# A STUDY OF SOME FACTORS THAT MAY AFFECT SEMEN QUANTITY AND QUALITY AS WELL AS LIBIDO IN FOUR BREEDS OF DAIRY BULLS.

BY

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II

## DECLARATIONS

This is my original work and has not been presented for a degree in any other University.

Signed.....Date.....28-10-1994.

Hilary Binta (B.V.M.)

This Thesis has been submitted for examination with our approval as University supervisors.

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# DEDICATION

This work is dedicated to my Mother Mrs. I. Akiiki Binta and my Father, the late Dr. P.B. Binta Amooti.

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# ABBREVIATIONS

Ab = Antibody

ABP = Androgen binding protein

ACTH = Adrenocorticotrophic hormone

Ag = Antigen

A.I = Artificial insemination

AV = Artificial vagina

BSA = Bovine serum albumen

BSE = Breeding soundness examination

C.I = Confidence interval

CMO = Carboxy methyl oxygen

Cp = Mallows' Cp statistic

c.p.m = Counts per minute

D = Spermatozoa concentration

DPX = Distrene Dibutylphthalate Xylol

EE = Electroejaculation

EL = Epididymal length

EV = Ejaculate volume

FAO = Food and Agricultural Organisation

FSH = Follicle stimulating hormone

GnRH = Gonadotrophin releasing hormone

$^{3}H$ = Tritium

LDRAT = live/dead ratio

LH = Luteinizing hormone

M = Spermatozoa motility

MW = Molecular weight

PPO = 2,5-diphenyl oxazole

PRIM = Primary abnormalities

QC = Quality control

r = Pearson correlation coefficient

RIA = Radioimmunoassay

R.P.M. = Revolutions per minute

RT = Reaction time

RTC = Recovery'total counts

SC = Scrotal circumference

SVD = Seminal vesicle diameter

SVL = Seminal vesicle length

SEC = Secondary abnormalities

S.E = Standard error

TL = Testicular length

$T_4$ = Testosterone

WHO = World Health Organisation

## ABSTRACT

Artificial insemination, an important applied reproductive technology which is used worldwide in animal breeding has been used in Kenya since 1935. The Central Artificial Insemination Station (C.A.I.S), Kabete was established in 1946. There are about 100 dairy bulls at the station which have been selected based on milk yields of their dams and grand dams.

Various factors, which include environment and the bull's anatomy may affect semen quantity and quality. Some work has been done to establish the factors that affect semen quantity and quality especially the effects of environment but scanty information is available about the associations betweeen some reproductive organs of tropical dairy bulls and semen quantity and quality. This study was thus initiated with the aim of studying the interrelationships between age, body weight, scrotal circumference (SC), the size of seminal vesicles, length of *cauda epididymis* and testicular length on semen quality and quantity. In addition, this study attempted to establish the relationship between plasma testosterone concentrations and reaction time in the four dairy breeds, namely, Ayrshire, Friesian, Jersey and Guernsey kept at C.A.I.S.

The study utilised a convenient sample of 98 bulls at the C.A.I.S. Nineteen of these bulls were less than 16 years of age and were not used for semen collection during the study period. Scrotal circumference, testicular length and length of the *cauda epididymis* were measured using a scrotal tape while the sizes of the seminal vesicles were estimated by rectal palpation. The semen samples were collected from the bulls twice a week using a standard artificial vagina. They were evaluated for volume, spermatozoa motility, concentration, morphology and viability.

Blood samples for testosterone assays were collected by middle coccygeal venipuncture and the levels of testosterone in plasma were determined by radioimmunoassay.

Statistical analyses of the gathered data involved computation of descriptive statistics, graphical, correlation and regression analysis as well as analysis of variance.

There were no significant ($p > 0.05$) differences in ages of the bulls of the four breeds but there were significant ($p < 0.05$) differences in the body weights with Friesian bulls being the heaviest and Jerseys the lightest. Scrotal circumference, seminal vesicle diameter, *cauda epididymal* length, testosterone concentration and reaction time did not differ among breeds ($p > 0.05$).

However, significant differences were observed between breed-specific means of seminal vesicle lengths ($p < 0.01$) and also those of testicular lengths ($p < 0.05$) of some breeds. Differences observed in the means of the volumes of the ejaculates and the ones of the overall means of the percent spermatozoa motility between breeds were not significant ($p > 0.05$). Guernseys had the highest ejaculate volumes and Jerseys the lowest. However, these were not signnificantly different ($p > 0.05$). This seemed to be related to the sizes of the seminal vesicles which were longer in Guernseys and hence capable of holding more secretions than those of Jerseys. Guernseys had also the greatest testicular lengths and the Jerseys the shortest. In contrast, the mean spermatozoa concentration for Jerseys was significantly ($p < 0.05$) higher than those of the other breeds.

In this study, the spermatozoa abnormalities found in the semen samples from all bulls irrespective of the breed were within the recommended ranges for normal fertile bulls. The most common head abnormality were detached heads (0.77 % in Ayrshires, 0.68 % in Friesians, 0.73 % in Guernseys and 0.96 % in Jerseys). For the midpiece, duplications were the most prevalent (0.21 % in Ayrshires, 0.13 % in Friesians, 0.05 % in Guernseys and 0.14 % in Jerseys). The predominant tail abnormalities were coiled tails in Ayrshires (1.47 %) and free tails, 1.36 % in Friesians, 1.15 % in Guernseys and 1.54 % in Jerseys.

Non significant and negative correlations were observed between scrotal circumference and spermatozoa motility and primary as well as secondary abnormalities. Ejaculate volume was positively correlated to scrotal circumference ($r = 0.32$), seminal vesicle length ($r = 0.36$) and diameter ($r = 0.41$) as well as *cauda epididymal* length ($r = 0.29$). The magnitudes and significance of coefficients between age or body eight and most of the

# CHAPTER ONE

## 1.0 INTRODUCTION

Artificial Insemination (A.I) in cattle was first performed in Kenya in 1935 (Anderson, 1948). Initially this was confined to individual farms where the semen was collected from bulls and used on cows in the same farms. The main purpose of this A.I. programme was to combat infectious reproductive diseases particularly epididymitis-vaginitis. A survey carried out during that time revealed that over 35% of bulls examined were sterile due to this disease (FAO, 1977). As a result of this, the emphasis of A.I. grew steadily to overcome the problem.

In 1946, the Central Artificial Insemination Station (C.A.I.S.) was established at Kabete, Kenya, with the aim of distributing disease-free semen. Initially, this distribution was carried out by European farmers around Limuru area in Kiambu District and later extended to other parts of the country (FAO, 1977). Since then, the A.I. services in Kenya have had the major aims of controlling diseases causing infertility, improving the productivity of the national herd and upgrading the low producing Zebu cattle. These objectives are designed not only to increase milk and meat production but also to make it economical and sustainable. Thus, the activities of this service should increase the income and improve the standards of living of the dairy farmers, particularly the small scale ones who are the majority in the high potential farming areas. It is hoped that this will in turn improve the nutritional standards of these farmers as well as those of the urban dwellers through increased milk supply. In addition, A.I. services have led to the reduction in foreign exchange expenditure on imports of cattle and cattle products and increased foreign exchange income through export of live animals, dairy products and semen. Currently the A.I station has over 100 dairy bulls comprising mainly Friesian, Ayrshire, Guernsey and Jersey breeds.

Various factors can affect the quality and quantity of semen harvested from a bull. These may be environmental, genetic, physiological and anatomical (Roberts, 1986). Almquist and Cunningham (1967) were unable to show any effects

of season on semen volume, but reported that it increased steadily with age up to two years. Igboeli and Rakha (1971) showed that the mean semen volume of Zebu bulls declined during the hot season. Although the overall amount of semen harvested from a given bull is influenced by the volume and spermatozoa concentration of each ejaculate (Amann *et al.*, 1974), Everett and Bean (1982) postulated that both genetic and environmental factors may affect the quality and quantity of such semen. A study done at C.A.I.S showed that a relationship exists between season and semen quantity as well as its quality (Njogu, 1989). However, the effects of reproductive anatomical organs on the quantity and quality of semen were not taken into account during that study.

Breeding soundness examination (BSE) is an important criterion used in selecting breeding stock. Hence to exploit maximum genetic potential in any livestock improvement programme, thorough BSE of the male prior to use is vital. The examination usually involves evaluation of the breeding history of the bull, a thorough physical and genital examination and analysis of a fresh undiluted semen sample (Elmore *et al.*, 1975). In evaluating a semen sample, particular emphasis is placed on volume, concentration, motility, morphology and percentage of live spermatozoa. While these characteristics bear an important relationship to reproductive potential, the latter may also be influenced by a bull's libido (Roberts, 1986).

Reaction time (time taken by the bull from exposure to the cow or teaser to mounting, ejaculation and dismounting) in association with testosterone levels may be an important component of BSE. Breed and seasonal effects on reaction time in bulls have been reported (Bhosrekar and Nagpaul, 1972; Nath *et al.*, 1980; Sharma *et al.*, 1982). However, these researchers did not relate their findings to the testosterone levels in bulls. Correlation studies of hormonal levels and libido in bulls have shown promising results in testing sires to be used for natural mating. Post *et al.* (1987) found a significant and positive relationship between testosterone ($T_4$) and luteinizing hormone (LH) concentrations with fertility following gonadotropin releasing hormone (GnRH) injections,

Post mortem studies have shown that a positive correlation exists between testicular weight, gonadal and extragonadal sperm reserves and sperm production (Amann, 1970). Due to this, testicular weight has been used as an estimate of sperm producing parenchyma (Amann, 1970). However, it is difficult to measure testicular weight in live bulls and hence scrotal circumference (SC) has been used as a proxy measure of testicular size (Coulter and Foote, 1976). In recent years, researchers have evaluated SC as a potential indicator of fertility in bulls. Results of several studies suggest that bulls with small SC have significantly lower fertility measures than those with large SC (Mickelsen and Paisley, 1979; Gipson *et al.*, 1985). SC is easier to obtain than sperm production or behavioral measurements and therefore it is a useful criterion for selecting bulls for early maturity (Morris *et al.*, 1989). The potential for selection programmes based on SC is further supported by the high heritability of this characteristic in bulls (Coulter *et al.*, 1976; Morris *et al.*, 1989). Breed and SC effects on seminal traits have been studied extensively in beef bulls (Elmore *et al.*, 1976; Mickelsen *et al.*, 1979; Smith *et al.*, 1981; Gipson *et al.*, 1985), but less information is available on dairy bulls (Hahn *et al.*, 1969). The fact that SC is highly correlated to body weight as well as puberty suggests that in the absence of an animal's actual weight, SC may be useful as an indicator of puberty.

The *cauda epididymis* contributes between 50 to 54 % of the total epididymal sperm reserves (Tegegne *et al.*, 1992) hence external estimates of the lengths of the epididymis could be an important anatomical factor influencing the quantity of semen collected from bulls.

Seminal vesicles contribute the greatest volume in the ejaculates of bulls (Roberts, 1986) and seminal fructose has been reported to reflect androgen stimulation of the vesicular glands (Fields *et al.*, 1979). The secretions of the vesicular glands play an important role in keeping spermatozoa viable (Mann, 1974). In a study involving yearling beef bulls, Berry *et al.* (1983) found no significant correlations between the size of seminal vesicles and libido scores. However, SC and size of the seminal vesicles were closely related. Information

is needed to show the association between seminal vesicle size, epididymis and SC with seminal traits representing components of semen quality. The information derived from such a study could be useful in selecting bulls with improved semen traits The objective of this study, therefore, was to investigate the interrelationships between age, gross anatomy, physiological and behavioral factors, semen quantity and quality in dairy bulls in Kenya.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Functional anatomy of the male genital system.

The male genital system consists of the testicles, a system of ducts, blood vessels, nerve supply, accessory glands and the penis (Figure 1), (Johnson *et al.*, 1970). The testes produce spermatozoa and testosterone, while the scrotum provides favourable temperatures suitable for the production of spermatozoa (Arthur *et al.*, 1986). The remaining structures namely: the epididymis, *ductus deferens*, accessory sex glands (ampullae, seminal vesicles, prostate and bulbourethral glands), the urethra and penis assist the spermatozoa to reach their ultimate goal, the female reproductive tract in a viable condition conducive for fertilization of the ova (Arthur *et al.*, 1986).

#### 2.1.1 Testis

The testicle is a gametogenic as well as an endocrine organ producing spermatozoa and androgens (Bone, 1982). Each testis consists of a mass of seminiferous tubules surrounded by a fibrous capsule called the *tunica albuginea* (Johnson *et al.*, 1970). Fibrous septae pass inward from the *tunica albuginea* to form a framework or stroma for support of the seminiferous tubules. These septae unite to form a fibrous cord the mediastinum testis. The *rete testis* consists of anastomosing canals within the mediastinum testis. The Leydig cells which secrete testosterone are located in the seminiferous tubules.

#### 2.1.2 Epididymis.

The epididymis lies upon the medial surface of the testicle and is composed of three parts namely: the head (*caput epididymis*), the body (*corpus epididymis*)

and the tail (*cauda epididymis*). The head is a long convoluted tube that connects the *vasa efferentia* of the testis with the *ductus deferens*. The tail of the epididymis continues as the *ductus deferens* which enters the spermatic cord. The epididymis serves as a place for spermatozoa maturation prior to the time they are expelled at ejaculation (Frandson, 1986). Spermatozoa are immature when they leave the testicle and must undergo a period of maturation within the epididymis before they are capable of fertilising ova.

### 2.1.3 Accessory sex glands.

The male accessory sex glands include the ampullae, seminal vesicles, prostate gland and bulbourethral glands. These glands produce the greater part of the ejaculate or seminal plasma which serves as a favourable transport medium for nutrition and a buffer against excess acidity in the female genital tract (Frandson, 1986).

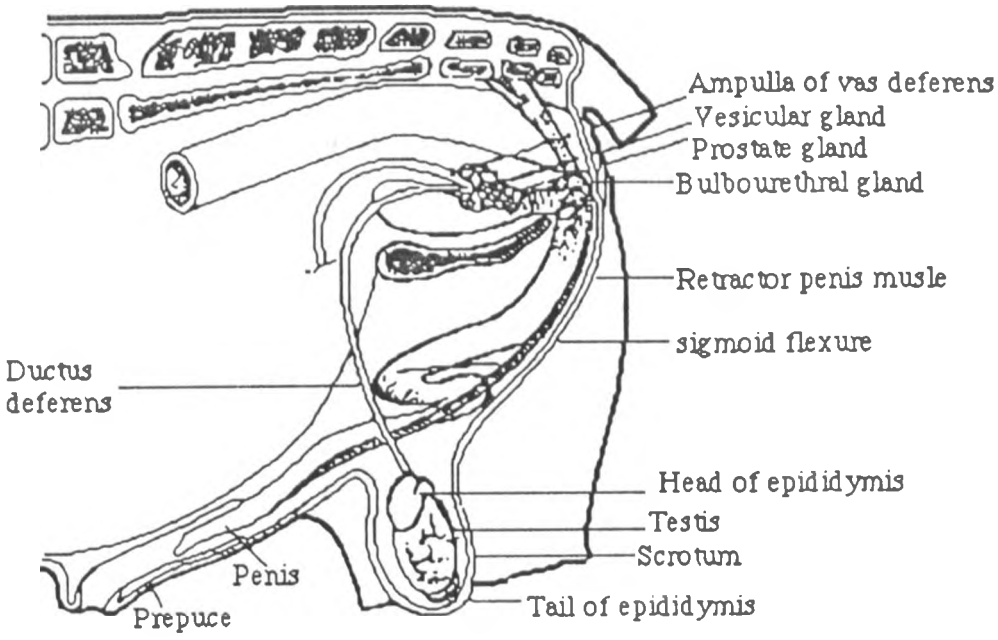



**Figure 1:** The reproductive tract of the bull (lateral section).
Source: Ashdown, (1987)

## 2.2 Testicular size and sperm production.

Testicular weight is an important trait that has been used to provide estimates of the amount of sperm producing parenchyma in the testis (Almquist and Amann, 1961; McMillan and Hafs, 1968a). However, testicular weight cannot be directly and easily measured in live breeding bulls and so to alleviate this difficulty, indirect measurements have been developed for use in these bulls. These include diameter and length of the testis, width of the paired-testes and scrotal circumference (Boyd and Van Demark, 1957; Willet and Ohms, 1957; Hahn *et al.*, 1969; Coulter and Foote, 1976; Elmore *et al.*, 1976; Lunstra *et al.*, 1978). The linear measurements are made with callipers while SC is measured with a self-releasing, flexible, plasticised cloth or a loop of metal tape (Coulter and Foote, 1979).

