, can be measured for heritability and if moderately to highly heritable can be selected to improve the general fertility of breeding bulls. An example of an indicator of fertility is scrotal circumference. As mentioned before the scrotal circumference is positively correlated with sperm production and any bulls with a scrotal circumference lower than 30 cm should be eliminated from the breeding herd. Scrotal circumference has an approximate heritability of 0,50 (Fuchs *et al,* 1992) and can be selected when selecting for increased reproductive efficiency. Weight or body size is positively correlated with puberty and testis size. The weight at puberty has a heritability of approximately 0,50 (Newman, 1989) and the age at puberty can also be selected for to an extent due to an estimated heritabiity value of 0,40 (Newman, 1989).

There is cumulating evidence proving the presence of genes that result in the production of enzymes. Gene mutations could alter these enzymes making them useless for their intended purpose. At least 40 hereditary disorders involving such enzyme dysfunction have been identified in humans and it is likely that the same may be found in animals (Salisbury *et al*, 1978). There are approximately 25 hereditary structural defects that can result in calves being stillborn or cause them to die soon after birth, have been identified. Increasing evidence shows that these defects may also be induced by various

teratogenic agents like radiation, drugs, dietary deficiencies or toxic compounds (Salisbury *et al*, 1978).

The person in charge of breeding programmes and selection of breeding bulls must be aware of the genotypic make up of the bulls under inspection and it is of the utmost importance that the heritabilities of important aspects pertaining to the expected fertility and reproductive performance of the offspring are known. The knowledge of the heritabilities of desirable traits is also important so that one can select for those traits that will be seen within one or two generations, thus speeding up genetic improvement. It is not economically viable to spend time and money in selecting for traits which will only be evident in several generations. Such traits can be selected for if it is positively correlated with another economical trait of high heritability. The effect of certain traits on each other must also be clear. For example, reproduction traits and production traits are mostly negatively correlated, such as rate of gain and rate of conception.

2.4 Pathological Causes of Reproductive Failure

The susceptibility of animals to diseases are also linked to management, such as housing, feeding and maintenance. For example, animals which share a drinking trough or feed from the same trough may share pathogens (Cupps, 1987). However, due to the large adverse effect diseases have on reproduction it is better that they be discussed under a separate section. Some of these diseases may occur suddenly and have a detrimental effect on

both production and reproduction. Others may be present in a herd without the manager's knowledge and the cause for reproductive failure may be assumed to be due to other factors. It must be remembered that the environment plays an important role in disease transmission in terms of the types of organisms or transmitters which pass the pathogens from one animal to another. The most common types of organisms which pose a problem in South Africa include biting flies, mosquitoes and ticks. These factors must be controlled to reduce the chance of pathogenic diseases spreading through a herd (Cupps, 1987).

It is important that the bulls, which are to be used for breeding, are regularly examined for the presence of any disease, especially those that are sexually transmitted. In an extensive system where natural mating is used this examination should be performed at least one month before the breeding season begins. If the bull is tested positive for a disease then the one-month period might allow sufficient time for recovery before the bull starts mounting females. Bulls which have just been purchased must, as a rule, be quarantined and tested for any diseases which he may transmit to the other bulls in the breeding programme or to the cows. In an artificial insemination system, more regular checks are done on bulls for semen quality as a part of the routine semen quality examination, testing for the presence of any sexually transmitted disease must be done to ensure that the disease is not distributed together with the semen straws which may even be destined for exportation. The farmers, especially in the dairy industry pay very high prices

for top quality semen from artificial insemination centres. It is the centre's responsibility to provide safe semen to their customers.

There are three groups of diseases which have an effect on reproduction; (a) bacterial diseases, (b) protozoan diseases and (c) viral diseases.

(a) Bacterial Diseases:

Vibriosis is a venereal disease which is caused by *Campylobacter fetus venerealis*. It is transmitted during natural mating by semen which is infected with the organism, but it can also be passed from one bull to another in an artificial insemination centre when using the same teaser bull (Bearden and Fuquay, 1997). The infected bull may leave the organism on the teaser bull, and if the non-infected bull touches the same teaser bull with his glans penis, he could become infected. Bulls can also become infected with the organism if he is mated with a female which is infected. The bull is merely the carrier of the disease and will show no clinical symptoms of the disease, which is why it is so important to do regular examinations. The clinical symptoms of the disease are seen in the females in the form of repeated breeding, irregular oestrous cycles, abortions, a cloudy oestrual mucous. Temporary infertility in bulls may result from this organism (Salisbury *et al*, 1978, Bearden and Fuquay, 1997). Bearden and Fuquay (1997) state that there have been reports of spontaneous recovery in some bulls. In a natural mating system this disease can have detrimental effects because it can be spread rapidly in the herd because one bull can mate up to 35 females before any repeat

breeding or abortions are apparent. If another bull is used because the first bull is assumed sterile the disease is spread to the new bull as well. This disease can be prevented by vaccination annually, two months before the breeding, or by using semen for artificial insemination from a disease free artificial insemination centre.

Streptomycin can be used to treat infected animals but extensive testing must be done to ensure that the bull is totally free of the organism before utilizing him again in a breeding herd. Leptospirosis is a bacterial disease caused by *Leptospira interrogens*. There is a concern that this disease can also be passed onto humans. There are several symptoms of the disease that have appeared in cattle and depending on the severity of the disease some or all of them may be apparent in cattle. Many of the clinical symptoms of the disease may be present in an infected female, but bulls may show symptoms of increased body temperature as high as 40,5ºC to 41,7ºC (Bearden and Fuquay, 1997), blood in the urine; loss of appetite and a loss of body weight. Bulls become infected with the organism by ingesting it from an infected area where other infected animals have shed the organism in their urine on pastures or in the water. Once ingested it enters the circulatory system and congregates in the kidneys. Bulls can infect females through their semen. If a bull infects a female the reproductive efficiency, due to abortions reduced production with an accompanying decrease in milk production. The main problem with this disease, besides the effect on the animal, is that animals which are treated may continue to shed the organism in their urine for up to three months before they are cured. The chances of more animals becoming

infected in this time increases and infected animals must be isolated from the healthy part of the herd until they are absolutely free of the *leptospira* organism.

The best method to diagnose Leptospirosis is through a serum agglutination test (Bearden and Fuquay, 1997). If the test is positive one should not assumed that the animal is infected with Leptospirosis, as the bull may have been vaccinated or he may have had the disease previously. It is for this reason that the test should be done again within two or three weeks and the concentration of antibodies present compared with the first test to see if there is an increase. One should test for all the known strains of *Leptospira* to ensure that the organism that is present will be confined to cattle or if it is the strain that can be transmitted to humans known as *Leptospira sejroe.* The disease is difficult to control because of the number of different types of animals which can be infected with *Leptospira* organisms. The best measure of control is to vaccinate the bulls, isolate and vaccinate newly purchased bulls, ensuring that potential organisms are contained before they join the rest of the herd. It may help to prevent them from drinking from farm ponds where the water moves slowly or is stagnant.

Brucellosis is the bacterial disease which is a problem worldwide and poses a concern to public health (undulant fever). It is caused by three strains of the organism *Brucella* (Salisbury *et al*, 1978). Humans are able to contract the disease (Bearden and Fuquay, 1997) from various sources like slaughterhouses, raw milk and other animal products. The most prominent

clinical sign of Brucellosis in cattle is an abortion after the fifth month of gestation. The testes of bulls may also become infected and swollen resulting possible in sterility in some cases (Salisbury *et al,* 1978, Bearden and Fuquay, 1997). Livestock can become infected with Brucellosis by ingesting contaminated feed or water. The female which aborts, sheds *Brucella* organisms with the aborted fetus and other animals can become infected by consuming contaminated pasture or the aborted fetus. Purchasing of new animals which are infected with brucellosis and placing them with the rest of the herd before testing for diseases is one of the main causes of introducing the disease into the herd (Salisbury *et al*, 1978). Bulls transmit this disease via their semen. It is for this reason that all bulls in an artificial insemination centre are regularly tested for the presence of *Brucella* organisms.

Testing for the presence of *Brucella* organisms is generally done with an agglutination test using blood serum (Bearden and Fuquay, 1997). The best means of preventing the occurrence of this disease in a herd is through vaccination usually in calfhood before puberty which gives the animal immunity. As for many vaccines it is not totally effective, but presents a good preventative measure. The best control measure if brucellosis is found in a herd is to destroy the infected animals and to regularly test the others to ensure that the organism has not spread.

(b) Protozoan Diseases:

Trichomoniasis is a venereal disease caused by the protozoan *Trichomonas fetus* and is transmitted through sexual contact including artificial

insemination. There are no clinical symptoms of the disease in bulls, however, if a large number of females, that have been served by the same bull, develop a pussy discharge from the reproductive tract and they are examined and found to have a pyometra. The bull should be tested by doing a preputial wash, as *Trichomonas fetus* usually lives on the penis and the prepuce in the bull. A sample should be sent to a laboratory for diagnosis. Unlike females infected which, can be treated with a period of sexual rest, infected males must be slaughtered because they will generally remain infected for life (Salisbury *et al*, 1978). There is a vaccine available for the prevention of Trichomonas for animals bred by natural mating (Bearden and Fuquay, 1997).

There are other protozoan diseases that affect reproduction but the effect is mainly evident in the female.

(c) Viral diseases:

Bovine viral diarrhoea (BVD) is the viral disease which can be transmitted by bulls through natural service and artificial insemination. It causes loss of appetite, emaciation, mild diarrhoea and irregular growth rates. An acute form of the disease is characterised by profuse diarrhoea, elevated body temperature and erosion of the gastro-intestinal tract (Bearden and Fuquay, 1997). The disease can occur in a mucosal form, which is most common and in addition to the symptoms seen in the acute form of the disease there is also ulceration of the oral cavity and mucous membranes of the digestive tract.

This form of the disease is lethal and animals can die within 14 days. BVD mostly affects animals between the ages of 8 and 18 months. The subclinical form of the disease is however the most common occurrence of the disease displaying no symptoms of infection (Bearden and Fuquay, 1997).

Bearden and Fuquay (1997) stated that as high as 50% of the tested animals in many herds actually exhibit antibodies against the virus. The disease can be controlled with the use of either a modified live virus or a dead virus vaccine. There are advantages and disadvantages of both types of vaccines. The modified live virus when administered, will provide the animal with immunity for one year, and with the aid of an annual booster lifetime immunity is achieved but this vaccine may cause abortions in some cows. The dead virus vaccine consists of a two – dose priming regimen which will induce immunity, but at least three vaccinations are required during a year to maintain immunity. The main advantage of using this type of vaccine is that it can be used on pregnant cows (Bearden and Fuquay, 1997).

Another viral disease which will have an adverse effect on reproduction is Infectious Bovine Rhinotracheitis-Infectious Pustular Vulvovagenitis (IBR-IPV). This disease is transmitted by viral particles from a contaminated environment coming into contact with the mucous membranes of the animal. IBR-IPV can affect the respiratory system and the digestive system, but its effect on the reproductive tract is of interest. When the bull is infected with this viral disease the glans penis usually develops pustules and the associated pain may be severe enough to prevent breeding. Kendrick and

McEntee as cited by Salisbury *et al* (1978) inseminated 12 heifers with semen containing IPV and only one conceived while 10 of the infertile animals showed histological evidence of endometritis and exhibited abnormally short oestrous cycles. This suggests that there is also a reduced fertilising ability of the semen of infected bulls. Vaccination is the recommended control measure with a modified live virus vaccine which may give lifetime immunity, but booster vaccinations are advisable (Bearden and Fuquay, 1997).