Scrotal circumference is a good indicator of both testicular weight and sperm output in growing bulls (Coulter and Foote, 1979). Published correlations between SC and the weight of paired-testes at or near slaughter in dairy bulls range between 0.89 and 0.95 (Van Demark and Mauger, 1964; Hahn *et al.*, 1969) and 0.95 in both Hereford and Angus bulls (Coulter and Foote, 1976). As bulls aged, these positive correlation coefficients decreased, reached zero and then became negative and significant (Willet and Ohms, 1957; Hahn *et al.*, 1969). This negative relationship in older bulls has been attributed to an increase in fibrotic tissue (Coulter and Foote, 1979).

### 2.2.1 Factors affecting testicular size and consistency.

Many factors including breed, age, body weight and the environment, for example level of nutrition may affect testicular size and consistency. Each of these factors are reviewed in the subsequent subsections.

2.2.1.1 Breed.

Scanty literature exists on breed effects on testicular characteristics. Breed differences in relation to SC and testicular consistency have been reported to occur in bulls belonging to Holstein, Angus and Brown Swiss breeds (Holy and Barba, 1972; Coulter *et al.*, 1975). It has been reported that testicular sizes of Angus, Simmental and Charolais bulls of the same age are similar but Angus and Simmental bulls may have greater SC at two years of age than Charolais bulls (Holy and Barba, 1972; Kupferschmied *et al.*, 1974; Coulter *et al.*, 1975; Coulter and Foote, 1979). Lunstra *et al.* (1978) reported that Brown Swiss bulls had larger testes whereas those of Herefords were smaller than those of Angus, Red poll and Angus-Hereford crosses. Similar comparisons are, however, not available for dairy bulls.

2.2.1.2 Age.

Available literature indicates that there are changes in testicular sizes associated with different ages in both dairy (Boyd and Van Demark, 1957; Willet and Ohms, 1957; Almquist and Amann, 1961; Amann and Almquist, 1962; Van Demark and Mauger, 1964; Hahn *et al.*, 1969; Coulter *et al.*, 1975; Coulter and Foote, 1976; Foote *et al.*, 1976) and beef bulls (Willet and Ohms, 1957; Coulter *et al.*, 1975; Almquist *et al.*, 1976; Elmore *et al.*, 1976).

Whereas testicular size increases gradually with age, it may decline in the older bulls (Iljinskaja, 1966). In addition, testicular consistency is high in young bulls but declines and remains relatively constant in mature bulls (Coulter *et al.*, 1975). There may be a slight increase in testicular consistency in the older bulls due to increase in fibrotic tissue (Coulter and Foote, 1979). Great variations in testicular weight, SC and consistency exist among bulls of the same age and breed. Ranges of 600 g in paired testes weight (Coulter and Foote, 1976), 15 cm in SC and 9 mm in average tonometer readings have been reported (Coulter *et al.*, 1975). These variations provide considerable opportunities for

selecting bulls of high reproductive and better productive traits based on the combinations of these testicular characteristics.

### 2.2.1.3 Body weight.

Coulter and Foote (1977) examined the association between body weight, SC and testicular consistency in Holstein bulls and reported a high and significant correlation coefficient of 0.81 between body weight and SC. In growing bulls, these two characteristics were positively correlated with age. When they held age constant, a partial correlation coefficient of 0.58 between body weight and SC was obtained. The simple and partial correlation coefficients between body weight and testicular consistency were -0.45 and -0.16. Kupferschmied *et al.* (1974) reported an overall simple correlation coefficient of 0.67 between body weight and SC in 167 Simmental bulls aged between 8.5 to 19 months.

### 2.2.1.4 Season.

Seasonal influences on SC and testicular consistency have been reported in temperate regions (Coulter and Foote, 1976). Highly significant seasonal differences were found between the best fitting regression equations for SC and testicular consistency traits measured in early Spring and Summer for Holstein bulls aged between 6 to 18 months (Coulter and Foote, 1976). In this study decreases in SC and increases in testicular consistency were observed. Fields *et al.* (1979) reported that testicular size decreased in non-adapted Hereford bulls in Summer months which was attributed to environmental stress. However, Wildeus and Hammond (1993) found no seasonal effects on scrotal circumference in Holstein bulls. This contrasts with observations in seasonal breeding rams (Islam and Land, 1977).

### 2.2.1.5 Nutrition.

Although testicular size and weight are generally influenced by the plane of nutrition fed to dairy bulls (Davies *et al.*, 1957; Van Demark and Mauger, 1964; Van Demark *et al.*, 1964), the exact relationships are unclear. For example, Coulter (1978) recorded no differences in testicular weight between bulls on different levels of energy consumption. However, Ndama *et al.* (1983) demonstrated that by providing protein supplementation to cross-bred Brahman bulls, they could positively influence testicular size and sperm production. In contrast, diets with 8, 10 or 14 % protein had no significant effect on the SC of young beef bulls (Sitarz *et al.*, 1977).

In a study involving weaned beef calves, Mwansa and Makarechian (1991) reported no significant differences on libido scores after feeding low or high energy feeds. However, bulls on the high energy feeding regimes had the highest scrotal circumferences but poorest semen characteristics. Tegegne *et al.* (1992) reported heavier weights and larger SC's at puberty in supplemented bulls than the controls. In contrast, Rekwot *et al.* (1987) and Barber and Almquist (1978) obtained lighter body weights and smaller SC's at puberty in supplemented than in control bulls.

## 2.3 Spermatogenesis.

Spermatogenesis is a reproductive process which occurs in the seminiferous tubules of the testis resulting in the production of spermatozoa (Duane and Hafez, 1987). Generally, it starts with embryonic colonisation of the germinal crests by primordial germ cells which is followed by differentiation and multiplication of stem cell spermatogonia (Bedford and Hoskins, 1990). Several mitotic divisions of the spermatogonia occur. After the last mitosis, the primary spermatocytes have an extended prophase during which DNA is formed to a degree corresponding to four haploid cells (Ortavant *et al.*, 1977). The cells then undergo two successive mitotic divisions which lead to the formation of

secondary spermatocytes and later to spermatids (Figure 2).

Spermatozoa production begins at the age of 4-7 months in bulls (Ortavant *et al.*, 1977) and reaches maximum production level at three years of age (McMillan and Hafs, 1968b; Johnson *et al.*, 1970). The initiation of spermatogenesis is more dependent on the size (body weight) than the age of the bull (Coulter and Foote, 1979). Underfed bulls produce low quality semen characterised by fewer spermatozoa per ejaculate (Meacham *et al.*, 1963; Van Demark *et al.*, 1964) and severe under nutrition causes depression of spermatogenesis resulting in decreased ejaculate volume, sperm motility and an increased percentage of abnormal cells (Foote, 1978). Spermatogenesis is divided into two phases namely: spermatocytogenesis and spermiogenesis (Ortavant *et al.*, 1977).

### 2.3.1 Spermatocytogenesis.

During spermatocytogenesis, primordial germ cells divide several times mitotically to form gonocytes. The gonocytes undergo differentiation just before puberty to form the type $A_o$ spermatogonia from which the other stem cells originate (Figure 2). Type $A_1$ spermatogonia divide successively to form type $A_2$, $A_3$, $A_4$ and intermediate spermatogonia. The latter divide to give rise to type B spermatogonia. It is from the type B spermatogonia that primary spermatocytes are formed. The primary spermatocytes undergo meiotic divisions to secondary spermatocytes. Primary spermatocytes have been found in the seminiferous tubules of bull calves as young as 71 days of age (Santamarina and Reece, 1957). However, no further development has been noted until puberty which occurs at 6-9 months of age (Hooker, 1944). The secondary spermatocytes divide to give rise to haploid cells known as spermatids. The entire divisional process from the spermatogonia to the spermatids takes approximately 45 days in the bull (Garner and Hafez, 1987).

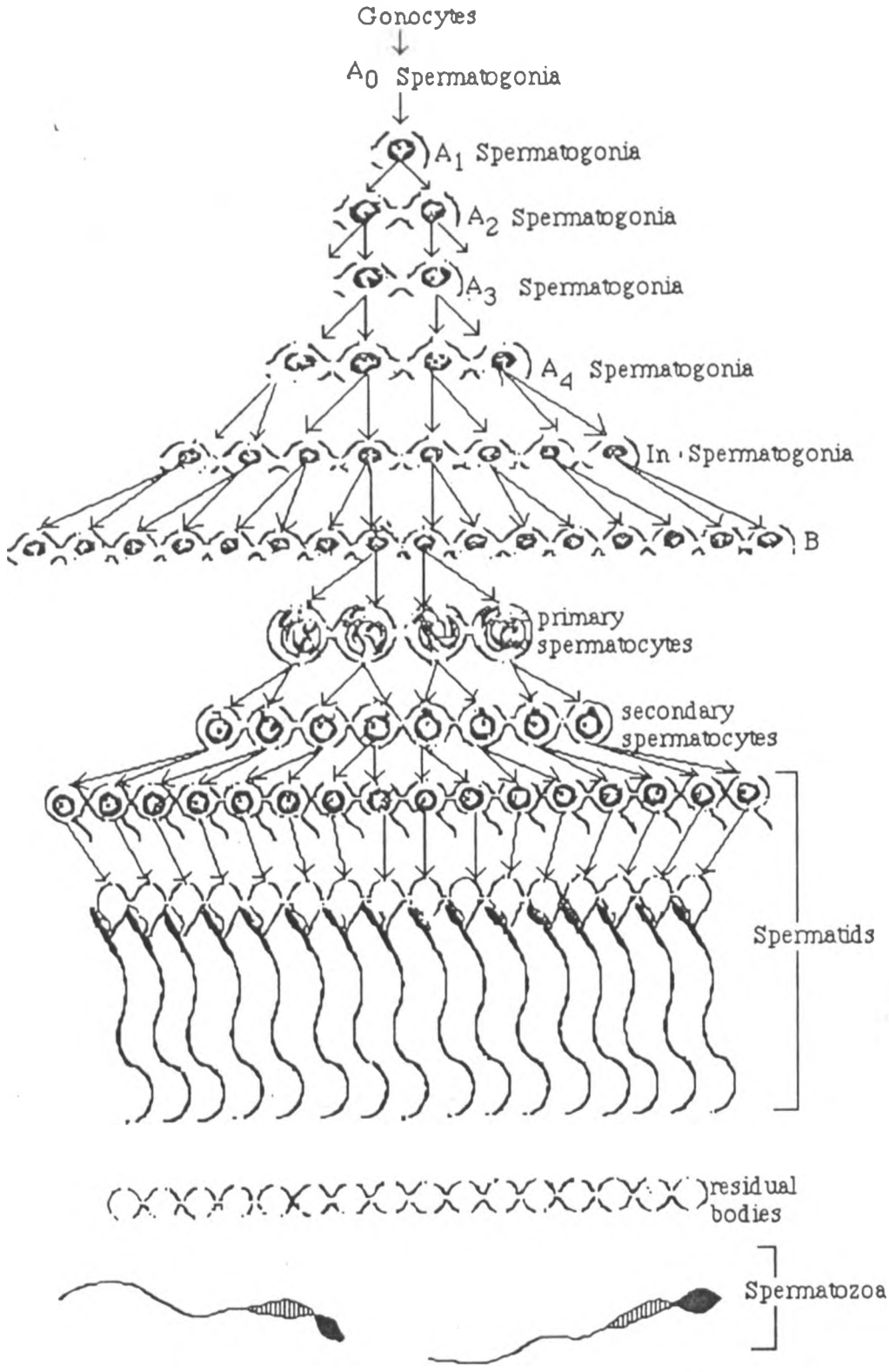



**Figure 2:** The representation of the various cell types that occur during spermatogenesis. Source: Bloom and Fawcett, (1975).

## 2.3.2 Spermiogenesis.

Spermiogenesis is the transformation of spermatids to mature spermatozoa (Duane and Hafez, 1987). Spermiogenesis is divided into four phases namely: Golgi phase, cap phase, acrosomal phase and the maturation phase.

**Golgi phase:** Pro-acrosomal granules are formed within the Golgi apparatus. These granules coalesce into a single acrosomal granule. During this phase, commencement of early stages of tail development also occur.

**Cap phase**: This is characterised by the spreading of the acrosomal granule over the spermatid nucleus and enclosure of the nuclear envelope by a thin double-layered membranous sac.

**Acrosomal phase**: In this phase, condensation of chromatin into dense granules and reshaping of the spherical nucleus into an elongated flattened structure occurs. Also the acrosome is condensed and elongated to correspond to the shape of the nucleus. There is displacement of the cytoplasm to the caudal aspect of the nucleus, formation of the annulus and aggregation of mitochondria to form the middle piece of the tail.

**Maturation phase**: This involves final transformation of elongated spermatids into spermatozoa. During the maturation phase, a fibrous sheath and underlying coarse fibres are formed around the axoneme. The annulus migrates distally to a point where it separates the middle piece from the principal piece of the tail. Mitochondria become tightly packed into a continuous sheath extending from the neck to the annulus.

**Spermiation**: This is the release of formed germ cells into the lumen of seminiferous tubules. Extrusion of the spermatozoal components continues until only a slender stalk of cytoplasm connects the neck of the spermatid to the residual body. Breakage of the stalk results in formation of the cytoplasmic droplet in the neck region of released spermatozoa (proximal droplet). The sertoli cells phagocytose the remaining residual bodies and any degenerating cells.

## 2.3.3 Hormonal control of spermatogenesis.

Testosterone in relatively small doses inhibits secretion of gonadotropins and thus suppresses spermatogenesis (Ewing *et al.*, 1973; Berndtson *et al.*, 1974). However, large doses of testosterone stimulate spermatogenesis, even though the secretion of pituitary gonadotropins is depressed (Ludwig, 1950). The reason why low doses of testosterone, adequate to maintain the functioning of accessory glands and libido, are insufficient to maintain spermatogenesis has been attributed to the anatomical location of the seminiferous tubules close to the source of endogenous testosterone in the Leydig cells (Bansal and Davies, 1986). The seminiferous epithelium is normally exposed to a much greater concentration of testosterone than the rest of the body and its function is not stimulated by testosterone levels which are sufficient to stimulate other androgen dependent tissues (Bansal and Davies, 1986).

Androgens are essential for normal Sertoli cell functioning as well as spermatogenesis (Steinberger, 1971). Sertoli cells, which form prior to puberty and do not divide thereafter (Lino, 1971) are the somatic elements of the seminiferous epithelium which are able to metabolise some steroids and elaborate a specific androgen-binding protein (ABP). This protein transports androgens to the germ cells of the seminiferous epithelium and to the epididymis (Ritzen and French, 1974). Sertoli cells support the process of spermatogenesis through a variety of specialised functions, for example the establishment of a blood-testis barrier (Dym and Fawcett, 1970), maintenance of high intratesticular testosterone levels (Means, 1977) and nourishment of developing germ cells (Mita *et al.*, 1982). Berndtson *et al.* (1987) showed that testes possessing a greater number of Sertoli cells are heavier and produce more spermatozoa than testes with fewer Sertoli cells.

The Leydig cells, which are highly differentiated cells, are specialised in the synthesis of androgens (Cox, 1984). Functionally, they are involved in synthesising testosterone and other bioactive substances involved in the paracrine and autocrine regulation of testicular function (Täkha, 1986). The

stimulatory effects of LH on androgen synthesis are mainly brought about by changes in the production of intracellular cAMP (cyclic adenosine monophosphate) by the Leydig cells, subsequent activation of protein kinase, the phosphorylation of specific proteins and the eventual increase in the activity of steroidogenic enzymes (Dufau *et al.*, 1984).

The hypothalamus secretes gonadotropin releasing hormone (GnRH) which stimulates the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary (Figure 3). These two hormones have synergistic actions on testicular function (Johnson and Ewing, 1971; Moger and Murphy, 1982). FSH modulates the functions of Leydig cells by inducing LH receptors and increasing steroidogenic capacity of the testis (Täkha, 1986). The functional state of the Leydig cells is predominantly determined by the pituitary secretion of gonadotropins, prolactin and possibly adrenocorticotrophic hormone (ACTH) (Liptrap and Raeside, 1978; Täkha, 1986).

Androgen receptors are present in the seminiferous tubules (Hannson *et al.*, 1975) and the seminiferous epithelium secretes inhibin under the stimulatory effect of FSH (Ganjam and Amann, 1976). This inhibin has a negative feedback effect on FSH but not on LH (Duane and Hafez, 1987). The rate of spermatogenesis cannot be affected singly by hormones such as gonadotropins or androgens but they do influence the number of germ cells produced during spermatogenesis (Ortavant *et al.*, 1977).

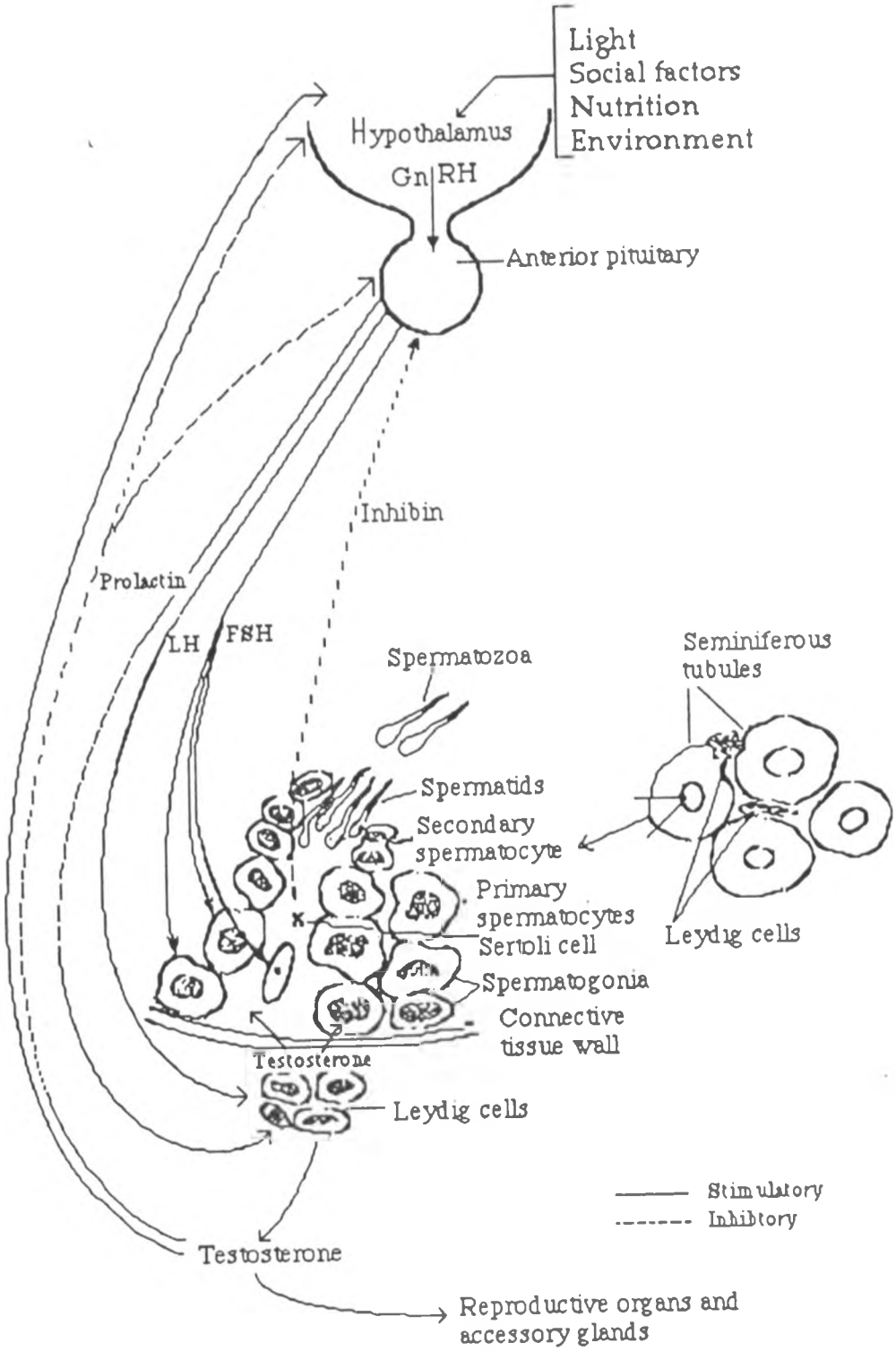



**Figure 3:** The representation of the hormonal interplays during spermatogenesis. Source: Noakes, (1988).

## 2.4 Male reproductive endocrinology.

Testosterone ($T_4$) produced by the Leydig cells in response to luteinizing hormone from the anterior pituitary is the major androgen in male animals (Steinberger, 1971). Testosterone is necessary for the normal functioning of spermatogenic epithelium, patterns of sexual behaviour and voice (Cox, 1984). Androgens stimulate the later stages of spermatogenesis, prolong the life span of epididymal spermatozoa and maintain secondary sex characteristics (Cox, 1984). The growth and secretions of the accessory sex glands (seminal vesicles, prostate and bulbo-urethral) are also dependent on androgens. Testosterone exerts a negative feed back control on the pituitary output of LH (Duane and Hafez, 1987).

Testosterone is transported in blood by an alpha globulin designated steroid-binding globulin and 97-99% of the circulating testosterone is bound by this globulin (Cox, 1984). The remaining testosterone is free to enter the target cells where an enzyme in their cytoplasm converts it to dihydrotestosterone. The latter acts on the nuclear receptors of these target cells (Reeves, 1987). Dihydrotestosterone is a metabolite of testosterone and may be the active androgen in the glands, the secretions of which are added to semen before ejaculation (Cox, 1984). Prolactin potentiates the effect of LH in spermatogenesis and testosterone production (Hafez and Thibault, 1975; Roberts, 1986).

Androstenedione has been identified in the bull testis and in spermatic vein blood (Lindner and Mann, 1960). It is classically believed to be the predominant testicular androgen in many species before sexual maturity (Eik-Nes, 1975). However, testosterone is the principal androgenic hormone in bulls (Lindner, 1969). The androgenic activity of androstenedione is very low relative to that of testosterone and is probably related to its rapid conversion to androgenically inactive compounds (Mann *et al.*, 1960; Skinner *et al.*, 1968, Lindner, 1969).

Episodic LH activity is responsible for testicular development which initiates puberty. Lacroix and Pelletier (1979) reported episodic patterns of

LH release after birth in bulls, the frequency and magnitude of the peaks increased up to 4 months of age and decreased thereafter. High plasma LH values have been observed in 6-12 month old Charolais (Lacroix *et al.*, 1977), Brown Swiss (Karg *et al.*, 1976) and Friesian bull calves (Thibier, 1975). These infrequent LH peaks have been shown to produce a large testosterone release.

A high frequency of LH peaks has been reported in 6 year-old bulls (Katongole *et al.*, 1971) which suggests a difference in intensity of pulsatile pattern in prepubertal calves and adult bulls. From birth until the end of puberty, basal plasma levels of LH remain constant or even decrease in peripheral plasma while testosterone shows increases beginning at the 20th week of age. The increase occurs biphasically in relation to age (Karg *et al.*, 1976). Evidence that various physiological processes occur in biphasic patterns in bulls was first obtained by McMillan and Hafs (1968), who described a similar phenomenon for fructose concentrations in seminal plasma. Rawlings *et al.* (1972) reported biphasic patterns of testosterone after determining the levels of this hormone in blood plasma and testicular tissues.

However, Lindner (1969) reported occurrences of erratic increases in peripheral plasma levels of testosterone from birth to 11 months of age, which then drop and stabilise at about 12 months of age. The cause of the decrease in androstenedione/testosterone ratio associated with puberty may be due to either a deficiency in androstenedione reductase or limited availability of the reduced form of the pyridine nucleotide both of which are required for the conversion of androstenedione to testosterone. Androstenedione, unlike testosterone, does not appear to possess any marked stimulatory activity on the male accessory sex organs (Mann and Rowson, 1960). Hence the main function of testicular androstenedione is not that of a biologically active androgen but a precursor of the highly active testosterone (Slaunwhite and Samuels, 1956). In addition, androstenedione functions as an intermediary in the biosynthesis of oestrogens (Baggett *et al.*, 1956).

It has been demonstrated that there is a positive correlation between the secretory activity of the seminal vesicles and that of testosterone in the testes

of mature bulls (Lindner and Mann, 1960). Thus the level of fructose and citric acid in the seminal vesicles can serve as a criterion for assessing androgen activity of the testes. The rate of testosterone release from the testes into the spermatic vein is proportional to the actual level of this hormone in the gonads of bulls. This presumably explains why the level of fructose and citric acid in the seminal vesicles is related to testosterone levels in the testes (Lindner and Mann, 1960).

## 2.5 Semen collection.

Success in extending the usefulness of superior sires and achieving the highest possible fertility in artificial insemination commences with proper semen collection and its preservation. The goal of semen collection is to have the maximum number of spermatozoa of the highest quality per ejaculation. Although the initial quality of semen is dependent on the physiological status of the bull, quality can be lowered by poor collection procedures (Salisbury *et al.*, 1985).

Generally, there are three basic techniques of collecting semen samples from bulls, namely: artificial vagina (AV), electroejaculation (EE) and digital manipulation (Arthur *et al.*, 1986). The most commonly used procedures are the AV and EE.

### 2.5.1 Artificial vagina (AV).

The AV is a device that is used routinely to collect semen from bulls for artificial insemination. The AV is introduced just as the partially protruded penis starts a forward thrust. Sexual preparation (teasing) is known to improve ejaculate quality (Foster *et al.*, 1970) and is routinely employed for bulls. However, its advantages are not consistent with species (McMillan and Hafs, 1967; Pickett *et al.*, 1973). The AV offers the fastest and most convenient method of collecting semen and provides for the greatest degree of sanitation. The AV is

constructed to mimic the vagina of the female (Figure 4). Most designs have a firm outer case and an inner latex liner, the two ends of which are turned back over the casing to form a water jacket. The liner is held in place by either heavy rubber bands, cord or tape. A cone-shaped piece of rubber holding a graduated pyrex test tube or centrifuge tube is slipped over one end of the casing. The rubber funnel and semen collection tube are covered with a pre-warmed (38°C) protector to help prevent breakage of the tube and reduce the possibility of cold shock to sperm cells. The size of the AV depends upon the size of the penis and therefore the age and breed of the bull. The design of the AV for a particular species needs to take account of the length of the penile shaft in order that most of the ejaculate passes directly into the glass collecting vessel rather than on to the rubber, since components of the rubber may be toxic to spermatozoa (Boucher *et al.*, 1958). Using a short AV, Foote and Heath (1963) showed that, on average, 12 % of the ejaculate or about 1.5 billion spermatozoa adhered to the surfaces of the rubber liner and cone. Mature bulls require an outer casing of 40 cm long and 6.4 cm in diameter while yearling bulls require a casing 30 to 35 cm long and 5.1 to 5.7 cm in diameter (Salisbury *et al.*, 1985).

The warm jacket of the AV is filled with warm water and air under slight pressure to stimulate the tactile receptors on the penis. In general, the optimum temperature of the AV is kept above that of the body temperature and varies among individuals and species (Faulkner and Pineda, 1975). The best temperature is 60°C. The latter provides internal AV temperatures between 50°C and 55°C during semen collection. McMillan *et al.* (1966) studied five different internal AV temperatures between 39°C and 56°C and obtained significantly higher ejaculate volumes and spermatozoa concentrations at the highest temperature of 56°C.

Potential negative effects of semen collection equipment upon spermatozoa have been recognised for many years (Beseth, 1962). In spite of various recommended procedures to minimize liner toxicity, collection of bovine semen continues to be associated with detrimental effects upon its quality (Flick and

Merilan, 1984). The sources of this toxicity are not well understood but hypotheses point towards excessive AV temperatures (Boucher *et al.*, 1958; McMillan *et al.*, 1966), heating and drying effects (Foote, 1964), chemical residues left from materials used in cleaning and leaching of the liner surface (Hafs, 1978).

Spermatozoa motility has been used to determine the level of toxicity associated with various medical rubber and plastic products including insemination pipettes, since it is a sensitive indicator of rubber toxicity (Flick and Merilan, 1984). Although conventionally, rubber equipment is used for semen collection, it has been shown that single liner-collection cones made of polyethylene are less toxic to bovine spermatozoa than rubber liner-collection cones (Flick and Merilan, 1988).

Semen collected with an AV is lower in both the volume and pH but higher in concentration and mass motility than that collected by electroejaculation (Faulkner *et al.*, 1967; Memon *et al.*, 1986). Although the viability of frozen semen which has been collected by an AV is generally superior to that collected by EE (Hopkins and Evans, 1989), non-return rates between the two types of semen samples do not differ (Hill *et al.*, 1956; Manton *et al.*, 1956).

### 2.5.2 Electroejaculation (EE).

This is achieved by inserting a probe or electrode into the bull's rectum and stimulating sacral and pudendal nerves to the reproductive organs by gradually increasing voltage in a rhythmic fashion for a period of 3-5 minutes. Prior to this, the probe is lubricated with water or a water soluble jelly. When inside the rectum, it is then held over the ampullae and stimulations are applied in an increasing pattern. At lower voltages, the bulbourethral glands are stimulated to discharge their secretions. This is followed by the copious discharges from both the seminal vesicles and prostate glands and finally semen output.

Electroejaculation is used in dairy bulls primarily to extend their potential reproductive life after their libido has waned or declined. It is also the procedure used in collecting semen from superior sires who are crippled, low in sexual activity or are unable to serve AV's (Almquist, 1968). Electroejaculation stimulates the release of additional accessory glandular fluids along with the sperm-rich fraction, while AV collections have less accessory glandular fluids. The efficiency of EE is enhanced by giving bulls a tranquillizer prior to semen collection (Dziuk *et al.*, 1954; Herrick, 1958; Wells *et al.*, 1966).