Bluetongue is a viral disease which affects both cattle and sheep. This virus can be transmitted by bull semen through natural mating and via artificial insemination. The clinical form of bluetongue affects the mouth and feet and causes abortions and weak or stillborn calves. Control measures include vaccination with a modified live virus vaccine which will provide immunity for 2 – 4 years (Bearden and Fuquay, 1997).

2.5 Physiological and Psychological causes of Reproductive Failure

Other than the sterility or reduced reproductive efficiency caused by pathological factors, physiological causes of reduced fertility are the most important and must be considered when assessing reproductive efficiency in bulls. Physiological factors can include age, seasonal variations including changes in light and temperature, or anything affecting the normal function of the endocrine balance in the animal, directly or indirectly (Salisbury *et al*, 1978, Bearden and Fuquay, 1997).

The effects of age on reproductive efficiency is difficult to measure due to the effect of environmental factors affecting reproductive efficiency and selection pressures for both the ability to produce and reproduce (Bearden and Fuquay, 1997). Generally, young bulls increase in fertility as they grow older and reach a fertility "peak" at about two years of age which may last until four years of age, after which there is a gradual decline with age. Some bulls may maintain a high level of fertility longer than average. The cause of reducing fertility with increasing age is not known but it may be due to a hormonal imbalance or deficiency (Bearden and Fuquay, 1997). Hahn, Foote and Seidel as cited by Salisbury *et al* (1978) reported that sperm output and the percentage of morphologically normal sperm at the time of collection was lower in older bulls compared to younger bulls. There are obviously exceptions to every rule, there are some old bulls that survive rigid selection in artificial insemination centres and are just as fertile as younger bulls.

The seasons of the year has been shown to have an effect on fertility but the effect is mainly due to the changes in length of daylight. Light appears to have a influence on fertility and it has been shown that in areas where there is a difference in the daylight length between seasons, the time when fertility is at its highest is in spring when the daylight hours increase (Salisbury *et al*, 1978). In South Africa however, cattle are not considered seasonal breeders although the semen quality may improve in the spring, which is why the October to December period is generally used as the breeding season. In sub-Saharan Africa the summer heat can counteract the benefit of the longer daylight hours and it can actually reduce fertility. Furthermore, humidity and

improper nutrition management can also complicate fertility even if the daylight hours are sufficient. Controlled environments can be used to overcome extremes in the environment but this is in most cases not economically viable.

Season also affects the type of nutrition the animal's has at its disposal and different nutrients are available at different times of the year. Management plays a role here because the manager must be aware of which nutrients are not available at a certain time of year and they must be supplemented to improve the reproductive efficiency of breeding bulls. All these factors undoubtedly influence the endocrine function in bulls but they are unfortunately not fully understood (Salisbury *et al*, 1978). It is assumed that bulls producing low numbers of spermatozoa may be deficient in follicle stimulating hormone (FSH) which stimulate spermatogenesis. Bulls with lowered libido and lack of energy are assumed to have an androgen deficiency which may be caused by a lack of luteinizing hormone (LH), however, real proof of these deficiencies are lacking (Salisbury *et al*, 1978).

Psychological causes of reduced reproductive efficiency has not been thoroughly researched (Salisbury *et al*, 1978, Bearden and Fuquay, 1997) but due to reports from some farm managers and results from the limited experiments which have been conducted to try and gather psychological data, it is becoming increasingly evident that animal handling techniques can have an effect not only on reproduction but production as well.

Bulls in artificial insemination centres have shown some psychological effects on performance. For instance some bulls develop inhibitions about certain teaser animals or a certain collection area (Bearden and Fuquay, 1997). If any pain is associated with collection of semen some bulls have exhibited a lowered libido often associated with the artificial vagina temperature being too high. Any stress or change in routine has shown to increase the percentage of abnormal sperm in some bulls. Thus animals should be handled in a manner which reduces stress as much as possible to avoid the effects of mistreatment on fertility.

2.6 Failure in mating performance

The first aspect when evaluating a bull to determine his value as a breeding bull, be it in an artificial insemination centre or for natural mating purpose, is his willingness to mate or his libido. A bull's mating performance can be affected by psychic disturbances, physical disabilities, environmental temperature and nutrition (Hafez, 1974).

Injuries or physical disabilities are probably the most common cause for reduced mating performance or reduced libido. As mentioned above, anything which may cause pain during copulation will decrease the bull's willingness to serve thus reducing the reproductive efficiency. There can be abnormalities of the penis which will result in failure to achieve intromission. The most common defects of the penis are deviations, fibropapilliomas and persistence of the penile frenulum (Hafez, 1974). The most common

deviation is a spiral deviation or "corkscrew penis". This can be seen in some normal bulls stimulated by electroejaculation and thus should only be diagnosed for bulls which repeatedly fail to achieve intromission (Hafez. 1974). Penile fibropapillomas are frequently seen in young bulls which are in the same pen. Young bulls often mount their penmates which causes abrasions on the glans penis, this allows viruses to invade the penile epithelium which causes cutaneous warts and genital fibropapillomas. Penile fibropapillomas appear as flat or raised masses with a crusty or horny surface and can become ulcerated and infected (Oxford Science Publications, 1988). These can be surgically removed but if removal is done when the lesions are first observed then regrowth is likely to occur. Vaccination is also possible to avoid this disease.

Persistent penile frenulum causes a deviation of the erect penis which prevents normal protrusion. This can be surgically corrected and most bulls exhibit a full recovery. Where the incidence of a penile frenulum has been high in certain herds, it has been suggested that there might be a genetic basis for this abnormality (Hafez, 1974). It is for this reason that surgical correction of this abnormality may not be advisable.

Injuries sustained to the scrotum or sheath can cause infection and inflammation. Injuries to the penis can occur during natural mating and during collection of semen for artificial insemination, one such common injury is the "broken penis". This can happen when the penis is bent at a sharp angle during the ejaculatory thrust, rupturing the corpus cavernosum penis (Figure

2.1). In some cases the penis has even been amputated by tight rubber bands lost from the artificial vagina during semen collection (Bearden and Fuquay, 1997)

Figure 2.1: Bull with a broken penis. Note the swelling between the sheath opening and the testes. (Taken from Bearden and Fuquay, 1997)

Abnormalities of the bull prepuce can also affect the ability of the bull to serve. The prepuce is longer in the Zebu type breeds compared to the European cattle breeds. Prolapse of the prepuce can result in injuries and subsequent infections which lead to scarring and constriction of the preputial orifice (Hafez, 1974). Such injuries are mainly sustained from walking in the bush and being entangled by or scraped against small bushes. Prolapse of the prepuce is heritable and this should be taken into account when selecting breeding bulls. A bull's inability to serve may also be due to abnormalities or injuries in other parts of the body other than in or on the reproductive tract. The legs of the bull must be strong and in good condition as they give him the ability to mount a female or a teaser, or in a natural mating system, to walk

and find cows which are in oestrous. Dislocations, arthritis, skeletal fractures, ruptured ligaments, tendons or muscles, foot rot, and overgrown hooves are all factors which can affect the bull's ability to mount even if he has a high libido. Abnormalities of the vertebral column, bones and joints in the hind limbs frequently impair the ability of older bulls to serve (Hafez, 1974). Advancing age usually gives rise to the more frequent occurrence of arthritis to the stifle, hock joints, knees and fetlocks.

2.7 The effect of poor semen quality on reproduction

The morphologically normal bull sperm (Figure 2.2) consists essentially of a head which contains the paternal hereditary material and a tail with the principle function of locomotion. The head can be described as a flattened ovoid structure made up of a nucleus covered anteriorly by an acrosome and posteriorly by a post nuclear cap. The tail is approximately 40-50µ long and is differentiated into three parts: the midpiece, the mainpiece and the endpiece. Movement is accomplished by waves generated in the implantation region (anterior end of the midpiece connecting the head) forming a whiplash type of movement. The midpiece which is the thickened region of the tail between the head and the mainpiece is the energy supplier for the sperm because it is rich in phospholipids, lecithin and plasmalogen (Hafez, 1974). The mainpiece is the longest part of the tail and is the main part responsible for propelling the sperm forward.

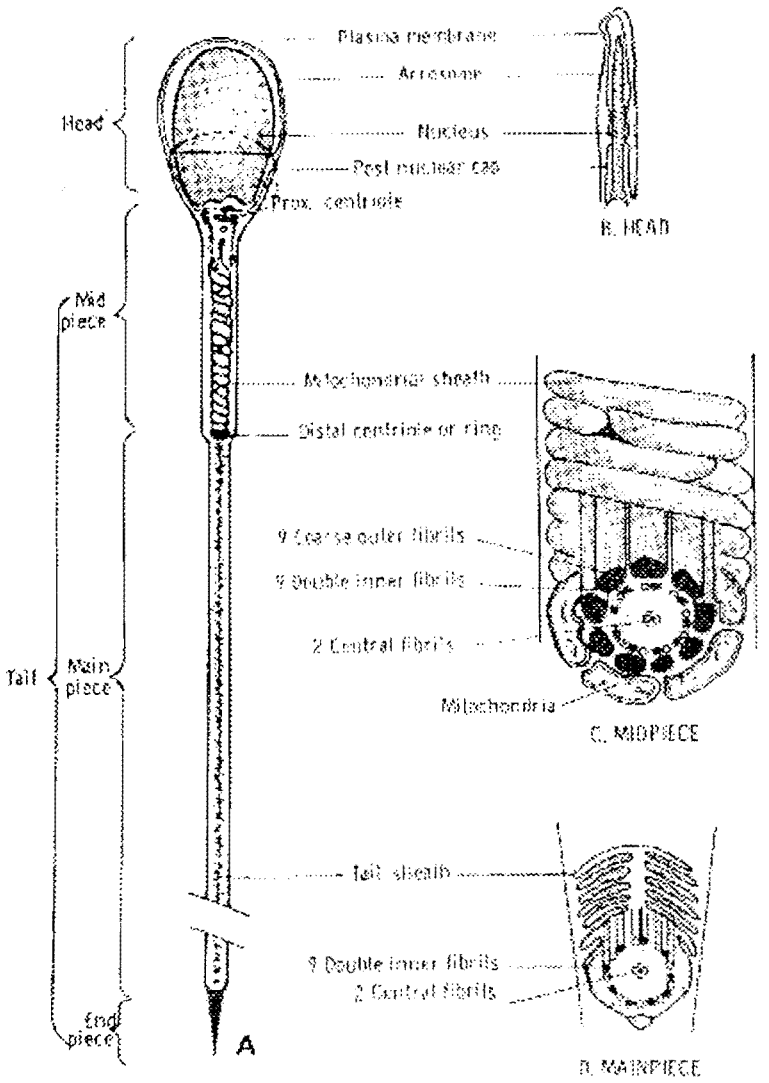

Figure 2.2: Structural Diagram illustrating the normal bovine sperm cell

(Taken from Bearden and Fuquay, 1997)

Any abnormality of the sperm, be it in the head or in the tail, will have an effect on the fertilising capability of the sperm. The spermatozoon may display one or a combination of defects which include; knobbed acrosome, pyriform or tapered heads, microephalic and macroephalic heads, nuclear vacuoles, abnormal DNA condensation, nuclear crests, detached heads, distal midpiece reflex, dag defect, mitochondrial aplasia, pseudodroplet,

corkscrew defect, bowed midpieces, abaxial tails, stumptails, cytoplasmic droplets and teratoid spermatozoa (Figures 3.1 to 3.20). The presence of one or more of these defects in the ejaculate of a bull indicate not only the fertility of the bull, but the presence of certain defects. This can help to identify where the problem in the reproductive tract of that bull exists, and possibly it can be corrected to improve the quality of the semen.