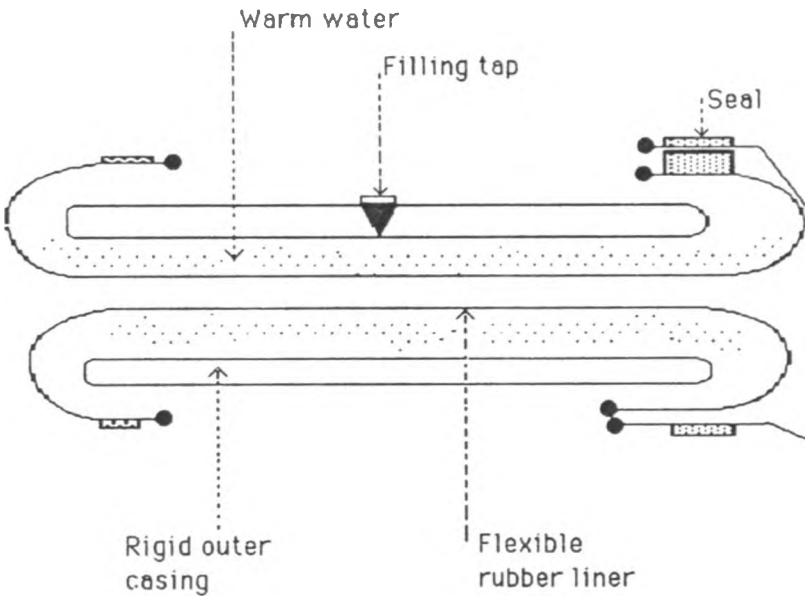
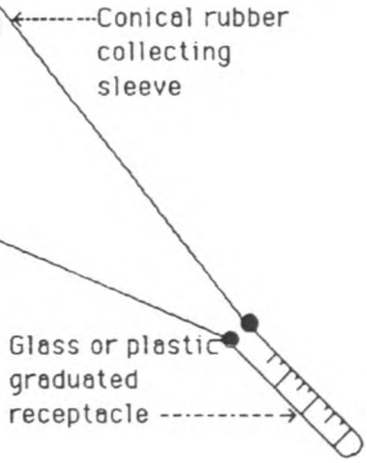



**Fig. 4:** Components of an artificial vagina
Source: Watson (1990).


Conical rubber
collecting
sleeve
Glass or plastic
graduated
receptacle

## 2.6 Semen composition.

Semen is the liquid or semi-gelatinous cellular suspension containing spermatozoa (gametes) and secretions from the accessory glands along the reproductive tract (Duane and Hafez, 1987). The fluid part of this suspension is known as seminal plasma (Salisbury *et al.*, 1985). Seminal plasma functions as a transport medium, protection, as well as a source of nutrients for spermatozoa (Cole and Cupps, 1977). In most species, the secretions of the various accessory glands constitute the bulk of ejaculated semen. The ejaculate volume varies from bull to bull. It varies with the sizes of the bulls, their vigour and frequency of use as well as the health status of their reproductive tracts (Killian and Amann, 1972). The function of the accessory glands and the concentration of the various specific constituents in semen for example fructose is dependent on testosterone levels (Mann, 1974, 1975). The proportionate volume occupied by sperm cells in semen containing $1.25 \times 10^9$ cells per millilitre is 10 percent (Salisbury *et al.*, 1985).

The epididymis contributes glycerylphosphoryl choline, carnitine, and sialic acid to the semen (Crabo, 1965). The glycerylphosphoryl choline is used as an energy substrate once the spermatozoa are in the female genital tract (Duane and Hafez, 1987).

The seminal vesicles are the primary sources of seminal fructose, citric acid and ascorbic acid (Salisbury *et al.*, 1985). The levels of seminal fructose reflect androgen stimulation of the vesicular glands (Fields *et al.*, 1979) while high ambient temperatures have been associated with increased seminal fructose (Glover, 1956; Moule and Waites, 1963).

The pH of freshly ejaculated semen depends on the varying proportions of the several secretions involved. Most normal samples vary from 6.5 to 6.9 (Anderson, 1942). The total phosphorus content of bull semen is positively correlated with the spermatozoa concentration, (Flerchinger and Erb, 1955). There is a higher concentration of sodium and magnesium in spermatozoa compared to seminal plasma while the converse is true for calcium (Cragle *et al.*, 1958;

Quinn *et al.*, 1965; Karagiannidis, 1976).

The protein in semen is contributed mainly by the seminal vesicles (Sexton *et al.*, 1971). Most of the amino acids in the semen originate in the testes or the epididymis (Hoopwood and Gassner, 1962). Free amino acids serve as alternative oxidizable substrates for the aerobic metabolism of spermatozoa (Mann, 1964b), in addition to being used in nucleic acid synthesis (Setchell *et al.*, 1967). Glutamate is the predominant amino acid in *rete testis* fluid, *vas deferens* and seminal plasma while alanine is the predominant amino acid in the accessory sex glandular fluid (Bhargva *et al.*, 1959; Al-Hakim *et al.*, 1970; Sexton *et al.*, 1971).

## 2.7 Semen evaluation.

Semen evaluation involves a critical examination of the ejaculates for specific characteristics before the semen is processed for storage or for immediate use. These characteristics include: appearance, volume, spermatozoa concentration and motility, spermatozoa morphology and the ratio of live to dead spermatozoa.

### 2.7.1 Ejaculate appearance and volume.

The appearance of the ejaculate is an important feature during evaluation in that it gives a gross indication of the state of the reproductive tract and therefore the quality of the semen (Hafez, 1987). The semen is examined for opaqueness (high opaque appearance is indicative of high sperm cell concentration), colour and admixtures. The volume of the ejaculate is recorded immediately after semen collection.

## 2.7.2 Spermatozoa motility.

The motility of spermatozoa is an important criterion for assessing the quality of an ejaculate. Normal bull spermatozoa move in a fairly straight path while rotating along their longitudinal axes (Gray, 1958). The vibrating tail movements get their energy from adenosine triphosphate (ATP) mediated by ATPase and divalent cations (Quinn and White, 1968). Regulation of the ATP is maintained by glycolysis and respiration (Salisbury *et al.*, 1985). Acetylcholine has been isolated from bull spermatozoa and is the coordinator of the contraction-relaxation process that is necessary for normal progressive motility (Nelson, 1972).

Current methods of assessing spermatozoa motility are primarily visual and results are usually expressed in comparative rather than absolute terms (Salisbury *et al.*, 1985). The motility is usually expressed as a percentage (Hafez, 1987). Although spermatozoa motility is a simple test for determining semen quality (Finn, 1979), absence of motility does not indicate absence of viability (Bedford and Hoskins, 1990).

Semen evaluation involves wave motion and progressive motility. Fresh undiluted semen exhibits the collective phenomenon called wave motion (Rothschild, 1949). The degree of this type of phenomenon has been reported be to be correlated with fertility (Bishop *et al.*, 1954). Its grading is based on the vigour of the swirls (Glover, 1968) and is best performed on a microscope equipped with a temperature controlled stage at 37°C (Roberts, 1986).

Progressive sperm motility is determined by microscopic examination for the percentage of progressively moving (forward motility) spermatozoa, but not those moving circularly, backward or side to side (vibratory) or in a static position (Almquist, 1968). Various methods have been used to objectively estimate motility of semen samples. Rothschild (1949) described a technique called Impedance Change Frequency (ICF) for objective measurement of motility which measures the electrical conductance of semen between two platinum electrodes immersed in the semen sample, while Glover (1968) described a shearing cell for

the measurement of motility. Bartoov *et al.* (1981) described a measurement system which enables simultaneous determination and objective evaluation of the intensity of the collective motility in fresh undiluted semen using a sperm motility analyzer. The latter is based on photoelectric effect.

Quantitative methods of assessing spermatozoa motility are reasonably accurate and repeatable, but each of them has a limited application. Those which are based on mass movement of spermatozoa require a suspension diluted in such a way that individual spermatozoa can be observed (Dott, 1975; Katz and Dott, 1975). Measuring the area change in frequency using an image analysing computer has also been used to objectively assess spermatozoa motility and results obtained are comparable to other methods for assessing motility (Dott and Foster, 1979).

It has been shown that ATP content of spermatozoa and motility are related (Mann, 1964b; Soderquist and Stalhammar, 1991), suggesting that measurement of ATP content would provide an objective method of motility estimation. Using a bioluminescent technique to measure the ATP content of bovine semen, Foulkes and McDonald (1979) found a linear relationship between motilities of ejaculated, frozen and thawed semen and their ATP content.

### 2.7.3 Spermatozoa concentration.

Spermatozoa concentration determination is important since the doses produced depend the concentration, while the percent motility and the volume of the ejaculate collected determine the dilution rate (Hahn *et al.*, 1969). The number of spermatozoa per unit volume of bull semen varies from zero in complete azoospermia to over 3 billion ($3 \times 10^9$) cells per cubic millilitre in dense samples (Salisbury, *et al.*, 1985). First ejaculates tend to be more concentrated than subsequent ejaculates (Foster *et al.*, 1970; Igboeli and Rakha, 1971).

Visual appearance of the ejaculates has been used for a long time to determine spermatozoa concentration (Salisbury *et al.*, 1985). However, it is more accurate to dilute 50-100 $\mu$L of a fresh semen sample with physiological

saline and determine the concentration by either a haemocytometer (Lasley, 1951) or a spectrophotometer. The disadvantage of using the haemocytometer is that it is time consuming (Lasley, 1951) whereas the main disadvantage of using a spectrophotometer is that there are considerable variations between spectrophotometers used in the determination of spermatozoa concentration (Foote, 1972).

### 2.7.4 Spermatozoa morphology.

Fully formed spermatozoa are elongated cells consisting of a flattened head, midpiece and a tail. The sperm head consists of a nucleus containing highly condensed chromatin which is composed of DNA. The anterior end of the nucleus is covered by the acrosome which contains hydrolytic enzymes (acrosin, hyaluronidase and acid hydrolases) involved in the fertilisation process (Mann and Lutwak-Mann, 1981).

The sperm tail is composed of the neck, middle, principal and endpieces (Hafez, 1987). The neck forms a basal plate that fits into the posterior portion of the nucleus. The middle piece is the region of the tail between the neck and the annulus. The central core of the middle piece consists of the axoneme which comprises nine pairs of micro tubules that are arranged around two central filaments. The axoneme and associated fibres are covered by mitochondria which generate energy needed for spermatozoa motility.

The principal piece continues posteriorly from the annulus and extends to near the end of the tail. The end piece is the portion of the tail posterior to the termination of the fibrous sheath. The protoplasmic (cytoplasmic) droplet which is usually detached from ejaculated spermatozoa is composed of residual cytoplasm containing remnants of the Golgi apparatus that were involved in the formation of spermatozoa. When the droplet is retained in the neck region, it is known as a proximal droplet, whereas when it is retained near the annulus it is called a distal droplet (Hafez, 1987).

Spermatozoa morphology is assessed by microscopic examination of a small drop of semen which has been mixed with a stain, smeared on a slide and air dried. Eosin-nigrosin and Haematoxylin-eosin are the most commonly used stains for spermatozoa examination, but other acceptable stains such as India ink and Wrights have been used (Hopkins and Evans, 1989). Spermatozoa morphology reflects the functional status of the testes and/or the excurrent duct system (Wolfe *et al.*, 1993). It is dependent on factors such as age, size of the testes, sexual preparation of the bull and method of semen collection (Howard and Pace, 1988).

Spermatozoa abnormalities, which involve the head, midpiece and tail are mainly classified as either primary or secondary. Primary abnormalities are those that occur during spermatogenesis such as double tails, abaxial midpieces or double heads (Carrol *et al.*, 1963). Secondary abnormalities are those that occur during the storage and transportation of spermatozoa within the excurrent ducts such as detached heads, coiled tails and distal cytoplasmic droplets (Salisbury *et al.*, 1985).

The frequency of abnormal spermatozoa increases with extremes of either heat (Anderson, 1941; Erb *et al.*, 1942; Rathore, 1970; Stephan *et al.*, 1971; Vogler *et al.*, 1991) or cold (Faulkner *et al.*, 1967; Wells *et al.*, 1972). Diseases mainly affecting the reproductive tract, congenital testicular hypoplasia, acquired testicular degeneration and epididymal dysfunction have also been associated with a high frequency of abnormal spermatozoa (Lagerof, 1936; Cupps and Briggs, 1965; Gustafsson *et al.*, 1972). Chronic infections like besnoitiosis can lead to not only loss of libido, but also elevated morphological sperm abnormalities, oligozoospermia and terminally to azoospermia (Sekoni *et al.*, 1992).

The main tail abnormalities are coiled and broken tails. Coiled tails include all forms of abnormally contorted tails and midpieces (Swanson and Boyd, 1962). The condition involves a sharp bend or looping of the posterior section of the midpiece with the tail of the sperm folded or coiled laterally and anteriorly to the head, rather than being straight out posteriorly. This

abnormality occurs during the passage of spermatozoa through the epididymis because these abnormalities are not found in the testes (Rollinson, 1951). It has been reported that simple coiled tails are the most common of all the abnormalities of spermatozoa (Rollinson, 1951). The physiological cause of coiled tails is not well known (Hafez, 1987). Wu and McKenzie (1955) suggested that the cause for the development of coiled tails may lie in the elasticity and contractility of spiral fibrils of the middle piece while Swanson and Boyd (1962) suggested that it could be caused by weakness of the spermatozoa or by effects of abnormal secretions of the genital tract such as decreased fructose and citric acid in semen, which indicates that accessory glands are possibly affected.

Coiled tails can also be produced in most semen samples by rapid chilling (cold shock) or by dilution with water (Salisbury *et al.*, 1942; Lasley and Bogart, 1944). Conditions which enhance coiling, favour the retention of the protoplasmic droplet even on motile straight-tail spermatozoa. Once the middle piece is kinked or coiled, it is difficult for the sperm to lose the droplet (Swanson and Boyd, 1962). The coiled-tail defect may be heritable and extensive use of such bulls would result in a serious problem of semen quality in later generations (Hafez, 1987).

Most epididymal spermatozoa possess proximal or distal cytoplasmic droplets on their midpieces. These spermatozoa are usually considered normal (Rao and Hart, 1948). However, the presence of large numbers of spermatozoa possessing proximal cytoplasmic droplets in semen is an indication of abnormal spermiogenesis or epididymal maturation (Ott *et al.*, 1982; Salisbury *et al.*, 1985; Thilander *et al.*, 1985; Barth and Oko, 1989). Migration of the cytoplasmic droplet has been identified as a correlative factor in the pathogenesis of the separation of sperm heads and tails in bulls (Hancock, 1955). The complement of hydrolytic enzymes found in the cytoplasmic droplet may also contribute towards the degradation of tail structures (Garbers *et al.*, 1970). Nevertheless, distal droplets are not considered to be a serious problem for normal fertility (Barth and Oko, 1989).

Abnormalities involving tail-coiling, breakage and disruption of axial filament complex or disintegration of mitochondria have been reported (Blom, 1966). The onset of some of these defects have been associated with sperm maturation in the epididymis (Koefoed-Johnsen and Pedersen, 1971; Wenkoff, 1978). Appearance of tailless spermatozoa is an early indication of testicular degeneration (Glover, 1961; Rob, 1967) and in some cases partial testicular hypoplasia (Williams, 1965). Amann and Almquist (1962) suggested that tailless sperm heads in bull semen may be preceded by spermatozoa with bent and broken tails. High prevalence of spermatozoa heads separated from tails have been reported as a common abnormality in semen from Guernsey bulls (Jones, 1962).