The knobbed acrosome defect can be described as a refractile or dark-staining area or peculiar thickening at the tip of the sperm head (Barth and Oko, 1989). It is usually seen, by light microscopy, in bull semen as an indentation or flattening in the apex of the acrosome and more uncommonly, as the name actually suggests, as a bead on top of the acrosome. The Friesian breed seems to have a relatively high incidence of this defect compared to other breeds (Barth and Oko, 1989). This defect can occur in any bull where the spermatogenesis cycle has been disturbed one way or another. If this is the case then it will be accompanied by other defects, resulting in reduced density and motility of sperm. It is suggested that the occurrence of knobbed acrosomes may be heritable, but the environment can also have an effect on their occurrence (Barth and Oko, 1989). If knobbed acrosomes are found together with other abnormalities such as nuclear vacuolation, then adverse environmental conditions may be the cause. Nutritional deficiencies, toxicity, inefficient testicular temperature control, or systemic illnesses are all stress factors which can cause the occurrence of knobbed acrosomes (Barth and Oko, 1989). Many authors (cited by Barth and Oko, 1989) have concluded that bulls which are severely affected by

knobbed acrosomes are sterile. In a trial, which used a Charolais bull with 83-99% of the spermatozoa having knobbed acrosomes and which served 23 of 49 cows, only produced 2 calves. The motility and sperm transport appeared to be unaffected, thus the problem lies when the sperm attempts to attach to the ova. Depending on the cause of the appearance of the knobbed acrosome defect bulls may recover to normal fertility. If the reason is genetic then the bull will always have a low fertilising capacity and his value as a breeder will decrease. If the cause is due to testicular degeneration or poor spermatogenesis then recovery is more likely upon removal of the responsible stressor (i.e. high temperature).

The most common abnormality of the sperm head is the pyriform shape, it is pear-shaped where the acrosomal region is rounded and the postacrosomal region is narrow (Barth and Oko, 1989). Sometimes the difference between the normal sperm head and a pyriformed one is difficult to distinguish and require a trained eye. There is a wide range of the appearance of this defect, from paddle-shaped to slightly pinched in the postacrosomal region. Most bulls have this type of defect in their semen and exhibit normal fertility levels. This defect may have a genetic or an environmental origin. Adverse influences may activate hormonal or metabolic events, either extrinsic or intrinsic, to the sertoli cells which would unfavourably affect spermatid development, resulting in a rounder sperm head (Barth and Oko, 1989). Pyriform heads seem to appear whenever the normal testicular function is disturbed, for instance, when heat regulation of the testis is affected or there is an endocrine imbalance. As long as the sperm head has an acrosome

which is intact and there is no other defect affecting the motility of the spermatid the sperm should have no problem in penetrating the zona pellucida, but it is evident that semen with a high percentage of pyriform heads are less fertile. The reason for this may be that the abnormally shaped nuclei cannot initiate embryonic cleavage (Barth and Oko, 1989).

If heredity as a cause for this defect has been ruled out and obesity, injury to the reproductive tract or illness is identified as the cause, then a bull may recover from this abnormality and once again produce normal semen, depending on the severity at which these stressors occurred. In the case of thermo-regulatory problems caused by testicular hypoplasia, recovery may not be possible.

Microephalic heads are sperm heads which appear smaller than normal, thus, similarly, macroephaly suggests a sperm head which is larger when compared to a normal sperm cell. These defects rarely occur in large numbers in bull semen therefore, information on these defects is scarce. Barth and Oko (1989) have observed that microephalic heads have an occurrence rate of less than 1% in the spermiogram of a fertile bull and although microephalic heads occur more frequently than the macroephalic defect, macroephalic heads do not usually exceed 5-7%, even when severe disturbances have occurred.

Even though there is a lack of information on these defects it can be assumed that the smaller or larger heads result in either a deficiency or excess in

genetic material and thus sperm cells with these types of defects may not be able to fertilise an ovum resulting in non-development of an embryo (Barth and Oko, 1989).

Nuclear vacuoles are craters or pouches that occur in the nuclear region of the sperm head and have been identified in bulls, rabbits, stallions, boars and even man. Bane and Nicander as cited by Barth and Oko (1989) described nuclear vacuoles to appear as sparkling, round or elongated white spots, mainly in the form of a string of beads like a necklace at the acrosome-postacrosomal sheath junction. They may also be found throughout the nucleus but not necessarily in a necklace form but as single structures. The incidence of this defect in bull semen can range from 1% to 100%. It was reported by Bane and Nicander (as cited by Barth and Oko, 1989) that nuclear vacuoles occurred in all bulls that were tested, apart from one, simultaneously with disturbed spermatogenesis, accompanied by decreased sperm concentration, decreased motility and high levels of other sperm abnormalities.

Infertility due to nuclear vacuoles has been demonstrated by Miller *et al* (as cited by Barth and Oko, 1989). Miller *et al* also showed that a bull which had a history of low fertility had up to 80% vacuolated sperm. It is suggested that single nuclear vacuoles may not adversely affect the fertilising ability of the sperm cell but may result in early embryonic death (Barth and Oko, 1989).

The cause of nuclear vacuolation is not known but the incidence of occurrence may increase following illness, injury, feed shortage and abnormal environmental conditions. Recovery of a bull which is affected by nuclear vacuoles is possible but his semen should be carefully monitored for the rest of his productive life.

Abnormal DNA condensation can occur when spermatogenesis is disrupted causing nuclear morphological abnormalities to occur. These abnormalities can only be seen by using Feulgen's stain for DNA. Nuclear vacuolation can be seen as a type of abnormal DNA condensation. Barth and Oko (1989) have observed a DNA abnormality which has not been reported on before. The abnormality occurs as clumping of the DNA ranging from very coarse to fine-grained. DNA clumping has been identified in a number of cases by Barth and Oko (1989) to significantly reduce the fertility of the semen even when no other stressors such as illness, abnormal environment or nutritional deficiency were present. Sperm cells with abnormal DNA condensation are however seen in very low numbers in normal bull semen but the cause of this defect and the possibility of recovery is not known.

Nuclear crests may be found in bulls with both normal and pathological semen, but the incidence is usually less than 0,2% (Barth and Oko, 1989). Nuclear crests appear as a ridge on the surface of the head extending from the anterior of the cell for variable distances posteriorly. Macroephalic heads usually accompany this defect in addition to other morphological abnormalities. The effect of this defect on fertility is contrasting. Poor fertility

relating to this defect could be dependent on the percentage of affected sperm present in the bull's semen, but bulls which have displayed this abnormality have shown a constant level of occurrence when tested regularly, indicating that recovery from this defect is poor.

Detached heads are commonly found in the semen of bulls exhibiting normal fertility (Barth and Oko, 1989) and they appear to be caused by a number of conditions: testicular hypoplasia, testicular degeneration or an inflammatory condition of the seminal vesicles, ampullae and the epididymides, conditions that cause the temperature of the testicles to increase such as illness, pain causing the bull to lie down for long periods of time or experimental testicular insulation. Very low fertility and even sterility is caused by large numbers of detached heads in the semen due to the inability of the sperm cells to be propelled forward. In two cases of Hereford breeds fertility was normal with between 30 and 40% detached heads present in the sperm (Bath and Oko, 1989). The detached heads are usually absent from any other morphological abnormality. This defect is also genetic, probably due to a recessive gene.

The distal midpiece reflex is the most common sperm tail abnormality in bull semen, bulls produce a small percentage of spermatozoa with this defect (Barth an Oko, 1989). The typical appearance of this defect is a J-shape in the distal portion of the midpiece. In most cases a cytoplasmic droplet is trapped in the bend and the principle piece projects past the sperm head and in live sperm a backward motion results. There can be many variations in the appearance of this defect (Figure 2.3) where the bend may only cause a kink

in the distal part of the midpiece, or there may be a second bend in the opposite direction of the first. This defect can be induced by hypotonic solutions, this must be kept in mind when evaluating semen samples. In the bull the distal reflex is produced in the corpus and cauda epididymis, due to the cytoplasmic droplet being in the distal portion at this stage, the sperm with the distal reflex defect will have a trapped cytoplasmic droplet. If during examination there is a sperm cell with this defect but it is lacking a cytoplasmic droplet then it may indicate to the examiner that the defect was artificially induced by, for example, a hypotonic solution.

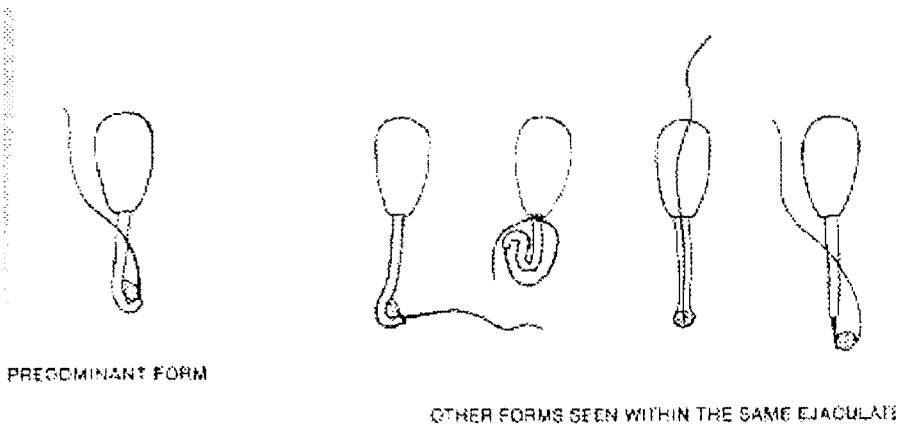

Figure 2.3: Variations in Distal Midpiece Reflex (Barth and Oko, 1989)

Blom (as cited by Barth and Oko, 1989) described the distal midpiece reflex to be a minor defect as it occurred at levels as high as 25% in the semen of bulls with normal fertility. It would seem fair to presume that the backward movement which is caused by the bending of the tail, there is no way that the sperm cell with this abnormality would be able to penetrate the zona pellucida. This could have a negative effect on fertility if the defect occurs at higher

frequencies. This defect can be induced by adverse environmental conditions but some bulls have this defect at varying levels continuously. If the environment is to blame then recovery is possible when the stress of the adversity has been relieved (Barth and Oko, 1989).

The Dag defect when observed through a light microscope, can be identified by the folding and coiling of the midpiece, the axis of the main fold is in the distal part of the midpiece. This folding and coiling is associated with the distal cytoplasmic droplet (Barth and Oko, 1989). The major feature of this defect is the fracturing or shattering of the midpiece associated with a disrupted arrangement of mitochondria.

It is suspected that this defect may be due to a heritable recessive gene and Barth and Oko (1989) suggest that the folding, coiling and dislocation of the axial fibres may be due to the malformation of the mitochondrial sheath late in spermatogenesis. There are few reports on the Dag defect being the cause of infertility and it usually constitutes less than 5% of the ejaculate sperm cells (Barth and Oko, 1989).

Sometimes a small gap can occur in the mitochondrial sheath known as mitochondrial aplasia. This defect appears not to have an effect on the fertilising ability of the sperm cell but these gaps can cause fractures and separation of the principle piece from the midpiece. When this occurs then the sperm cell loses its motility and thus cannot fertilise an ovum. It is usually

the movement of the principle piece that causes it to break at the point of the gap in the mitochondrial sheath.

The rare sperm defect known as the pseudodroplet is characterised by a local thickening somewhere along the midpiece and is often accompanied by a bend or fracture at the same place. The incidence of this defect can vary between 7 and 26% (Barth and Oko, 1989), the frequency of which can increase with age at the same time as motility and fertility decline. Very little about this defect is known and it has even been suggested by Blom that it is heritable (Barth and Oko, 1989).

The irregular distribution of mitochondria along the mitochondrial sheath is known as the corkscrew defect. In some cases a proximal droplet accompanies it. The defect, when observed under a light microscope, will appear to have a mitochondrial sheath with a corkscrew shape. This defect has been linked to a progressive degeneration of the testis and coincides closely with high levels of nuclear fission products. Barth and Oko (1989) state that this defect mainly occurred between 1960 and 1965, thus it may have been caused by nuclear fallout from the atmospheric testing of nuclear bombs in that period, today this defect is very rare. The effect on fertility was high, as the affected sperm were either dead or immobile.

Bowed midpieces can often be found in semen smears and appear as rainbow- or U-shaped. This defect differs from the midpiece reflex defect in that the tail does not fold. In most cases this defect occurs when a cell has

lost all but its motility and makes one last bend before it dies (Barth and Oko, 1989). This defect can often be caused by an incorrect semen smear preparation, thus one must be careful when making a diagnosis and to always take care when preparing a smear.

Defects which occur in the principle piece usually involve the coiling or bending of the principle piece. The coiled principle defect is identified as the tight coiling of the principle piece at various levels distal to the annulus, resulting in these parts being enveloped by a common cytoplasm and membrane. The main reason for this defect having a negative effect on fertility is that motility is impaired. This defect is rare and if found in the ejaculate the number of affected cells are low (Barth and Oko, 1989).

The abaxial tail defect occurs when the tail is attached to the head at and angle and is often accompanied by accessory tails. Extrinsic factors do not appear to affect the incidence of this defect, otherwise the appearance of this defect would be more common. Williams and Savage cited by Barth and Oko (1989) repeated an incident where a bull was considered sterile, has semen with a large number of abaxial tails.

Another defect which involves the tail is the stumtail defect but its incidence in cattle is very low. At first glance of this defect under a light microscope, the semen sample may appear to have a high percentage of detached heads. In actual fact the tail is present but it is in the form of a small stump at the base of the sperm head. This defect is often found in conjunction with Daglike

midpieces and pyriform heads. To be certain it is a stumptail defect and not a detached head defect, is the absence of tails in the semen samples because the tail was never formed. The cause of this defect is unknown but it may be linked with the genetic makeup of the bull and could even be of genetic origin(Barth and Oko, 1989).

Cytoplasmic droplets are often found on the tails of a small percentage of ejaculated semen. It is a round formation of cytoplasm and can occur at one of two positions on the tail: in the proximal region of the midpiece, known as a proximal droplet and in the distal region, where it is known as a distal droplet. In the semen of normal mature bulls cytoplasmic droplets will not be found as they are shed during the epididymal transit and ejaculation. In most cases the presence of a cytoplasmic droplet will indicate that the bull is immature but abnormal spermatogenesis can also be responsible. In the case of an immature bull, the number of cytoplasmic droplets present will decrease as the bull matures. If poor spermatogenesis is responsible then the cytoplasmic droplet defect will be accompanied by other sperm defects.

It appears that if the cytoplasmic droplet occurs in low numbers in the semen then the effect on fertility is minimal. The cytoplasmic droplet may however cause the tail to bend around it causing another type of defect which would definitely affect its fertilising capability. Recovery is possible in male animals depending on which defect is associated with the cytoplasmic droplet (Barth and Oko, 1989).

The final defect that has been recognised in bull semen is that of teratoid spermatozoa. In most cases the tail is completely coiled and lies superimposed on the sperm head. In other cases only the bent midpiece lies superimposed on the head and the principle piece projects away from the abnormal form (Barth and Oko, 1989). In all cases of this defect the mitochondrial sheath is swollen and disrupted. Teratoid sperm usually occur at a frequency of less than 1% but where spermatogenesis has been severely disrupted they can be present at a rate of up to 25%. Sperm cells with this deformity have no fertilising capabilities but as long as their incidence in the semen of breeding bulls is low the overall fertility of the bull should not be affected.

2.8 Conclusions

From the above factors it is clear that keeping a bull fit for breeding entails many factors. All these factors have to be kept in mind when determining the possible causes of reproductive failure, the problem may not always be with the female. Management plays a key role in maintaining a bull fit for breeding, it involves the monitoring of all these factors which may negatively influence reproduction in the male. Reproductive failure of the male or female can have adverse economic implications, for example, in a dairy system, if the female does not calve then she will not produce any milk to sell and in a beef production system, if no calves are born then there is no weaner calves for sale. Never forget the role of the male in reproduction.

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CHAPTER 3

3.1 Material and methods

3.1.1 Experimental design and analyses

Eight Holstein-Friesian bulls aged between 2 ½ and 3 years were required from the experimental farm at the University of Pretoria, South Africa. They were all half brothers from the same sire and were of similar weights. The bulls were randomly divided into two groups of four. The control group was fed a diet of concentrates and *Eragrostis tef* hay as roughage with no vitamin E supplementation. The treatment group was fed the same diet as the control group but the diet was supplemented with Rovimix E provided by Roche Animal Nutrition and Health. The vitamin E supplementation was calculated according to previous research results (Kozicki *et al,* 1981 and McDowell *et al*, 1996) and the suppliers recommendations. The recommendation was that the bulls receive 1000 IU per day but due to the bioavailablity of the supplement being 50% the effective vitamin E supplementation was 500 IU per day per animal which is in accordance with the literature (McDowell *et al*, 1996). Both groups had access to fresh, clean water *ad libitum*.

All the animals were fed the control diet for approximately six months before the treatment commenced to ensure that the bulls were properly adapted to the diet. Thereafter four of the bulls were treated for 90 days with 100IU of vitamin E. Semen was collected every second week via artificial vagina. The artificial vagina was used because it is quick and a method available for

collection of semen (Bearden and Fuquay, 1997). Collections were done every second week to allow for a week of rest between collections to ensure that libido remains high and that the quality of the semen samples are not affected by exhaustion. The treatment commenced in October 1998 and was ended in December 1998 which falls in to the normal breeding season. The average temperature over the three experimental months reached a maximum of 23.3°C and an average minimum of 13°C with an average rainfall of 100.7mm (Pretoria Weather Bureaux). A complete semen evaluation of each bull was conducted after each collection, including quantification of volume, colour, motility, concentration, contaminants and morphology. Eosin and nigrosin was used for staining dead and live sperm. Eosin is referred to as a differential stain in that it cannot pass through living cell membranes but it can pass through non - living cell membranes. A background stain such as nigrosin helps make the unstained sperm heads visible.

The semen characteristics were divided in to three categories, namely the percentage normal spermatozoa (Figure 3.1), the percentage major semen defects, and percentage minor semen defect.

Major defects are those that relate to impaired fertility or to an abnormal condition in the epididymis (Blom as cited by Salisbury *et al* 1978) and include:

Teratoid sperm (TERAT) which is where the midpiece lies over the sperm head in a bent or partially coiled form (Figure 3.2),

- Knobbed acrosomes (AKR) which is characterised by a localised swelling or bead on the apical ridge (Figure 3.3a and 3.3b),

- Pyriform heads (PEER) where the head narrows in the post acrosomal region (Figure 3.4),
- Nuclear vacuoles(KERN) or the "Diadem defect" which appears as a dark necklace along the anterior edge of the posterior nuclear cap (Figure 3.5),
- Nuclear ridge or fold (VOU) (Figure 3.6),
- Macroephalic heads (MAKR) where the sperm head is larger than normal(Figure 3.7),
- Microephalic heads (MIKR) where the sperm head is smaller than normal (Figure 3.8),
- Abnormal loose heads (ABN) where the head is detached and another abnormality is present (Figure 3.9),
- Double forms (DUBB) which consist of any double whether it be double tails (Figure 3.10) or two heads which is a more uncommon representation of the defect,
- Degenerative head (DEG) where the acrosome is loose.
- Corkscrew midpiece (KRTR) where the midpiece is shaped like a corkscrew (Figure 3.11),
- Stumptails (STMP) this is characterised by a very short stump attached to the base of the nucleus (Figure 3.12),
- Midpiece reflex (MIDS) which is shown by the severe bending of the midpiece (Figure 3.13),
- "Dag defect" (DAG), the tails are either coiled, folded or somehow disrupted (Figure 3.14),
- Broken flagellum (GEBR) where the tails are broken or detached in any way (Figure 3.15),

- Proximal cytoplasmic droplet (PPD) which is when the cytoplasmic droplet is retained in the proximal midpiece position (Figure 3.15) and,
- Pseudo cytoplasmic droplet (PSD) where the cytoplasmic droplet is located near the centre of the midpiece (Figure 3.16).

Minor semen defects are defects that should only be of concern when the occurrence of any of the minor semen defects exceeds 10 – 15% (Blom as cited by Salisbury *et al* 1978) because they are not considered detrimental to the fertility of the semen. The minor semen defects which were taken into account were:

- Normal loose heads (NRM) which is when the sperm head is detached from the tail but there is no sign of other defects on the head,
- Degenerative or loose acrosome (DLA), this defect is considered to be very similar to the major defect, degenerative head,
- Abaxial implantation (ABAK), this defect is characterised by abaxial tails being attached to the head at an angle (Figure 3.17),
- Mitochondrial aplasia (MIT) which is characterised as a fracture in the midpiece (Figure 3.18),
- Curled principle or end piece (KRUL) which is the bending or coiling of the sperm tail (Figure 3.19) and,
- Distal droplet defect (DPD) which is evident when the cytoplasmic droplet is in the distal part of the midpiece (Figure 3.20).