A midpiece abnormality termed the pseudo-droplet defect has been demonstrated as a major defect in the semen from Friesian bulls (Blom and Birch-Andersen, 1968). The defect, which is located near the centre of the midpiece, appears as a rounded or elongated thickening that contains dense granules surrounded by mitochondria and its incidence increases with age.

Blom (1966) observed coiled, folded and disrupted tails (Dag effect) in semen from two subfertile Jersey full brothers soon after sexual maturity. Further ultrastructure studies showed that the tail axial fibres were abnormal, translocated or missing and that the coiled tails were enclosed in a common cell membrane. The defect involves the principal tailpiece most frequently and the midpiece only occasionally (Koefoed-Johnsen and Pederson, 1971).

### 2.7.5 Ratio of live to dead spermatozoa.

Estimation of the percentage of dead and live spermatozoa in an ejaculate is achieved by mixing a drop of semen on a warm slide at 32°C with a warm eosin-nigrosin stain (Hancock, 1951). The dead spermatozoa take up the eosin stain whereas the live ones do not. This difference is assessed against a dark background imparted by nigrosin and a count made of dead spermatozoa. A high percentage of dead spermatozoa usually indicates severe testicular disease (Arthur *et al.*, 1986).

Since the first description of supravital staining of spermatozoa (Lasley *et al.*, 1942), several stain combinations have been proposed (Hackett and Mcpherson, 1965). Essentially, a cell with a functional plasma membrane excludes the stain, while a cell with impaired membrane function takes it up (Watson, 1990). A second dye in the mixture provides a coloured background to differentiate the unstained cells. The simplistic assumption is that the stain distinguishes live and dead spermatozoa, permitting a count of the live spermatozoa at the time of staining (Watson, 1990).

The difficulty in this interpretation is the tendency for some cells to be partially stained (Campbell *et al.*, 1956). A more cautious interpretation is therefore that any stained area of a cell implies a region of defective membrane and the cell should be classified as impaired (Watson, 1990). However, Aalseth and Sacke (1986) found that partially stained spermatozoa had intact acrosomes and hence considered them viable. The results of these staining techniques are, however, subject to variations resulting from different stain batches (Mayer *et al.*, 1951), temperature and duration of staining (Campbell *et al.*, 1956). Diluent composition may also affect the proportion of the stained cells (Dott, 1975). Species differences exist in the ease of distinguishing stained from unstained cells. Furthermore, smears are normally temporary and must be examined within a short period following staining (Watson, 1990). However, Dott and Foster (1975) showed that inclusion of formaldehyde in the diluent preserves the original staining characteristics of diluted spermatozoa for up to 3 weeks allowing staining to be undertaken at convenient times for assessment of the smears. The percentage of unstained cells under controlled conditions is correlated with fertility in bulls and rams (Bishop *et al.*, 1954; Hulet *et al.*, *1964*; Linford *et al.*, 1976).

## 2.8 Libido and sexual behaviour in bulls.

The term libido is commonly used to describe sex drive in male animals (Fraser, 1960). It can also be defined as the willingness and eagerness of a

male animal to mount and attempt service of a female (Chenoweth, 1981). The most obvious expression of libido is in copulatory behaviour which occurs post pubertal. However, this behaviour may be mimicked by pre-pubertal males under influence of oestrogens (Chenoweth, 1981).

Various methods have been used to assess libido in bulls. These include libido score method (Osborne *et al.*, 1971; Chenoweth and Osborne, 1975), serving capacity test (Blockey, 1976) and response to GnRH challenge which is measured by LH and $T_4$ concentrations (Byerley *et al.*, 1990). In addition, observations on the time elapsed between exposure of a bull to a suitable stimulus and the first copulation (provocation time or reaction time) has also been used to assess libido (Mercier *et al.*, 1949; Fraser, 1960).

### 2.8.1 Factors affecting libido.

Libido is affected by various factors which include: genetic, breed, hormones, nutrition, season and sexual preparation.

#### 2.8.1.1 Genetic.

Variations in libido have been reported among different lines of bulls (Chenoweth, 1981). Studies with monozygous twin bulls have shown that much of the variations observed among bulls are controlled by genetic factors (Olson and Petersen, 1951; Bane, 1954). A study of sexual inhibition of rams found out that inbreeding did not appear to be associated with this trait, although some lines showed a greater prevalence of sexual inhibition than others (Hulet *et al.*, 1964).

#### 2.8.1.2 Breed differences.

Differences in libido among bulls have been reported (Amann and Almquist, 1976; Chenoweth, 1981). Chrichton and Leishman (1985) using a serving capacity

test reported that *Bos indicus* bulls displayed fewer services than those of *Bos taurus*. Short-horn and Guernsey bulls have slower excitability than Friesian bulls under A.I conditions (Smith, 1951). Further differences also exist between Friesians of European and those of American origin (Bonadonna, 1956). Zebu bulls exhibit marked sexual sluggishness and have tendencies of mounting only cows in full oestrus (Hafez, 1960).

### 2.8.1.3 Hormonal influences.

Although it is generally accepted that testosterone is the principal hormone that influences male sexual activity, there exists no consensus on its mechanisms of this influence (Chenoweth, 1981). Two theories have been advanced in which the first states that sexual performance is related directly to the levels of circulating androgens. This hypothesis is mainly supported by work carried out on rats in which it was shown that sexual performance is related to the amount of androgens administered parenterally (Beach and Fowler, 1959). The second theory states that differences in sexual performance are determined by individual differences in activity and responsiveness of the tissues which mediate this to threshold levels of androgens (Chenoweth, 1981). In castrated bulls, it has been reported that exogenous testosterone does not improve sex drive above pre-castrate levels irrespective of the dosages used, provided a certain threshold level is attained (Blockey and Galloway, 1978).

Based on the work in guinea pigs, Grunt and Young (1952) hypothesized that; the presence of testosterone above a certain threshold level is necessary in maintaining serving capacity. They noted that the action of testosterone on the tissues which mediate serving capacity was limited by somatic responsiveness and differences in activity between individuals to the threshold levels of testosterone. Therefore, available reports show that there is no apparent relationship between testosterone levels and libido in bulls (Bindon *et al.*, 1976; Foote *et al.*, 1976).

2.8.1.4 Nutrition.

Conflicting reports exist concerning the effects of nutrition on libido in bulls especially in the late pre-pubertal and early post-pubertal periods. For example, Zoder *et al.* (1969) found no significant correlations between average daily weight gains and mating behaviour. In contrast, Flipse and Almquist (1961) reported negative effects of a high level of TDN (total digestible nutrients) intake on reaction time. On the other hand, in another study, rams fed on a high protein supplement displayed more intense sex drive than those fed on low protein supplement (Salamon, 1964). This observation contrasted that of Maree *et al.* (1989) who reported no impact of levels of nutrition on libido.

2.8.1.5 Season.

The effect of season on libido in bulls is poorly documented. A review of earlier work reported the occurrence of wide variations in libido in the tropical and subtropical areas. It noted that libido was greatly reduced in these areas during the hotter periods of the year (Anderson, 1948). In general, libido in domestic male animals is lower in Summer months than in other seasons (Hafez, 1959). Nath *et al.* (1980) reported lower average reaction times in Winter compared to Summer in Holstein-Friesian bulls. Similarly, Sharma *et al.*(1982) reported significant seasonal effects on reaction time in Jersey bulls in India. However, studies carried out in Sweden revealed that libido did not vary with seasons in young bulls (12-26 months) of the Swedish Red and White breed (Hültnas, 1959).

2.8.1.6 Sexual preparation.

Psychological stimulation of males has been shown to increase motivation of inactive animals as well as semen quality (Hale and Almquist, 1960; Hafs *et al.*, 1962; Hale, 1966).

Sexual preparation (teasing) of males is provided by allowing the bull to mount the teaser animal several times, moving the location of the teaser animal, allowing the bull to observe other bulls mount and by restraining the bull near the teaser before semen collection (Katz and McDonald, 1992). Although false mounting is apparently an efficient and effective method of sexual preparation in terms of sperm output, it increases the possibility of injury to bulls. When assessing male behaviour, it is important to avoid confounding sexual motivation or willingness to mate (libido) with sexual performance which depends upon both psychological and physical factors, genital abnormalities and infections, locomotory difficulties, muscular coordination and sexual experience. High sexual performance requires both high motivation and competent physical abilities, while low performance may not reflect low motivation, but rather poor ability to express this motivation (Katz and McDonald, 1992).

## 2.9 Principles of Radioimmunoassays

Radioimmunoassay (RIA) is a type of binding assay in which an antibody is used as the binder. A binding assay is a procedure in which quantitation of a material depends on progressive saturation of a specific binder by that material and subsequent distribution between bound and free phases (Chard, 1987). Determination of the distribution between bound and free phases usually depends on physical separation of these phases, the distribution being followed by incorporation of a tracer consisting of a small amount of ligand labelled with a radioactive isotope (Kent and Niswender, 1979).

The principles of RIA are based on the fact that in the absence of unlabelled antigen or hormone (H) the labelled radioactive hormone (H*) has maximal opportunity to react with a limited number of antibody binding sites (Ab). Antibodies have bivalent binding sites so one antibody molecule can bind two molecules of a hormone. If some of the limited antibody combining sites are allowed to react with labelled hormone, fewer sites will be left to react with the labelled hormone, which results in a decrease in antibody-bound

radioactivity. Radioimmunoassay allows rapid measurement of large numbers of samples containing low concentrations of hormones (Chard, 1987).

The standard curve is a basic requirement for quantitation of the ligand in unknown samples. It involves incubation of fixed amounts of tracer ligand and binder with different amounts of unlabelled ligand. Plotted as percentage of tracer bound against serial dilutions of the ligand on a log scale, this gives a sigmoid curve. When the sample is substituted for the standard, and using the same fixed concentrations of binder and tracer, the value determined for the distribution of the bound and free phases will be equivalent to some value on the horizontal scale of the standard curve and can be read by extrapolation.

### 2.9.1 Separation of bound and free ligand.

Charcoal is commonly used to separate the bound from the free hormone. It is usually pre-treated with dextran. Coating of charcoal with dextran blocks large pores which might accommodate the binder while hindering the adsorption of the free ligand of low molecular weight. The most commonly used of the available charcoal are the Norit range (Norit SX1) with a maximum particle size of 60 $\mu$m (Chard, 1987).

### 2.9.2 Extraction of ligand from biological fluids.

The extraction procedures are in two categories. (1) Those in which the ligand in the sample is adsorbed to particulate materials and then separated from the unadsorbed components. (2) Those in which the sample is treated with an immiscible organic solvent which extracts the ligand (Kent and Niswender, 1979). Adsorption to particles has been widely used in situations where the ligand must be concentrated prior to assay as in case of small peptide hormones (Chard, 1987). Partition with organic solvents is commonly employed where the separation of ligand from other components as in case of steroid hormones is a requirement.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study location.

The study was undertaken at the Central Artificial Insemination Station (C.A.I.S) at Kabete in Kiambu District, Kenya between November 1993 and February 1994. The station lies in an area approximately 1° 16'S and 34° 44'E at an altitude of 1,932 metres above sea level. The area has a bimodal rainfall pattern with a mean annual rainfall of about 1000 mm. The long rainy season occurs from March to June and the short one from October to December. In this area, July is the coldest month of the year. Mean minimum and maximum temperatures are 10°C and 26°C respectively.

### 3.2 Study animals.

A total of 98 bulls comprising Ayrshires (n=27), Friesians (n=30), Guernseys (n=19) and Jerseys (n=22) were used in this study. Apart from the young bulls (n=19) which were not yet mature for semen collection, the others had previous semen collection experience.

### 3.3 Feeding and management practices.

Bulls were housed individually. They were penned at night and allowed to graze on paddocked pasture during the day. They were supplemented with 3-6 kg of concentrates (Bull cubes, A.B.C. Foods Company Ltd) per day depending on the body weight as twice daily ration (in the morning and the afternoon). Good quality hay made of Rhodes grass and clean drinking water were provided *ad libitum* and 2-3 kg of Nappier grass once a day. All bulls were weighed once every month and exercised twice a week by walking them over a distance of about

half a kilometre. Standard health measures were regularly taken. These included deworming, hoof trimming and spraying with a standard acaricide twice a week against ectoparasites (Steladone$^{R}$, Kenya Swiss Ltd).

## 3.4 Measurement of testicular and seminal vesicle sizes.

Bulls were individually restrained in a crush to enable the above measurements to be taken. The sizes of the seminal vesicles were estimated by rectal palpation (Plate 1). The lengths and diameters were estimated to the nearest 0.1 cm using pre-measured fingers.

In determining the scrotal circumference, the testicles were first palpated to ensure normal consistency. They were then pulled firmly down into the lower part of the scrotum so that they were side by side and scrotal wrinkles which could inflate the measurement eliminated. With the left hand holding firmly at the proximal end of the scrotal attachment, the circumference was measured with a flexible looped scrotal tape (Lane Manufacturing Inc., Denver, Colorado) (Plate 2). The looped tape was slipped over the scrotum and tightened around the largest circumference. Tension was placed on the tape until moderate resistance was provided by the testes. The procedure was repeated to confirm the results (Plate 3). The testicular length of the right testis and the length of the *cauda epididymis* were also measured using the same scrotal tape (Plate 4).

## 3.5 Blood samples.

Blood samples were collected once from the middle coccygeal vein, using disposable 10 ml syringes and 1.5" x 18 gauge needles and immediately transferred to heparinised universal bottles and chilled at 4°C until centrifugation. All samples were collected between 1400 and 1700 hr. They were centrifuged (3000g x 10 min) at 4°C within 2 hours of collection. Centrifugation was carried out in 15 ml conical centrifuge tubes (Gallen Kamp Griffin). The resultant plasma was stored in plastic vials at -20°C until hormonal assays were performed.

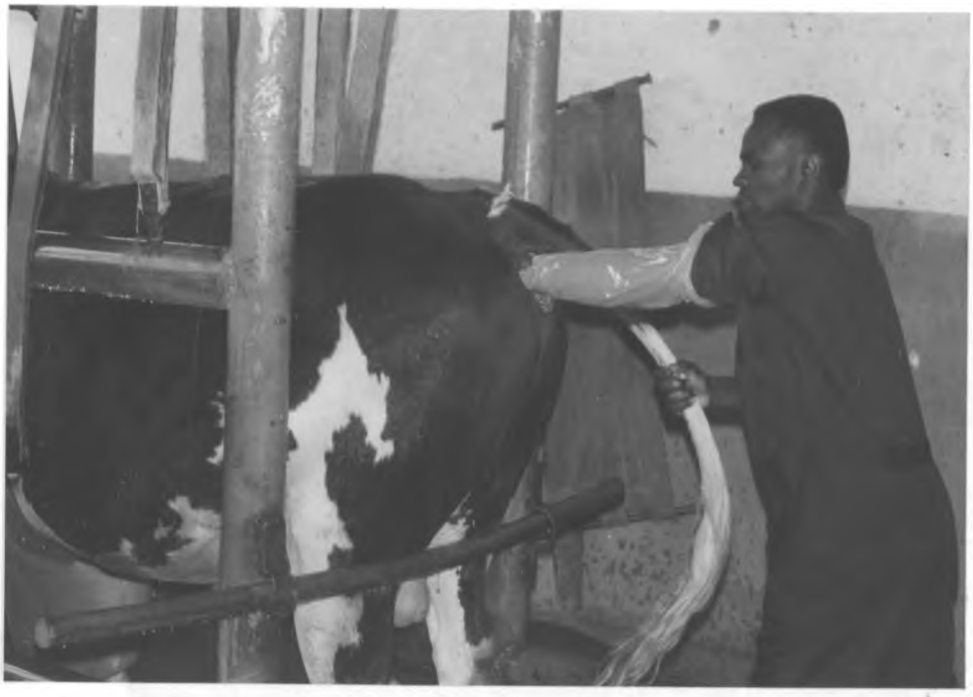


Plate 1. Determination of sizes of seminal vesicles by rectal palpation.


Plate 2. Scrotal tape used to take testicular measurements.

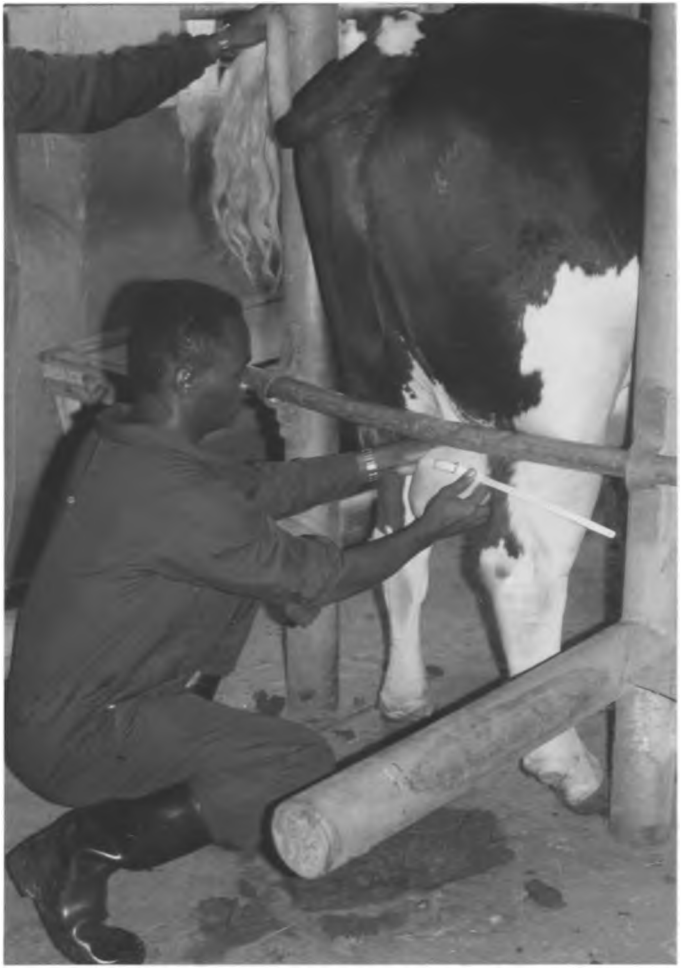


Plate 3. Measurement of scrotal circumference

Plate 4. Measurement of testicular length.

## 3.6. Preparation of the Artificial Vagina for semen collection.

The day prior to semen collection, the AV's were placed overnight in an oven set at 61°C. A few minutes before collection, warm water at 50°C was placed between the outer casing and the inner liner of the AV to create gentle pressure necessary for stimulation and ejaculation. The inner latex rubber liner of the AV was lubricated using sterilized petroleum jelly. Some air was blown into the space containing the water to augment the pressure effect. A clean sterilised rubber cone was attached to a sterilised and graduated glass test tube with a label indicating the name, code number and breed of the bull. The glass tube was protected from light by a sock of cloth. The AV was then ready for use. After semen collection, the AV's were first cleaned in soapy cold water and then in boiling water for a period of 20 min. Subsequently, they were hang over wooden racks in a lockable cupboard to dry.

## 3.7 Semen collection.

The semen samples were collected between 0730 hr and 0900 hr twice a week. Bulls were given a body wash with clean water and allowed to dry for at least 30 min prior to semen collection. They were then dressed with plastic aprons to prevent contamination of semen with hair. Semen collection was carried out in an open space about 50 metres from the laboratory using a 30 x 7.5 cm AV. For smaller bulls, 25 x 7.5 cm AV's were used.

Two teaser animals (steers) were used simultaneously. They were restrained by tying them to strongly fixed posts and placed in such a way as to allow minimal movement while ensuring easy mounting and semen collection from the right side of the bull. Prior to mounting, bulls were restrained in the presence of the teaser animals or allowed one or two false mounts before being allowed full mounts with subsequent ejaculation (Plate 5).

A clean rubber glove in the left hand was used to direct the penis into the AV while the right hand was used to hold the AV (Plate 6). All bulls for semen

collection on a particular day were tied within 20 metres of the collection area and allowed full view of the collection activity.

The reaction time (time taken from the time each bull was presented to the teaser to the time when ejaculation occurred) was taken using a standard sportsman's stop watch. Immediately after ejaculation, the semen contained in the glass tube was taken to the laboratory and placed in a water bath at 37°C before its evaluation.

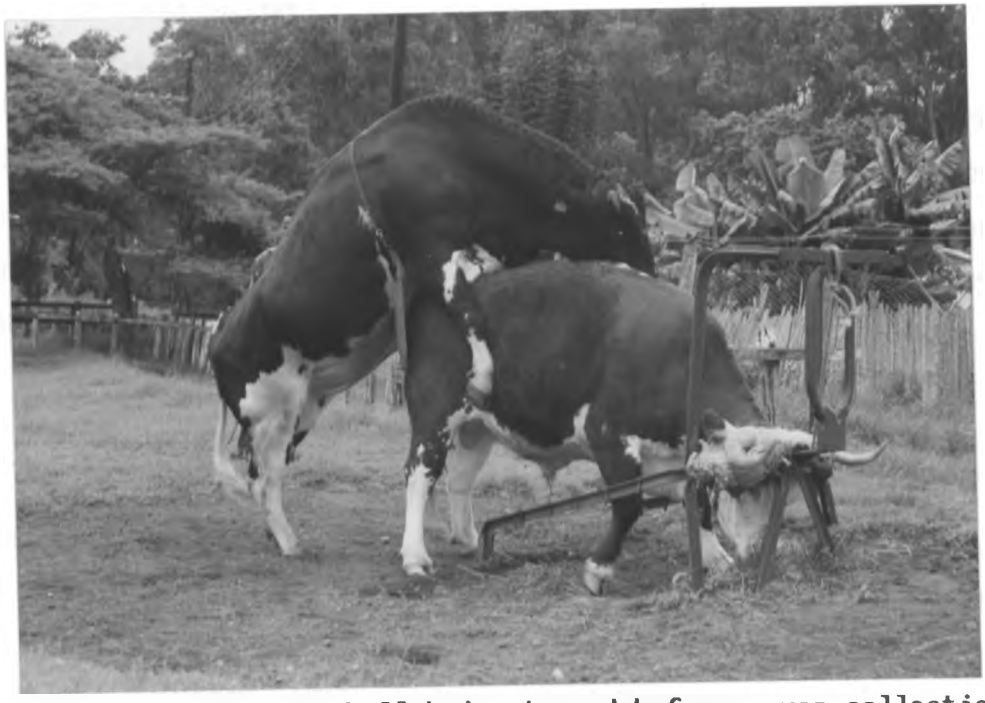


Plate 5. A Friesian bull being teased before semen collection.

Plate 6. Collecting semen from a bull with an artificial vagina.

## 3.8 Semen evaluation.

The collection tubes containing semen samples were transferred to the laboratory and placed in a warm water bath at 37°C. Evaluation involved determination of ejaculate volume, motility, spermatozoa concentration, ratio of live to dead spermatozoa and morphology. The ejaculate volume was recorded to the nearest 0.1 millilitre.

### 3.8.1 Motility.

Individual progressive motility of each semen sample was determined by placing a drop of diluted semen on a pre-warmed microscope slide (25.4 x 76.2 mm). It was then covered with a clean and pre-warmed cover slip (22 mm x 22 mm). The slide was examined under a warm stage microscope at 32°C. Motility was subjectively determined to the nearest 10% at x 250 magnification.

### 3.8.2 Spermatozoa concentration.

This was determined by mixing 0.05 ml of semen with 4.95 ml of 10% sodium chloride. The mixture was placed in a photoelectric calorimeter. The readings obtained were later translated into spermatozoa concentration per cubic millilitre ($mm^3$) using a standard chart.

### 3.8.3 Determination of ratio of the live to dead spermatozoa.

A drop of freshly ejaculated semen was placed on a warm glass slide using a sterile glass rod. A drop of pre-warmed (32°C) eosin-nigrosin stain was added and the two mixed gently using a sterile glass rod. Staining was allowed to take place for 2 min. A smear was made using the edge of a clean glass slide and allowed to air dry. The stained smears were used to determine the ratio of live to dead and percentage of the live spermatozoa, after counting 400 individual

cells at x 1000 magnification using a light microscope (Leitz Wetzlar, Germany). The spermatozoa were seen against a dark background of nigrosin. Normal live spermatozoa were identified as those which had completely unstainable heads while dead spermatozoa were stained pink by eosin.

### 3.8.4 Determination of spermatozoa morphology.

A smear of fresh semen was made on a warm slide. It was air dried and then fixed in methanol for 3 to 5 min. The fixed smear was later stained with Haematoxylin-Eosin stain and eventually mounted using DPX mountant. All smears made were evaluated for any abnormalities using a Leitz microscope under oil immersion at (x 1000) magnification. A total of 400 spermatozoa from different fields on the microscope slide were examined. The abnormalities were classified as head (free heads, pear shaped, abnormal contours, microcephalic, narrow base), midpiece (proximal droplets, distal droplets, abaxial attachment, duplications, swollen) and tail (free, looped, tightly coiled, broken). They were further classified as primary or secondary abnormalities.

## 3.9 Hormone assays.

### 3.9.1 Equipment.

Beta counter (Tricarb Liquid Scintillation Analyzer, Model 1500, United Technologies Packard, U.S.A.).

Pipettes: Manual pipettes of sizes 100 $\mu$L, 200 $\mu$L, 500 $\mu$L and 2 ml.

Test tubes, plastic assay tubes of 2.5 ml each and glass extraction tubes.

Vortex-Genie Mixer (Scientific Industries INC. Bohemia, N.Y. U.S.A.).

Centrifuge (International Equipment Company, U.S.A). The refrigerated centrifuge was capable of maintaining temperatures between 4 to 20°C.

Magnetic stirrer.

3.9.2 Reagents.

Antiserum: Monoclonal Antiserum against testosterone was raised in mice after injection with Testosterone-3CMO-BSA (Antigen). This freeze dried antiserum was kindly provided by Dr. F. Kohen (Israel) through WHO. The final dilution in the assay tube was 1:210, 000.

Tracer: This solution was made up of Testosterone 1, 2, 6, 7 $H^3$-T in toluene: ethanol (9:1 v/v ). The stock solution had a concentration of 10 $\mu$ Ci/ml. (Amersham International pic. Amersham, U.K.).

Scintillant: PPO (2,5-diphenyl oxazole) 35g in 4 litres of Toluene.

Assay buffer: Phosphate buffer, pH 7.4.

Standards: Provided by the WHO Matched Reagent Programme.

3.9.3 Preparation of reagent solutions.

3.9.3.1 Tracer.

Fresh working tracer solution was prepared by taking 150 $\mu$L of the stock solution into a tube and the solvent evaporated. It was then redissolved in 15 ml of phosphate buffer for about 30 minutes. The resultant solution contained (100 Ci/ml).

3.9.3.2 Antiserum.

The freeze dried antiserum was kept at 4°C before use. At the time of assay, the antiserum was reconstituted with 10 ml of phosphate buffer. The mixture was allowed to stand for 5-10 minutes and mixed with a magnetic stirrer before use.

### 3.9.3.3 Dextran charcoal reagent.

Prior to use, the charcoal reagent was chilled to 4°C and well mixed using a magnetic stirrer. The charcoal suspension was made of 0.625g charcoal, 0.0625g dextran and 100 ml of phosphate buffer. Both the charcoal (activated) and dextran were provided by the WHO Matched Reagent Programme. Dextran was dissolved in 100 ml of buffer in a stoppered container, then charcoal was added and the mixture shaken vigorously with a magnetic stirrer for 30 seconds. The charcoal was always stored at 4°C. Prior to use, the suspension was stirred vigorously.

### 3.9.3.4 Hormonal assay buffer.

2.70 g Sodium dihydrogen phosphate (hydrated) $NaH_2Po4.H_2O$ (MW 156) (Merck, Darmstadt, Germany).

11.6 g Disodium hydrogen phosphate (anhydrous) $Na_2HPo_4$ (MW 142) BDH (Chemicals Ltd. Poole, England).

8.8 g Sodium chloride (Nacl).

0.1 g Sodium azide ($NaN_3$); MW 65.01 (E.T. Monks and Co. Ltd).

1.0 g Gelatin (provided by the WHO Matched Reagent Programme).

All constituents except gelatin were dissolved in 750 ml of double distilled water. The gelatin was added to 50 ml of warm water before being added to the other reagents. The final volume of the mixture was made to 1 litre and the pH maintained at 7.4. After preparation, the buffer was stored at 4°C.

### 3.9.4 Extraction.

On the day of the assay, plasma samples (unknowns) were thawed at room temperature. Aliquots of plasma (200 µL) were transferred to clearly labelled Pyrex extraction tubes capable of holding 20 ml. To each of these tubes was added 5.0 ml of fresh diethyl ether and 5.0 ml of ether was added to two empty

tubes (ether blanks). The contents of each tube were vortex-mixed for 1 minute and the ether allowed to settle. The extraction tubes were kept at 4°C for about 1 hour to allow the aqueous layer to freeze. The organic layer containing the hormone was decanted off into another set of tubes. The extracts were left in a well ventilated room to allow the ether to evaporate overnight. When the ether extracts were dry, 2.0 ml of phosphate buffer were added to each tube. Then 100 µL of the tracer and 100 µL of antiserum were added, vortex-mixed, left for 5-10 minutes and then vortex-mixed again. After this, 200 µL of charcoal reagent were added to the unknown samples.

### 3.9.5 Standard Curve.

The testosterone standard provided by the WHO was in ethanolic solution at a concentration of 220 nmol/L. The ampoule was broken open and 3 x 100 µL aliquots were carefully transferred to bottles which were capped tightly. The aliquots were stored at 4°C until needed. At the time of the preparation of the standard curve, 10 ml of the phosphate buffer were added, the mixture heated to 40°C for 30 minutes, mixed vigorously using a magnetic stirrer and allowed to cool to room temperature. At this point the solution contained 2.2 nmol/L testosterone.

A rack was set up containing 5 test tubes capable of holding about 10 ml each. Two ml of buffer were carefully added to all the five tubes followed by 2.0 ml of the 2.2 nmol/L testosterone solution to tube 1 and the mixture in the tube vortexed. Again, 2.0 ml of the solution in tube 1 were transferred to tube 2 and vortexed. The above process was repeated up to the fifth tube.

To obtain the dose levels shown on the next page, duplicates of 500 µL aliquots were transferred from the tubes directly into assay tubes. The assay tubes were placed on a rack and left to incubate overnight at 4°C before the charcoal reagent was added.

| Testosterone standard | |
|---|---|
| fmol/tube | |
| 1100 | Standard solution |
| 550 | Tube 1 |
| 275 | Tube 2 |
| 138 | Tube 3 |
| 69 | Tube 4 |
| 34 | Tube 5 |
| 0 | Buffer |

Note: Each tube contained 500 $\mu$l of solution.