Some of the three categories were calculated and expressed as a percentage

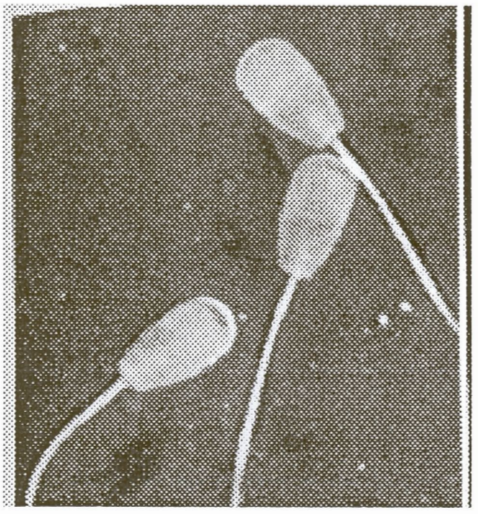

Figure 3.1: Normal bovine spermatozoa*

Figure 3.2: Teratoid forms*

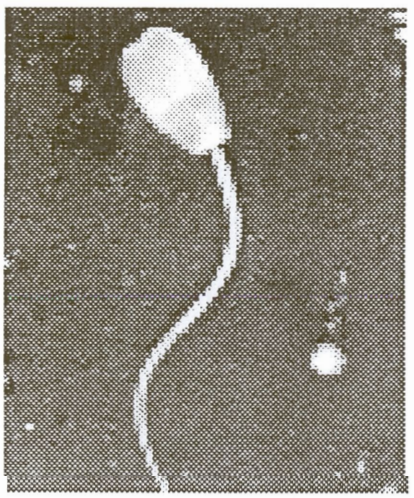

Figure 3.3a: The most common appearance of the knobbed acrosome defect. *

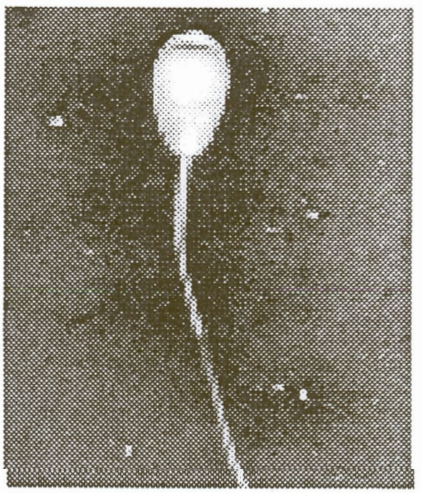

Figure 3.3b: Less common appearance of the knobbed acrosome defect.*

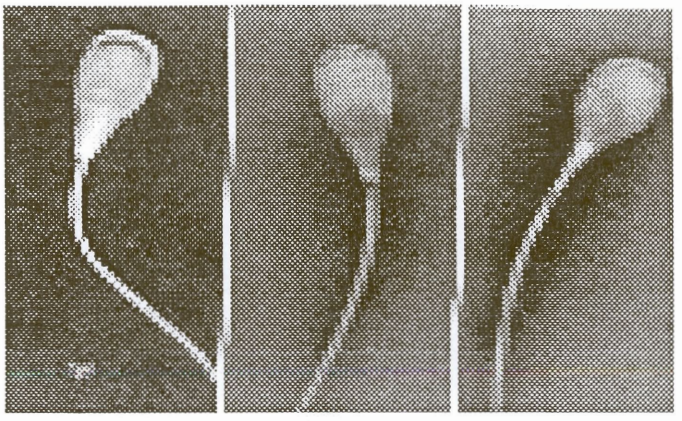

Figure 3.4: Three different forms of pyriform heads which may be observed.*

Figure 3.5: Nuclear Vacuoles in the Equatorial region ("Diadem defect")*

Figure 3.6: Nuclear ridge or fold*

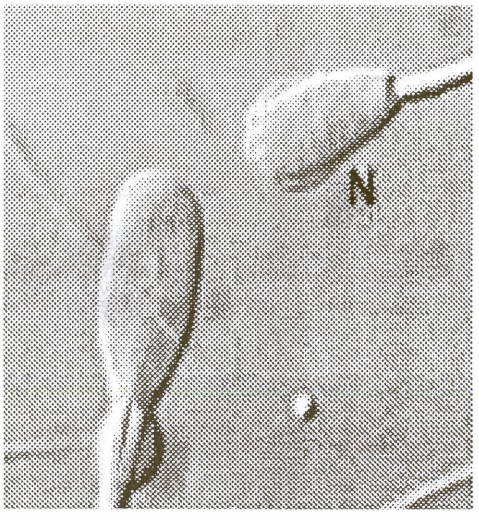

Figure 3.7: Macroephalic head coupled With double tails and the cytoplasmic Droplet defects, N is an example of a Normal normal cell to compare size. *