### 3.9.6 Charcoal separation.

To each tube (standards and unknowns) was added 200 $\mu$L of charcoal reagent at 4°C. The mixture was vortex-mixed (30 sec) and allowed to stand for 30 minutes at 4°C. The contents of the tubes were centrifuged at 2,500 R.P.M. using a refrigerated centrifuge for 10 minutes at 4°C. The supernatants were decanted carefully into scintillation vials, while maintaining a minimum delay between centrifugation and decanting.

### 3.9.7 Counting.

Four ml of scintillant were added to each supernatant in the scintillation vials. The mixtures were left overnight to equilibrate at room temperature. Counting of the percent bound testosterone was done using a Beta counter (Tricarb liquid scintillation analyzer, Model 1500, United Technologies Packard, U.S.A.) for 2 minutes per sample.

### 3.9.8 Assay sensitivity.

To check for the sensitivity of the assay, at least 20 tubes for zero standard (Bo) were set up. The mean values of the Bo's were obtained. The standard deviation (S.D.) of these counts were subtracted from the mean, expressed as percent of the bound hormone and then read back on the standard curve to give the first value of the sensitivity at one S.D. Subsequent subtractions of S.D. gave values at two and three S.D. All the assays had a sensitivity of 0.3 nmol/L at 2 S.D.

### 3.9.9 Reproducibility (quality control).

Three sets of duplicate quality control (QC) tubes were included in every assay at convenient points. Each set comprised of goat plasma sample (L) extracted at 200 $\mu$L, bull plasma (A) extracted at 200 $\mu$L and B extracted at 400 $\mu$L. The bull plasma used was a pooled sample obtained from the same bulls under study. The goat sample served as the internal quality control. These samples were subjected to the same extraction process described in sub section 3.7.4. The QC values for all the six assays were used to compute the intra (within batch) and interassay (between batch) coefficient of variation. An intrassay coefficient of variation of 7% and an interassay coefficient of variation 8.7% were obtained.

### 3.10 Extraction efficiency.

This was determined by adding 100 $\mu$L of tritiated testosterone (1000 c.p.m.) to randomly selected samples from the unknowns during the extraction stage. The samples were extracted with 4 ml of ether, vortex-mixed and then frozen to separate the aqueous from the organic layer. The organic layer was decanted off followed by evaporation and reconstitution of the residue with 2 ml of buffer. Total count tubes were prepared by adding 100 $\mu$L of testosterone

tracer into two tubes. To each of the total count tubes and recovery rate tubes was added 4 ml of scintillant, incubated at room temperature and then counted in a Packard Beta counter. The counts obtained were expressed as percentage recoveries by the following formula:

$[(y \times 4)/RTC] \times 100$ where: y = c.p.m. for any individual sample.

RTC = total counts/100 $\mu$L.

All recoveries were 95 % and above.

## 3.11 Assay validation.

The validity of the assay used in measuring testosterone in bovine plasma samples was established as described by Chard (1987) by comparing the dilution curves of endogenous testosterone with the standard curve for parallelism. A plasma sample (200 $\mu$l) of known testosterone concentration (70 nmol/litre) was extracted using 5 ml of ether. The ether was evaporated and the residue reconstituted with 4 ml of phosphate buffer. The solution was serially diluted to four points (doubling dilutions). The above dilutions were treated in the same way as the standards (section 3.7.5) and then counted in a Beta counter.

A rack containing labelled extraction pyrex tubes was set up and volumes of 25, 50, 100, 200 and 300 $\mu$L of the same plasma sample added. Each was extracted using 5 ml of ether. The mixtures were incubated at 4°C, the ether evaporated and the residues reconstituted with 2 ml of buffer. The tracer and antiserum were added. The samples were mixed with charcoal, scintillated and the percent bound testosterone determined using a Beta counter. The sample graphs (logit-log) were drawn on the same paper with the standard so as to compare their parallelism (Figure 5).

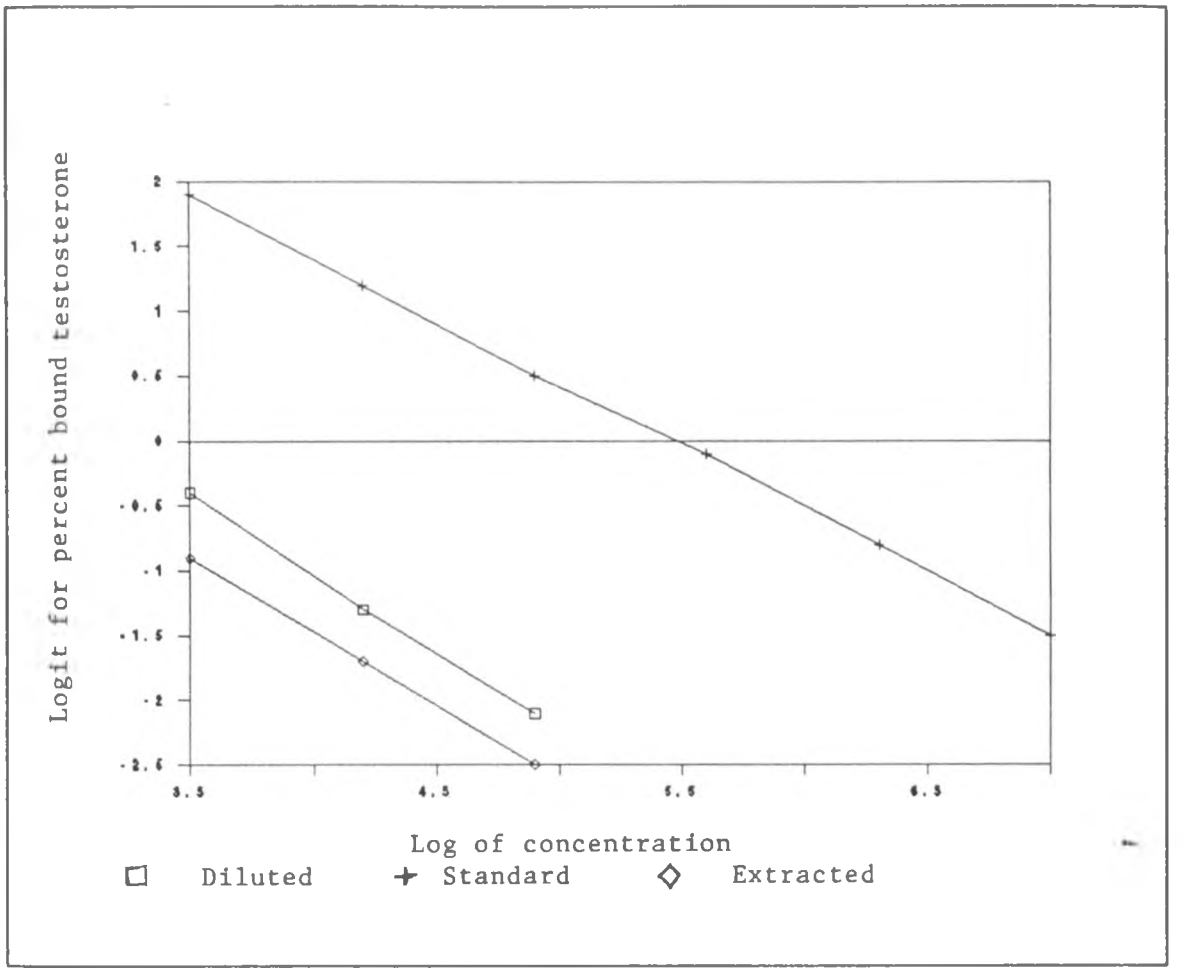
## 3.12 Calculation of results.

The results from the Beta counter were entered into a WHO Immunoassay Data Processing Programme (PR-Edwards) Version 5.4 Middlesex Hospital Medical school, Nassau street, London. The final results used to draw the logit-log graph were calculated as percent bound (%B) = (B/Tc) x 100.
Where B = mean counts per minute and Tc = total counts.

## 3.13 Statistical Analysis.

The data for each individual bull was entered in a data base package, dBASE IV Plus (Ashton-Tate Corporation, U.S.A.). Statistical analyses were done using statistical packages, SAS (PC-SAS version 6.03, SAS Institute Inc., Cary, NC, U.S.A) and Statistix (Version 4.0, Analytical Software, 1958 Eldridge Avenue, U.S.A). The analyses involved computation of descriptive statistics, graphical analysis, analysis of variance (ANOVA), regression and correlation analyses.

**Figure 5.** Logit-log graphs for validation of testosterone assay in in bulls.



# CHAPTER FOUR

## 4.0 Results.

## 4.1 Descriptive statistics and pair-wise comparisons.

### 4.1.1 Age.

The ages of the bulls ranged from 6 to 137 months with a mean of 43.8 months. Overall, the results of the analysis of variance were not significant ($p = 0.1533$) indicating that there were no differences between any two mean ages of the breeds. Table 1 summarises the mean ages of the four breeds. These mean ages were as follows: Ayrshires 35.3 months, Friesians 49.1 months, Guernseys 54.0 months and Jerseys 38.0 months.

### 4.1.2 Body weight.

The weights of the bulls ranged from 70 kg to 945 kg with a mean of 505.8 kg. The results of the analysis of variance were significant ($p = 0.0007$) indicating that differences existed between some pairs of the breed means. Table 2 shows the mean body weights and the summary results of the pair-wise comparisons of these weights. At the 5 percent significance level, the mean body weight of Ayrshire bulls, 437.2 kg, was significantly different from that of Friesians of 626.7 kg. Similar differences existed between the mean body weight of Friesians and that of Jerseys (381.3 kg) and between Guernseys (552.8 kg) and Jerseys.

Table 1.

Breed-specific means of age (months).

| Breed | n | Age ± S.E. |
|---|---|---|
| Ayrshire | 27 | 35.3 ± 5.29 |
| Friesian | 30 | 49.1 ± 5.53 |
| Guernsey | 19 | 54.1 ± 8.20 |
| Jersey | 22 | 38.0 ± 7.67 |

Overall, no significant ($p = 0.153$) differences between breeds.

Table 2.

Breed-specific means of body weight (Kg).

| Breed | n | Body weight (± S.E.) |
|---|---|---|
| Ayrshire | 26 | 437.2 ± 50.4 $^{bc}$ |
| Friesian | 30 | 626.7 ± 40.6 $^{a}$ |
| Guernsey | 19 | 552.8 ± 56.8 $^{ac}$ |
| Jersey | 22 | 381.3 ± 35.1 $^{b}$ |

$^{abc}$ Means with different superscripts are different at the 5 percent significance level.

### 4.1.3 Scrotal circumference (SC).

The measurements of the scrotal circumferences ranged from 20 to 46.9 cm with a mean of 35.5 cm. The SC for Ayrshires ranged from 20.5 to 44.5 cm, Friesians 20 to 45.2 cm, Guernseys 21 to 46.9 cm and Jerseys 21 to 45.7 cm. Table 3 summarises the means of the scrotal circumferences for the four breeds. Friesian bulls had the largest SC (37.2 cm) followed by Guernseys (36.7 cm) and Ayrshires (33.8 cm). Jerseys had the least SC of 33.7 cm. The results of the ANOVA of these circumferences showed no significant ($p = 0.1202$) differences between breeds.

### 4.1.4 Testicular length.

The result of ANOVA on the testicular lengths was significant ($p = 0.0013$) indicating that differences existed between some pairs of the means of these lengths. Table 4 shows the means of the testicular lengths as well as the results of the pair-wise comparisons of these means. The mean testicular length (13.3 cm) of Guernseys was significantly higher than that of both Ayrshires (11.5 cm) and Jerseys (10.6 cm). However, the mean testicular lengths of Ayrshires, Friesians and Jerseys were not significantly different. Similarly, the differences between the means of Friesians and Guernseys were not significant at the 5 percent significance level.

Table 3.

Breed-specific means of scrotal circumference (cm).

| Breed | n | Scrotal circumference (± S.E.) |
|---|---|---|
| Ayrshire | 25 | 33.8 ± 1.45 |
| Friesian | 30 | 37.2 ± 0.92 |
| Guernsey | 19 | 36.7 ± 1.60 |
| Jersey | 19 | 33.7 ± 1.48 |

Overall, no significant differences ($p = 0.1202$) between breeds.

Table 4.

Breed-specific means of testicular length (cm).

| Breed | n | Testicular length (± S.E.) |
|---|---|---|
| Ayrshire | 25 | 11.52 ± 0.49 [a] |
| Friesian | 30 | 12.42 ± 0.33 [ab] |
| Guernsey | 19 | 13.26 ± 0.52 [cb] |
| Jersey | 19 | 10.57 ± 4.55 [a] |

[abc] Means with different superscripts are different at the 5 percent significance level.

### 4.1.5 Epididymal length.

Table 5 shows the means of the *cauda epididymal* lengths for the four breeds. The mean of Ayrshires was 2.3 cm, Friesians 2.6 cm, Guernseys 2.5 cm and Jerseys 2.2 cm. Overall, there were no significant ($p = 0.152$) differences between the various pairs of the means of the *cauda epididymal* lengths.

### 4.1.6 Measures of the seminal vesicles.

The seminal vesicle lengths ranged from 3.0 cm to 11.0 cm with a mean of 6.5 cm. The results of the ANOVA of these lengths were significant ($p = 0.08$). This indicated that differences existed between some pairs of these means. The means of the four breeds are summarised in Table 6. At the 10 percent significance level, the mean seminal vesicle length (5.7) of Jerseys was significantly different from that of Friesians (6.7 cm) and that of Guernseys (7.0 cm). No significant difference existed between the means of Jerseys and Ayrshires.

Table 7 summarises the means of seminal vesicle diameters as well as the results of their pair-wise comparisons. There were no significant ($p = 0.145$) differences between any pairs of the means of these diameters in which Ayrshires had a mean of 2.3 cm, Friesians 2.2 cm, Guernseys 2.7 cm and Jerseys 2.3 cm.

Table 5.

Breed-specific means of *cauda epididymal* length (cm).

| Breed | n | Length (± S.E.) |
|---|---|---|
| Ayrshire | 25 | 2.18 ± 0.18 |
| Friesian | 30 | 2.56 ± 0.08 |
| Guernsey | 18 | 2.53 ± 0.21 |
| Jersey | 18 | 2.21 ± 0.15 |

Overall, no significant differences ($p = 0.152$) between breeds.

Table 6.

Breed-specific means of seminal vesicle length (cm).

| Breed | n | Length ± S.E. |
|---|---|---|
| Ayrshire | 18 | 6.5 ± 0.40 $^{ac}$ |
| Friesian | 29 | 6.7 ± 0.30 $^{a}$ |
| Guernsey | 14 | 7.0 ± 0.26 $^{a}$ |
| Jersey | 15 | 5.7 ± 0.27 $^{bc}$ |

$^{abc}$ Means with the different superscripts are significantly different at the 10 percent significance level.

Table 7.

Breed-specific means of seminal vesicle diameter (cm).

| Breed | n | Diameter (± S.E.) |
|---|---|---|
| Ayrshire | 18 | 2.28 ± 0.21 |
| Friesian | 29 | 2.19 ± 0.16 |
| Guernsey | 14 | 2.75 ± 0.17 |
| Jersey | 15 | 2.27 ± 0.14 |

Overall, no significant differences ($p = 0.145$) between breeds.