Figure 3.8: Microephalic head

Figure 3.9: Abnormal loose head.
A detached head with a pyriform base*

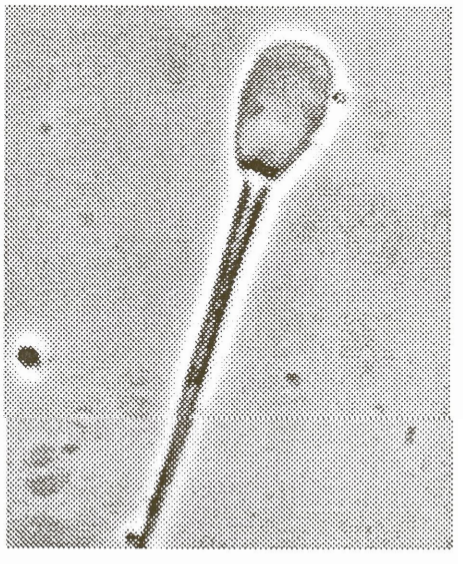

Figure 3.10: Double tails*

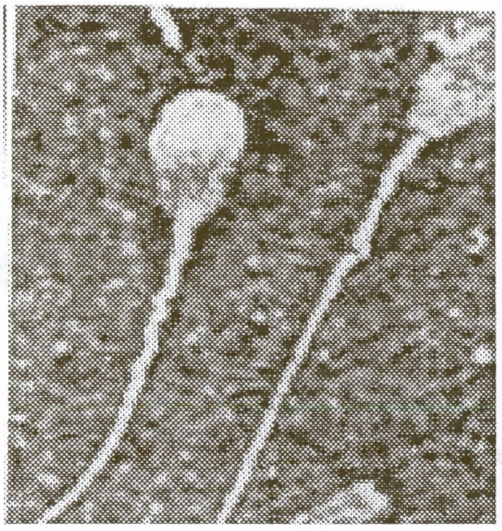

Figure 3.11: Corkscrew defect
Produced from india ink smears.*

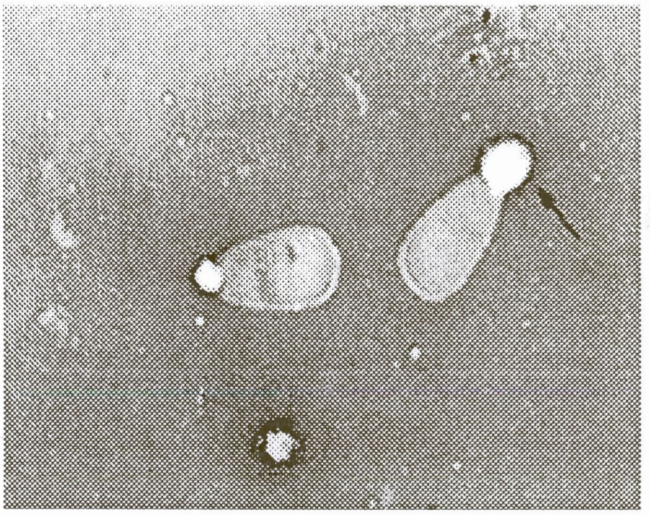

Figure 3.12: Stumptail defect.*

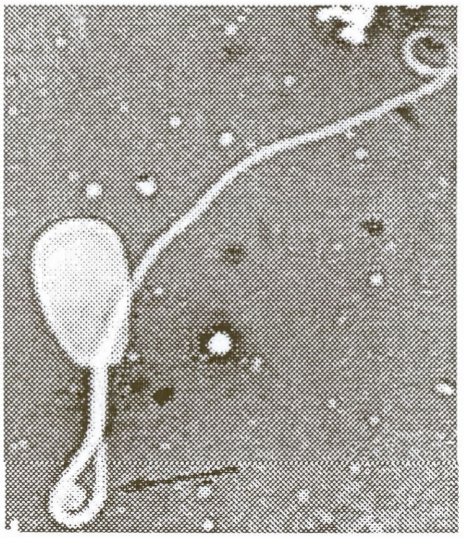

Figure 3.13: A commonly seen form
Of the midpiece reflex abnormality.*

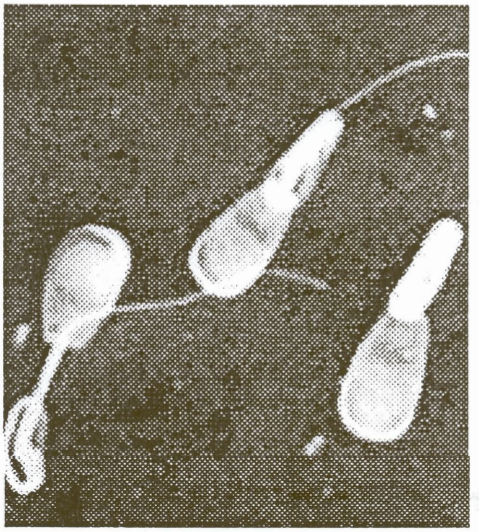

Figure 3.14: The "Dag defect" *

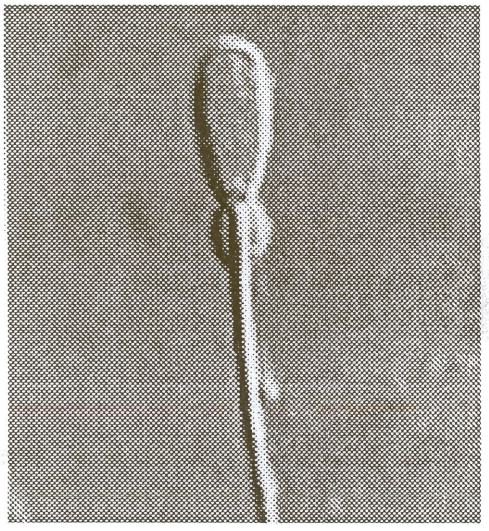

Figure 3.15: Proximal cytoplasmic droplet*

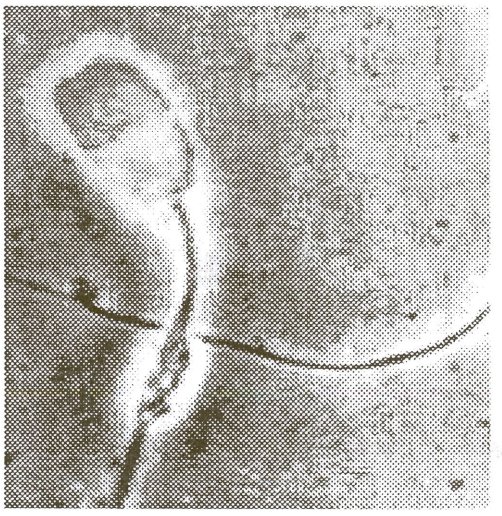

Figure 3.16: Thickening along the midpiece known as the pseudodroplet defect.*

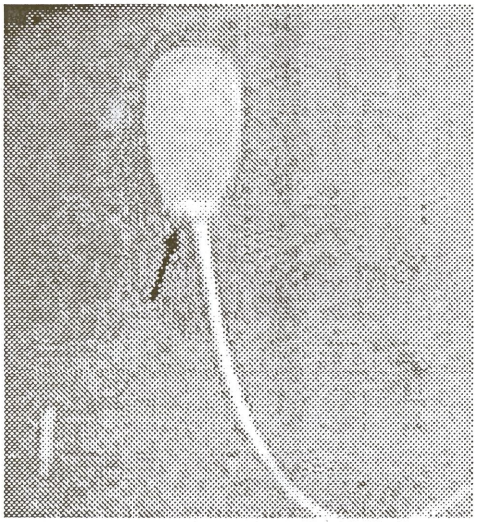

Figure 3.17: Abaxial tail defect

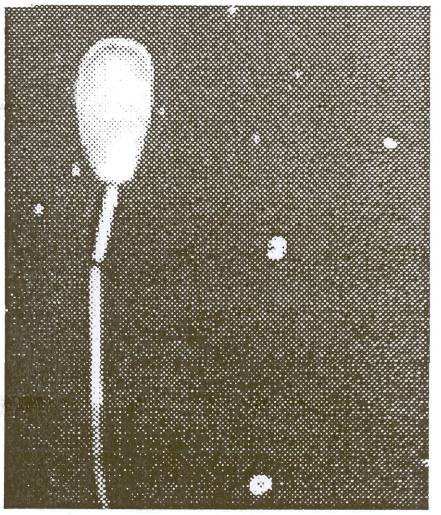

Figure 3.18: Mitochondrial aplasia showing how it can lead to a fracture in the midpiece.*

Figure 3.19: Bent or curled principle piece.*

Figure 3.20: Cytoplasmic droplet in the distal portion of the midpiece.*

(* Figures obtained from Barth and Oko, 1989. Abnormal Morphology of Bovine Spermatozoa)

3.1.2 Statistical Analyses

The effect of vitamin E supplementation and the length of the experimental period were analysed to study the effect of the treatment and treatment period on semen quality and sperm morphological abnormalities over a 90-day period. The data was analysed within and between months but the true effects of the treatment will probably only be evident in the third month after the spermatogenesis cycle has been allowed to complete. The third month is assumed to show the correct effect of the treatment because there may be carry over effects of previous spermatogenesis cycles within the first 60 days of treatment. The results were tabled and were entered into SPSS 11.0 for windows (copyright SPSS Inc. 1987 – 2001) where a GLM multifactor analysis of variance procedure was followed.

3.2 Results and Discussion

3.2.1 General Semen Morphology

Semen quality was evaluated in terms of the percentage normal sperm, while the defects were divided in two categories namely percentage major semen defects and percentage minor semen defects. The effect of Vitamin E supplementation for 30, 60 and 90 days on average percentage normal, major and minor semen defects are summarised in Table 1. The average percentage normal sperm was higher (84,08 ± 11.91%) in bulls fed the diet supplemented with vitamin E compared to those fed the control diet (78,92 ±

11,12%). Both the percentage major and minor defects were lower in bulls supplemented with vitamin E compared to those fed the control diet (Table 1). The effect of dietary vitamin E supplementation on the percentage normal sperm or major and minor semen defects was not statistically significant (Table 1).

Bulls supplemented with vitamin E had 6,13% more normal spermatozoa compared to those in the control group. The supplemented bulls also had 24,12% less major semen defects and 25,9% less minor semen defects when compared to the results obtained from the bulls in the control group. Although statistically not significant, the results do suggest that improvements can be expected subsequent to dietary supplementation with vitamin E. It is known that the bioavailability of the product used was approximately 50%, but it is possible that a higher dose could have yielded more favourable results. A possibility also exists that a more accurate evaluation of the effect of vitamin E supplementation might be possible if more animals were used because in this trial only 4 bulls per group were available. The fact that they are of the same sire reduces the effect of genetic differences, even though the heritability of most male reproductive traits is relatively low, except for scrotal circumference which has a heritabillity of 0,50.

Table 1. **Average percentage of normal, major and minor semen defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation.**

	PNORM			PMAJOR			PMINOR		
Treatment	Average	± SD	Sig.	Average	± SD	Sig.	Average	± SD	Sig.
C	78.92	± 11.12		15.92	± 15.64		5.17	± 4.86	
R	84.08	± 11.91	0.444	12.08	± 11.75	0.528	3.83	± 4.15	0.499

PNORM = Percentage Normal Spermatozoa

PMAJOR = Percentage Major Semen Defects

PMINOR = Percentage Minor Semen Defects

C = Control Group

R = Experimental Group

SD = Standard deviation

Sig. = Significance

The significance of the experimental period and the effect vitamin E treatment has on reproductive traits for the 30, 60 and 90 days were examined and were found to be statistically non-significant (Table 2). The interaction between the supplementation of the diet with vitamin E and the experimental period was also examined and was found to be non-significant.

Table 2. **Significance of the summary statistics (average & ± SD) of the percentage normal, major and minor semen defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation.**

	Significance		
Defects	Treatment period (P=F)	Treatment (P=F)	Interaction* (P=F)
PNORM	0.941	0.444	0.453
PMAJOR	0.835	0.528	0.503
PMINOR	0.764	0.499	0.482

* Interaction between treatment and time
PNORM = Percentage normal spermatozoa, PMAJOR = Percentage major semen defects, PMINOR = Percentage minor semen defects

The effect of treatment within months (e.g. 30, 60 and 90 days of treatment) was investigated (Table 3) to determine if any significant effects of the vitamin E supplementation were evident on fertility. No significant effects of supplementation were apparent in months 1, 2 or 3, however in month 1 the average percentage normal spermatozoa was higher (85,5 ± 13,33%) in the bulls which were supplemented. In month 2 the average percentage normal spermatozoa in the bulls supplemented with vitamin E was higher in the control bulls (86,5 ± 4,36%) resulting in the supplemented bulls having 7,80% less normal semen than the control group. Presumably the only reason for this slightly lower percentage normal spermatozoa in the supplemented bulls is that the spermatogenesis cycle was not completed, and therefore not able to show the effects of the vitamin E supplementation. This is confirmed by the

increase in the amount of normal spermatozoa in month 3 where the supplemented bulls had 14,37% more normal spermatozoa than the bulls which did not receive vitamin E supplementation. The spermatogenesis cycle in the bull is estimated to be between 56 to 63 days (Bearden & Fuquay 1997), thus it is expected that the effects of vitamin E supplementation on semen quality will only be fully evident in the third month (90 days) of the experimental period. It may have been beneficial to lengthen the experimental period to see if the semen quality improves even more with continued vitamin E supplementation.

The average percentage major semen defects showed a similar trend in the three-month experimental period, with statistically non-significant effects being reported. However, the bulls supplemented with vitamin E showed differences in the number of major semen defects when compared to the bulls that received the control diet (Table 3). In month 1 the supplemented bulls had 30% less major semen defects present compared to the bulls in the control group. In month 2 there were 38,60% more major semen defects in the bulls which received the supplemented diet when compared to the control bulls in the same month. The percentage major semen defects decreased in the bulls supplemented with vitamin E by 31,58% from month 2 to 3 months, but when compared with the control bulls, they had 58,97% more major semen defects.

Table 3. **Summary statistics (average & ± SD) of the percentage normal, major and minor semen Defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation.**

		% NORMAL SPERMATOZOA			% MAJOR SEMEN DEFECTS			% MINOR SEMEN DEFECTS		
Treatment	**Date**	**Average**	**±**	**SD**	**Average**	**±**	**SD**	**Average**	**±**	**SD**
C	1	75.75	±	23.26	17.5	±	15.67	6.75	±	7.68
	2	86.5	±	4.36	8.75	±	2.5	4.75	±	2.36
	3	74.5	±	22.99	4.00	±	4.08	21.5	±	23.06
R	1	85.5	±	13.33	12.25	±	11.81	2.25	±	2.22
	2	79.75	±	14.17	14.25	±	15.31	6.00	±	6.68
	3	87.00	±	17.73	9.75	±	10.9	3.25	±	1.89

C = Control group

R = Experimental group

% = Percentage

Similarly, the percentage minor semen defects were lower in the supplemented bulls (2.25 ± 2.22%) than the bulls that received the control diet in month 1. This shows a 66.67% difference in the appearance of minor semen defects between the control bulls and the supplemented animals. In month 2 the minor semen defects increased by 20,83% in the bulls supplemented compared to the control animals. In the third month of the experimental period the percentage minor semen defects decreased by 45,83% in the animals supplemented with vitamin E when compared to the bulls in the control group, the minor semen defects were 84,88% lower in the semen of the bulls supplemented with vitamin E compared to the bulls fed the control diet.

The average values obtained for the percentage major and minor semen defects do however have very high standard deviation values (Table 3). This suggests that the values for both the bulls supplemented and the bulls that received the control diet deviated greatly between the individuals. The small number of animals in the experimental period is most probably the reason for this large deviation in individual values.

From the results presented in table 3, it can be concluded at this stage that there is no significant effect of supplementing the diet of a breeding bull with vitamin E on the semen quality, when compared to bulls that did not receive any vitamin E supplementation which are of similar size, age and genetic composition. Therefore, even though on average there were no statistically significant effects on the percentage major and minor semen defects, a

question was raised as to whether any of the individual semen defects, either major or minor, were significantly affected which could possibly affect the quality of the semen of bulls supplemented with vitamin E.

3.2.2 Major Semen Defects

The summary statistics of the major semen defects for 30, 60 and 90 days subsequent to vitamin E supplementation are summarised in Table 4. Major semen defects have been shown to relate to impaired fertility or to an abnormal condition in the epididymis (Blom as cited by Salisbury *et al* 1978), thus it is important to look at each of these defects individually. The relationship between the experimental period and the major semen defects only showed to have a statistically significant effect on the presence of teratoid (P = 0,05) semen and macroephalic spermatozoa (P = 0,01). The experimental period did not have any significant effect on any of the major semen defects. The effect of the vitamin E supplementation (Table 4) exhibited statistically significant effects on macroephalic spermatozoa (P = 0,02), abnormal loose heads (P = 0,04) and the presence of spermatozoa with degenerative heads (P = 0,02).

The effect on semen quality 30, 60 and 90 days subsequent to dietary vitamin E supplementation in Table 5 shows whether the vitamin E treatment was positively or negatively correlated. In the case of the significant effect of supplementation on the Macroephalic spermatozoa, the bulls which were supplemented with vitamin E, had on average less macroephalic spermatozoa (0,25 ± 0,45%) when compared to the bulls in the control (0,33 ± 0.89%). Therefore the bulls in the control group had 24,24% more macroephalic

spermatozoa than the bulls which received vitamin E supplementation (Table 5). The abnormal loose head defect was positively correlated with vitamin E supplementation, because it was not detected in the semen of the bulls on the experimental diet, but it was present in the semen of the bulls that did not receive any vitamin E supplementation (0,42 ± 0,51%). The occurrence of

Table 4. Summary statistics of the major semen defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation

		Significance		
Defect	**Average ± SD**	**Treatment period**	**Treatment**	**Interaction***
Teratoid sperm	1.29 ± 0.488	0.051 *	0.088	0.419
Knobbed Acrosome	3.62 ± 2.79	0.976	0.315	0.832
Pyriform Heads	3.65 ± 3.33	0.904	0.29	0.704
Nuclear Vacuole	9.6 ± 16.23	0.546	0.704	0.375
Nuclear Ridge	1.14 ± 0.378	0.461	0.506	0.892
Macroeph-alic sperm	0.167 ± 0.482	0.010*	0.025*	0.1
Microepha-lic Sperm	2 ± 1.41	0.51	0.222	0.510
Abnormal heads	1 ± ND	0.424	0.042*	0.390
Double Forms	1.14 ± 0.38	0.641	0.506	0.892
Degenera-tive heads	1.63 ± 1.06	0.422	0.017*	0.232
Corkscrew	ND	ND	ND	ND
Stumptail	ND	0.279	0.321	0.161
Midpiece Reflex	2.06 ± 1.12	0.662	0.487	0.688
Other midpiece defects	1.00 ± 0.00	1.00	0.398	0.487
Dag defect	1.17 ± 0.39	0.676	0.222	0.229
Broken Tails	1.00 ± 0.00	0.25	1.00	0.026**
Proximal Droplet	3.57 ± 6.88	0.34	0.43	532
Pseudo-droplet	0.88 ± 0.28	0.615	0.174	0.615

* = Defects which have a significant effect
** = Significant interaction
SD = Standard deviation

spermatozoa with degenerative heads was 20% more in the bulls receiving the vitamin E supplementation (1,25 ± 0,50%), when compared to the bulls in the control group. The standard deviations of these statistically significant effects of treatment show that there is an increased variation between the individuals. The standard deviation values for the above three significant effects were high. In the case of the macroephalic defect and the abnormal loose heads defect the standard deviation value was actually higher than the average percentage of occurrence, this suggests that the occurrence of these defects may deviate greatly between individuals and that there are no or few differences in terms of treatment. As mentioned above the small number of animals in the experiment may be the cause of this large variation between individuals. On average the bulls that were supplemented with vitamin E, had numerically less major semen defects present in their semen than the bulls in the control group.

A major defect not detected in the semen of the bulls, not receiving vitamin E supplementation, was semen with pseudo droplets, but this defect was detected in the bulls that received vitamin E supplementation. The pseudo droplet is located near the centre of the midpiece and appears as rounded or elongated thickenings that contain dense granules surrounded by mitochondria (Salisbury *et al,* 1978).

Blom, as cited by Salisbury *et al* (1978), demonstrated that the pseudo droplet defect was a major semen defect in 5 Friesian bulls and because 2 of these bulls were half brothers a heritable base for this defect could be suspected.

Since no pseudo droplets were present in any of the control animals, the heritability argument is questionable. Blom (cited by Salisbury *et al* 1978)also found that the incidence of affected sperm increases with the age of bulls along with their gradual decline in fertility. This defect also showed a high standard deviation and a low average of occurrence so it may have only occurred in one bull in the supplemented group of bulls.

Table 5. Descriptive statistics and the significance of major semen defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation

Defect	C			R			
	Average	±	SD	Average	±	SD	Sig.
Teratoid Sperm	0.58	±	0.79	3.08	±	4.78	0.08
Knobbed Acrosome	1.33	±	1.67	0.25	±	0.62	0.32
Pyriform Heads	2.25	±	2.05	ND			0.29
Nuclear Vacuole	4.92	±	15.48	ND			0.7
Nuclear Ridge	0.42	±	0.51	0.83	±	0.29	0.51
Macroephalic Sperm	0.33 [a]	±	0.65	0.25[b]	±	0.45	0.03[c]
Microephalic Sperm	0.33	±	0.89	0.08	±	0.29	0.22
Abnormal Heads	0.42 [a]	±	0.51	ND[b]			0.04[c]
Double Forms	0.42	±	0.67	0.08	±	0.29	0.51
Degenerative Heads	1.00[a]	±	1.20	1.25[b]	±	0.50	0.02[c]
Corkscrew	ND			ND			-
Stumptail	0.42	±	1.16	0.83	±	0.29	0.32
Midpiece Reflex	1.58	±	1.73	1.17	±	0.83	0.49
Other midpiece defects	0.17	±	0.38	0.33	±	0.49	0.40
Dag Defect	0.75	±	0.75	0.42	±	0.51	0.22
Broken Tails	0.17	±	0.39	0.17	±	0.39	1.00
Proximal Droplet	3.00	±	7.59	1.17	±	1.80	0.43
Pseudodroplet	ND			0.17	±	0.39	0.17

c = a,b differed (P< 0.05) number / average in a row with different superscripts
ND = Not detected
SD = Standard deviation
C = Control Group
R = Experimental Group

Semen with corkscrew (KRTR) midpieces was not detected in either the control bulls or those which received the vitamin E supplemented diets. It would seem that in general vitamin E supplementation lowers the occurrence of major semen defects, but not largely when compared to the bulls which had no vitamin E supplementation.

3.2.3 Minor Semen Defects

Minor semen defects seem to be less important, and they should only be of concern when the occurrence of any of the minor semen defects exceeds 10 – 15% (Blom as cited by Salisbury *et al* 1978). However, the less minor or major semen defects then he better the overall fertility of the bull. The effects of vitamin E treatment on the percentage minor semen defects are investigated in tables 6 and 7.

Table 6. Summary statistics of minor semen defects of Friesian bulls 30,60 and 90 days Subsequent to dietary vitamin E supplementation.

		Significance		
Defects	Average ± SD	Treatment Period	Treatment	Interaction
Normal Loose heads	2.5 ± 2.39	0.861	0.832	0.368
Degenerative loose acrosome	2.78 ± 2.10	0.95	0.316	0.857
Abaxial implantation	2.67 ± 2.08	0.187	0.115	0.187
Mitochondrial aplasia	ND	ND	ND	ND
Curled endpiece	1.00 ± 0.00	0.301	0.521	0.075
Distal droplet	2.70 ± 3.68	0.381	0.608	0.448

ND = Not Detected
SD = Standard deviation

Table 7. **Descriptive statistics and the significance of minor semen defects of Friesian bulls 30, 60 And 90 days subsequent to dietary vitamin E supplementation.**

	C	R	
Defects	Average ± SD	Average ± SD	Significance
Normal loose heads	0.92 ± 2.31	0.75 ± 1.14	0.83
Degenerative heads	2.75 ± 4.27	1.50 ± 0.58	0.32
Abxial implantations	0.67 ± 1.50	ND	0.11
Mitochondrial aplasia	ND	ND	-
Curled endpiece	0.17 ± 0.39	0.08 ± 0.29	0.52
Distal sroplet	0.83 ± 1.03	1.42 ± 3.7	0.61

ND = Not detected
C = Control group
R = Experimental group
SD = Standard Deviationb

The effects of the minor semen defects 30, 60 and 90 days subsequent to dietary supplementation on semen quality show no statistically significant effects of the experimental period or the vitamin E supplementation on any of the minor semen defects (Table 7). The results in Table 6 indicate no significant effects,between bulls fed vitamin E and those nor receiving vitamin E supplementation for minor semen defects. Spermatozoa with Mitochondrial Aplasia were not detected in either of the two groups in the experiment and four of the five remaining minor defects had lower averages in the bulls which were supplemented with vitamin E than the bulls which were fed the control diet with no vitamin E supplementation. It can be assumed that vitamin E supplementation can reduce the occurrence of minor semen defects in the semen of bulls. However, once again there is the presence of high standard deviations, most of which are higher than the average values indicating that these values are unstable and deviated greatly between the individuals.

3.2.4 Effects after 60 days of supplementation

The second month of the experimental period was investigated separately to determine if an increase or decrease in the amount of major and minor semen defects and the increase in the percentage normal spermatozoa in the bulls which received the vitamin E supplementation compared to the same group in the first month of the experimental period had occurred. The results of the percentage normal spermatozoa and the percentage of major and minor semen defects show no statistically significant effects of the supplementation of vitamin E (Table 8).

The same trend was evident as that which was seen in the percentage normal spermatozoa and the percentage major and minor defects for the entire experimental period (Table 3). There was a statistically significant effect found for macroephalic heads (P = 0.05) (major semen defects Table 9). The defect occurred at an average of 1% in the bulls that received the control diet and it was not detected in the bulls that received vitamin E supplementation. The bulls in the control group had more undetected major semen defects compared to the supplemented bulls, this explains the trend which was seen in table 3 for the second month of the experiment. The reason for this however, is not known. An interesting effect was evident, there were some major defects which had exactly the same average occurrence in the control group and the group which had vitamin E supplementation, such as the amount of other midpiece abnormalities and the occurrence of the "Dag Defect". These could be the result of using half brothers and the effect of their age. According to Salisbury, van Demark and Lodge (1978) the occurrence of

midpiece abnormalities increases with age. The "Dag Defect" may be hereditary but it is uncertain. However Blom observed the "Dag Defect" in the semen of two Jersey full brothers.

No statistically significant effects on the percentage minor semen defects were evident 60 days subsequent to supplementation of vitamin E. The average Spermatozoa with normal loose heads and distal droplets had a greater occurrence in the bulls that were fed vitamin E (Table 10). Both Mitochondrial aplasia and spermatozoa with curled end pieces were not present in either the control group of bulls or the bulls that were fed the supplemented vitamin E diet.

Table 8. Descriptive statistics and the significance of percentage normal, major and minor semen Defects in Friesian bulls 60 days subsequent to dietary vitamin E supplementation.

	% Normal Spermatozoa			% Major Semen Defects			% Minor Semen Defects		
Treatment	Average	± SD	Sig.	Average	± SD	Sig.	Average	± SD	Sig.
C	86.50	± 4.36		8.75	± 2.50		4.75	± 2.36	
R	79.75	± 14.17	0.398	14.25	± 15.31	0.505	6.00	± 6.68	0.736

C = Control group
R = Experimental Group
SD = Standard deviation
Sig. = Significance
% = Percentage

Table 9. **Descriptive statistics and the significance of major semen defects of Friesian bulls 60 days subsequent to dietary vitamin E supplement.**

Defect	C			R			
	Average	±	SD	Average	±	SD	Sig.
Teratoid sperm	0.75	±	0.957	ND			0.168
Knobbed acrosome	0.75	±	0.957	2.75	±	4.27	0.396
Pyriform Heads	2.00	±	2.16	5.00	±	5.47	0.347
Nuclear Vacuole	0.75	±	1.5	4.00	±	7.35	0.41
Nuclear Ridge	0.50	±	0.577	0.25	±	0.50	0.537
Macroephalic Sperm	1.00 [a]	±	0.82	ND[b]			0.050[c]
Microephalic Sperm	ND			ND			0.00
Abnormal Heads	ND			0.25	±	0.50	0.356
Double Forms	0.50	±	0.577	0.25	±	0.50	0.537
Degenerative heads	0.50	±	0.577	0.25	±	0.50	0.537
Corkscrew	ND			ND			0.00
Stumptail	ND			0.25	±	0.50	0.356
Midpiecce reflex	1.50	±	1.73	1.00	±	0.82	0.620
Other midpiece defects	0.25	±	0.50	0.25	±	0.50	1.00
Dag Defect	0.75	±	0.96	0.75	±	0.50	1.00
Broken Tails	ND			ND			0.00
Proximal Droplet	1.25	±	0.50	1.00	±	0.82	0.620
Pseudodroplet	ND			0.25	±	0.50	0.356

c = a.b differed (P<0.05) number / average in a row with different superscripts
ND = Not Detected
C = Control Group
R = Experimental Group
SD = Standard Deviation
Sig, = Significance

Table 10: **Descriptive statistics and the significance of minor semen defects of Friesian bulls at 60 days subsequent to dietary vitamin E supplementation**

Defect	Control Group			Experimental Group			
	Average	±	SD	Average	±	SD	Sig.
Normal loose heads	0.50	±	1.00	1.00	±	1.41	0.585
Degenerative loose heads	3.00	±	2.16	1.50	±	1.29	0.278
Abaxial Implantations	0.25	±	0.50	ND			0.356
Mitochondrial aplasia	ND			ND			0.00
Curled endpiece	ND			ND			0.00
Distal droplet	1.00	±	1.41	3.50	±	6.35	0.471

SD= Standard deviation
ND = Not Detected

The third month of supplementation was investigated separately to determine if there were any statistically significant effects once the spermatogenesis cycle had been completed in order to determine the effects of the vitamin E supplementation on the semen. The results in Table 11 confirm the effect that was shown in table 3, there were no statistically significant effects on the percentage normal spermatozoa and the percentage major and minor semen defects 90 days subsequent to vitamin E supplementation. The percentage normal spermatozoa had a higher average in the semen of the bulls that had vitamin E supplementation. Subsequently, the average occurrence of major and minor semen defects were less in the supplemented bulls than in the control group. According to these results, the effect of vitamin E on the semen quality, when measured in terms of the presence of semen defects compared to the control group of bulls, can improve the quality of the semen of A.I. bulls, even if the improvement is only marginal.

Table 11. **Descriptive statistics and the significance of the percentage Normal, Major and minor semen defects in Friesian bulls subsequent to 90 days of dietary vitamin E supplementation**

	C			R			
Defect	Average	±	SD	Average	±	SD	Sig.
PNORM	74.50	±	22.99	87.00	±	10.03	0.357
PMAJOR	21.50	±	23.06	9.75	±	1.90	0.392
PMINOR	4.00	±	4.08	3.25	±	1.89	0.750

C = Control group
R = Experimental group
SD = Standard deviation
Sig. = Significance
PNORM = Percentage normal spermatozoa
PMAJOR = Percentage major semen defects
PMINOR = Percentage minor semen defects

The third month of the experiment showed vitamin E supplementation to have a statistically significant effect on spermatozoa with abnormal loose heads (P = 0,02). Abnormal loose heads were detected in the control bulls at an average of 0,75 ± 0,50% whereas the supplemented bulls showed no evidence of this defect (Table 12). The results on the semen of the bulls fed the vitamin E supplement, indicate that five of the major semen defects that were detected in the second month of the experiment were not evident in the third month. These were Spermatozoa with nuclear ridges, abnormal loose heads, double forms, degenerative heads and stumptails. Thus, resulting in a decrease the percentage of major semen defects and increasing the percentage of normal sperm which increases the fertilising ability of the bulls. The amount of major semen defects which were not detected remained constant in the control group from month two to month three but the type of defect which was not detected changed. The majority of the major semen defects had lower averages of occurrence in the bulls which received vitamin E supplementation when compared to the bulls which were fed the control diet.

Table 12 **Descriptive statistics and the significance of the major defects of Friesian bulls subsequent to 90 days of dietary vitamin E supplementation.**

	Treatment						
	C			R			
Defect	Average	±	Std Dev.	Average	±	Std. Dev.	Sig.
Teratoid sperm	ND			ND			-
Knobbed acrosome	2.00	±	2.16	2.25	±	3.86	0.914
Pyriform Heads	2.00	±	2.83	3.75	±	4.27	0.520
Nuclear Vacuole	13.50	±	27.00	2.00	±	3.37	0.43
Nuclear Ridge	0.25	±	0.50	ND			0.356
Macroephalic Sperm	ND			ND			-
Microephalic Sperm	0.25	±	0.50	ND			0.356
Abnormal Heads	0.75 [a]	±	0.50	ND[b]			0.024[c]
Double Forms	0.25	±	0.50	ND			0.356
Degenerative heads	0.75	±	0.96	ND			0.168
Corkscrew	ND			ND			-
Stumptails	1.25	±	1.89	ND			0.235
Midpiece reflex	1.00	±	2.00	1.25	±	0.50	0.816
Other midpiece defects	ND			0.50	±	0.58	0.134
Dag Defect	0.50	±	0.58	0.50	±	0.58	1.00
Broken Flagellum	0.50	±	0.58	ND			0.134
Proximal droplet	0.50	±	1.00	0.75	±	1.50	0.791
Pseudodroplet	ND			0.25	±	0.50	0.356

ND = Not Detected
C = Control group
R = Experimental group
Sig. = Significance

The percentage minor semen defects showed no statistically significant effects 90 days subsequent to the dietary vitamin E supplementation (Table 13). There was a general decline in the average occurrence of minor semen defects in both the control group and the bulls that were supplemented from month 2 to month 3. Abaxial implantations and mitochondrial aplasia did not occur in the control group or in the experimental group of bulls. The

supplemented bulls had slightly lower averages of minor semen defects when compared to the bulls in the control but were not statistically significant.

Table 13. **Descriptive statistics and the significance of the minor semen defects in Friesian bulls 90 days subsequent to dietary vitamin E supplementation.**

	Treatment						
	C			R			
Defect	Average	±	Std Dev.	Average	±	Std Dev.	Sig.
Normal loose heads	0.25	±	0.50	1.00	±	1.41	0.356
Degenerative loose acrosomes	2.75	±	4.27	1.50	±	0.58	0.583
Abaxial implantation	ND			ND			-
Mitochondrial Implantation	ND			ND			-
Curled Endpiece	0.50	±	0.58	ND			0.134
Distal Droplet	0.50	±	1.00	0.75	±	0.96	0.73

ND = Not Detected
C = Control Group
R = Experimental group
Sig. = Significance

3.2.5 Interactions

The only significant interaction between treatment and the treatment period which was evident in this study was observed for broken tails (p = 0.026) in Table 4. Broken tails were high in the bulls which were in the group that was supplemented with vitamin E in month 1 (30 days) of treatment and the graph (figure 1) shows that the defect was not detected in month 2 (60 says) or month 3 (90 days). In the control group the bulls showed no evidence of broken tails in the beginning of the trial, but after 60 days the occurrence of broken tails began to increase. The bulls in the control had an average of 0,50% broken tails after 90 days compared to 0,0% in the group of bulls supplemented with dietary vitamin E

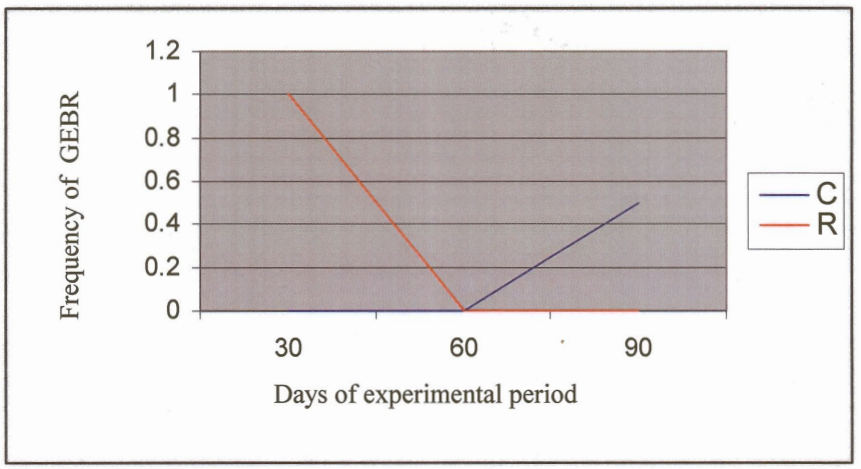

Figure 1: The interaction between treatment and treatment period on broken Flagellum.

3.3 Conclusions

Feeding vitamin E as a dietary supplement does not have a significant effect on the general semen morphology of Holstein-Friesian bulls. Dietary vitamin E supplementation slightly decreased the incidence of head abnormalities, which may improve the fertilising capability of the spermatozoa but the economic implications of the added cost of the supplement is uncertain. Although there was no statistically significant effect of the vitamin E supplementation the results are interesting. It appears that the supplementation can reduce the percentage of major and minor semen defects to a more acceptable level. Although the effects of the vitamin E supplementation on semen morphology were not significant, there may be an effect on the freezability of the semen and the length of time that the semen may be stored. There is very little information on the freezibility and storage

of dietary vitamin E supplementation of semen, with warrants further investigation.

Some of the morphological abnormalities which were present may have been caused by handling procedures and preparation of the semen smears, for example the appearance of bent midpieces may not be due to poor spermiogenesis on the part of the bull but due to a hypotonic solution. The nigrosin-eosin solution used for staining in this experiment was reported by Bishop *et al* (1954) to be hypotonic to bull semen. Swanson and Bearden as cited by Maule (1962) found that if the nigrosin-eosin solution included an isotonic citrate buffer, constant results would be obtained (Maule, 1962). Loose heads may also occur because of breakage in preparing the microscope slides.

The morphology of an individual bull's semen may change from time to time or from season to season, thus it is important to continue monitoring each bull's semen and to examine every collection from bulls that tend to react occasionally to unknown factors with a susequent increase in abnormal cells sperm cells (Salisbury *et al*, 1978).

3.4 Critical Overview

The limited number of animals that were available for this experiment may have had an influence on the results. Understandably the cost associated with obtaining bulls is high and to obtain bulls of similar ages and which are related is extremely difficult, and is therefore a limiting factor. The problem of

having a limited number of animals is that the results of one animal has a significant affect on the average, for instance, in this trial the effect of the performance of each bull constituted 25% of the average per group. A trial with more animals may have reduced the high standard deviations which were seen for most of the effects and more reliable results may have been obtained.

The manner in which semen was collected may have also been responsible for a number of limitations of the experiment due to the procedure being very technical and complex. Although the total number of bulls in the experiment from a statistical point was low, it is difficult to collect semen from so many bulls and it resulted in a stressful environment. Individual bulls react differently to stress and it may have affected the results which were obtained.

The experiment was only conducted in one season (summer), it would have been interesting to test the effects of vitamin E supplementation in other seasons for instance in winter with lower ambient temperatures. The season would also affect the protein and energy levels in the feed, vitamin E may have a greater influence in the absence of these two elements.

This experiment did discover that there is definitely a possibility that the effect of vitamin E in reproduction may be larger than previously thought and I think that it has opened the doors for further investigation, such as, the effect that vitamin E would have on the freezability of the semen. This may have great implications for the artificial insemination industry.

3.5 References

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