### 4.1.7 Testosterone concentration.

The testosterone concentrations ranged from 0.12 to 20.35 ng/ml with a mean of 4.97 ng/ml. The breed-specific testosterone concentrations ranged between 0.99-10.88 ng/ml for Friesians, 0.4-13.6 ng/ml for Jerseys, 0.37-18.6 ng/ml for Guernseys and 0.12 - 20.35 ng/ml for Ayrshires. The means of these testosterone concentrations and results of their pair-wise comparisons are summarised in Table 8. Ayrshires had the highest mean (5.39 ng/ml), followed by Guernseys (5.26 ng/ml), Friesians (4.90 ng/ml) and Jerseys (4.33 ng/ml). Overall, no significant ($p = 0.79$) differences were observed between any pairs of these means.

Table 8.

Breed-specific means of testosterone concentration (ng/ml).

| Breed | n | concentration (± S.E) |
|---|---|---|
| Ayrshire | 25 | 5.39 ± 0.96 |
| Friesian | 30 | 4.90 ± 0.47 |
| Guernsey | 18 | 5.26 ± 1.07 |
| Jersey | 21 | 4.33 ± 0.66 |

Overall, no significant differences ($p = 0.79$) between breeds.

4.1.8 Reaction time.

The reaction times ranged from 7 to 426 sec with a mean of 62.9 sec, approximately 1 minute. Table 9 summarises the means of the breed-specific reaction times and the results of their pair-wise comparisons. Guernseys had the longest mean reaction time of 89.4 sec followed by the Jerseys of 66.9 sec, then the Friesians with 53.8 sec and the Ayrshires with 51.9 sec. However, no significant ($p = 0.419$) differences were observed between any pairs of these means.

Table 9.

Breed-specific means of reaction time (sec).

| Breed | n | time (± S.E) |
|---|---|---|
| Ayrshire | 16 | 51.91 ± 8.73 |
| Friesian | 28 | 53.79 ± 8.70 |
| Guernsey | 14 | 89.43 ± 28.4 |
| Jersey | 15 | 66.93 ± 24.2 |

Overall, no significant differences ($p = 0.419$) between breeds.

### 4.1.9 Ejaculate characteristics.

The means of the various components used to determine semen quantity and quality are summarised in Table 10. These included ejaculate volume and characteristics of spermatozoa (i.e., motility, concentration, abnormalities and viability).

#### 4.1.9.1 Ejaculate volume.

The ejaculate volumes for the bulls ranged from 1.0 to 9.0 ml with a mean of 4.1 ml. Guernseys had the highest mean volume of 4.9 ml followed by that of Friesians of 4.5 ml, then that of Ayrshires of 3.6 ml and lastly that of Jerseys of 3.5 ml. However, no significant ($p = 0.107$) differences were observed between any pairs of these means.

#### 4.1.9.2 Spermatozoa motility.

The spermatozoa motility ranged from 20 to 80% with an overall mean of 69.3%. Table 10 shows the means of the spermatozoa motility observed for the four breeds. Spermatozoa motility was lowest in Ayrshires (65.6%) and highest in Jerseys (72 %). However, no significant ($p = 0.158$) differences were observed between the any pairs of the mean spermatozoa motilities.

#### 4.1.9.3 Spermatozoa concentration.

Spermatozoa concentrations for the bulls ranged from 0.64 to 3.12 x $10^9$ $mm^3$. with a mean of 1.471 x $10^9$ $mm^3$. It was highest in Jerseys, followed by Ayrshires, Guernseys and lowest in Friesians. The results of analysis of variance were significant ($p = 0.04$) indicating that differences existed between some pairs of the breed means. These means and results of their pair-wise comparisons are summarised in Table 10. At the 5 percent significance level, the

mean spermatozoa concentration of Jersey bulls ($1.68 \times 10^9$ mm$^3$) was significantly different from those of Ayrshires ($1.55 \times 10^9$ mm$^3$), Friesians ($1.32 \times 10^9$ mm$^3$) and Guernseys ($1.42 \times 10^9$ mm$^3$). No significant differences were observed between any other pairs.

### 4.1.9.4. Spermatozoa abnormalities.

The abnormalities were divided into head, mid-piece and tail abnormalities which were further classified as primary and secondary abnormalities. These abnormalities are also summarised in Table 10 which shows their mean percentages observed in the semen samples examined.

#### 4.1.9.4.1 Head abnormalities.

The head abnormalities observed in the semen samples examined in this study included free or detached heads, pear shaped, abnormal contours, microcephalic and heads with narrow bases. The head abnormalities accounted for an overall mean of 0.24 % of all the spermatozoa abnormalities in Ayshires, 0.22 % in Friesians, 0.35 % in Guernseys and 0.27 % in Jerseys. In general, free or detached heads were the most frequent abnormalities seen in all the breeds. The highest mean percentage (0.96 %) of the free heads was observed in semen samples from Jerseys, followed by that of Ayrshires (0.77 %), Guernseys (0.73 %) and then Friesians (0.68 %). However, the analysis of variance showed that there were no significant ($p = 0.912$) differences between any pairs.

#### 4.1.9.4.2 Midpiece abnormalities.

These abnormalities comprised of proximal and distal droplets, abaxial attachments, duplications and swollen midpieces. The overall mean percent of the midpiece abnormalities was 0.11 % in both Ayrshires and Friesians, 0.04 % in Guernseys and 0.52 % in Jerseys.

Duplications of the midpiece was the most common abnormality in the semen samples from the four breeds. They accounted for 0.21 % in Ayrshires, 0.14 % in Jerseys, 0.13 % in Friesians and 0.05 % in Guernseys. The analysis of variance showed that there were no significant ($p = 0.133$) differences between any pair of means. The pattern of the means of the percentages of spermatozoa with swollen midpieces was similar to that of the duplications of the midpieces. The highest percent of swollen midpieces was recorded in Ayrshires (0.21 % ), followed by that of Jerseys (0.13 %), Friesians (0.12 %) and the lowest in Guernseys (0.08 %).

The highest mean percentage (0.71 %) of spermatozoa with distal droplets was found in semen samples from Jersey bulls, whereas in the other breeds the mean percentages were less than 0.1 %. The mean of 1.54 % of the abaxial midpieces was the highest and this was observed in semen samples from Jerseys while the least of less than 0.1% was observed in all other breeds. Results of the analysis of variance of these mean percentages for the specific midpiece abnormalities showed no significant differences at the 5 percent significance level.

#### 4.1.9.4.3 Tail abnormalities.

The tail abnormalities observed in this study included free, looped, tightly coiled and broken tails. The overall mean percent of these abnormalities was 0.85 % in the semen samples from Ayrshires, 0.56 % in those from Friesians, 0.53 % in those from Guernseys and 1.2 % in those from Jerseys. The most frequent of the tail abnormalities in semen samples from Ayshires were coiled tails with 1.47 % of them recorded. However, in the semen samples from the other breeds, the most frequent abnormality was looped tails, with 1.36 % of them recorded in Friesians, 1.15 % in Guernseys and 2.27 % in Jerseys. At the 10 percent significance level, the overall mean percent of free tails in semen samples from Jersey bulls was significantly different from all the others.

4.1.9.5. Primary and secondary abnormalities.

The mean percentages of the primary abnormalities (e.g Pearshaped heads, microcephalic heads, abaxial midpieces, double tails) was 2.74 % in semen samples from Ayrshires, 1.54 % in those from Friesians, 1.40 % in those from Guernseys and 1.39 % in those from Jerseys. There were no significant ($p = 0.42$) differences between any pairs of these proportions.

The mean percentages of the secondary abnormalities (e.g detached heads, proximal and distal cytoplasmic droplets) was 3.02 % in samples from Ayrshires, 2.81 % in samples from Friesians, 2.81 % in Guernsey samples and 7.23 % in Jersey samples. No significant ($p = 0.12$) differences existed between any pairs of these proportions. Overall, the mean percentages of the secondary abnormalities were relatively higher than those of the primary abnormalities.

4.1.9.6 Ratio of live to dead and percentage of live spermatozoa.

The ratios of the live to dead spermatozoa in individual semen samples examined ranged from 0.4 to 35.4 with an overall mean of 4.2. The mean ratio was 4.21 for Ayrshires, 3.47 for Friesians, 4.00 for Guernseys and 5.70 for Jerseys. There were no significant ($p = 0.57$) differences between any pair of these means.

The percentages of the live spermatozoa in all the semen samples ranged between 27 % and 97.3 % with an overall mean of 73.4 %. The breed-specific means were 74.3 % for Ayrshires, 70.0 % for Friesians, 76.0 % for Guernseys and 76.5 % for Jerseys. Results of the analysis of variance were not significant ($p = 0.402$) which indicated that there was no significant difference between any two means.

Table 10. Breed-specific least square means for semen characteristics of the four dairy breeds at the C.A.I.S.
(Mean ± S.E)
Breed

| Characteristic | Ayrshire | Friesian | Guernsey | Jersey |
|---|---|---|---|---|
| **Volume** (ml) | 3.6 ± 0.42 | 4.5 ± 0.32 | 4.9 ± 0.5 | 3.5 ± 0.32 |
| **Density** ($mm^3$ x $10^9$) | 1.55 ± 0.13[a] | 1.32 ± 0.06[a] | 1.42 ± 0.07[a] | 1.68 ± 0.13[b] |
| **Motility** (%) | 65.6 ± 3.2 | 69.6 ± 1.3 | 70.0 ± 1.7 | 72.0 ± 0.9 |
| **Percent live spermatozoa** | 74.3 ± 3.90 | 70.0 ± 2.80 | 76.0 ± 3.0 | 76.5 ± 2.6 |
| **Live/Dead ratio.** | 4.21 ± 0.74 | 3.47 ± 0.73 | 4.00 ± 0.59 | 5.70 ± 2.3 |
| **Primary abnormalities** (%) | 2.74 ± 1.20 | 1.54 ± 0.24 | 1.40 ± 0.21 | 1.39 ± 0.34 |
| **Secondary abnormalities** (%) | 3.02 ± 0.73 | 2.81 ± 0.47 | 2.81 ± 0.39 | 7.23 ± 3.64 |
| Morphology | | | | |
| (1) Head abnormalities | | | | |
| Free heads | 0.77 ± 0.31 | 0.68 ± 0.13 | 0.73 ± 0.19 | 0.96 ± 0.55 |
| Pearshaped (%) | 0.11 ± 0.04 | 0.09 ± 0.03 | 0.12 ± 0.06 | 0.14 ± 0.06 |
| Abnormal contours (%) | 0.03 ± 0.02 | 0.05 ± 0.03 | - | 0.04 ± 0.03 |
| Microcephalic (%) | 0.24 ± 0.06 | 0.26 ± 0.09 | 0.20 ± 0.06 | 0.17 ± 0.08 |
| Narrow base (%) | 0.04 ± 0.02 | 0.01 ± 0.01 | - | 0.04 ± 0.03 |
| Total | 0.24 ± 0.05 | 0.22 ± 0.05 | 0.35 ± 0.07 | 0.27 ± 0.08 |
| (2) Midpiece abnormalities | | | | |
| Proximal droplets (%) | 0.08 ± 0.03 | 0.21 ± 0.08 | 0.02 ± 0.02 | 0.06 ± 0.03 |
| Distal droplets (%) | - | 0.06 ± 0.02 | - | 0.71 ± 0.69 |
| Abaxial attachment (%) | 0.07 ± 0.03 | 0.03 ± 0.02 | 0.05 ± 0.03 | 1.54 ± 0.05 |

| Table 10 (continued) | Ayrshire | Friesian | Guernsey | Jersey |
|---|---|---|---|---|
| Duplications (%) | 0.21 ± 0.06 | 0.13 ± 0.03 | 0.05 ± 0.03 | 0.14 ± 0.05 |
| Swollen (%) | 0.21 ± 0.07 | 0.12 ± 0.04 | 0.08 ± 0.03 | 0.13 ± 0.05 |
| Total (%) | 0.11 ± 0.02 | 0.11 ± 0.01 | 0.04 ± 0.0 | 0.52 ± 0.13 |
| (3) Tail abnormalities | | | | |
| Free (%) | 0.34 ± 0.09[c] | 0.16 ± 0.06[c] | 0.28 ± 0.10[c] | 1.54 ± 0.95[d] |
| Looped (%) | 1.26 ± 0.34 | 1.36 ± 0.42 | 1.15 ± 0.25 | 2.77 ± 1.38 |
| Tightly coiled (%) | 1.47 ± 1.04 | 0.49 ± 0.10 | 0.60 ± 0.14 | 0.45 ± 0.15 |
| Broken (%) | 0.32 ± 0.14 | 0.23 ± 0.08 | 0.08 ± 0.03 | 0.10 ± 0.05 |
| Total (%) | 0.85 ± 0.15 | 0.56 ± 0.14 | 0.53 ± 0.17 | 1.22 ± 0.30 |

[a b] Means in the same row with different superscripts differ significantly ($p < 0.05$).
[c d] Means in the same row with different superscripts differ significantly ($p < 0.1$).

## 4.2. Results of the correlation analyses.

The results of the correlation analyses of the quantifiable variables determined in the bulls in this study are given in Table 11. This matrix-table gives the zero-order correlation coefficients. Ages of the bulls were significantly and positively correlated with the body weights ($r = 0.77$, $p = 0.001$). In addition, age was significantly and positively correlated with the following: scrotal circumference ($r = 0.70$, $p = 0.0001$), testicular length ($r = 0.59$, $p = 0.0001$), seminal vesicle length ($r = 0.60$, $p = 0.0001$), seminal vesicle diameter ($r = 0.59$, $p = 0.0001$), epididymal length ($r= 0.62$, $p = 0.0001$), ejaculate volume ($r = 0.32$, $p = 0.005$) and testosterone concentration ($r = 0.49$, $p = 0.002$).

Similar patterns of significant ($p < 0.01$) and positive correlation coefficients were observed from pairs formed from the following: body weights, scrotal circumferences, testicular lengths, seminal vesicle lengths, seminal vesicle diameters, epididymal lengths, ejaculate volumes and testosterone concentration. However, correlations between testicular length and ejaculate volume and those obtained between epididymal length and seminal vesicle length as well as seminal vesicle diameter were not significant ($p > 0.05$).

The percent live spermatozoa were significantly and positively correlated to progressive motility ($r = 0.33$, $p = 0.005$) and to the live/dead ratio ($r = 0.59$, $p = 0.0001$).

All other correlations which have not been described in this section were either not significant ($p > 0.05$) or had very low magnitudes of correlation coefficients ($r < \pm 0.5$) or both.

Table 11. Pearson correlation coefficients between various variables studied in the dairy bulls at C.A.I.S.

| | Age | Wt | SC | TL | SVL | SVD | EL | EV | D | M | RT | $T_4$ | PRIM | SEC | % LIVE | LORAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 1 | 0.77* | 0.70* | 0.59* | 0.60* | 0.59* | 0.62* | 0.32* | -0.01 | -0.01 | -0.07 | 0.49* | -0.09 | -0.06 | 0.01 | -0.12 |
| Wt | | 1 | 0.83* | 0.75* | 0.64* | 0.60* | 0.62* | 0.35* | -0.02 | -0.10 | -0.05 | 0.47* | -0.13 | -0.03 | -0.08 | -0.16 |
| SC | | | 1 | 0.79* | 0.52* | 0.61* | 0.69* | 0.29* | -0.01 | -0.04 | -0.12 | 0.38* | -0.08 | -0.03 | -0.08 | -0.15 |
| TL | | | | 1 | 0.57* | 0.46* | 0.50* | 0.13 | -0.06 | -0.03 | -0.07 | 0.29* | -0.13 | -0.05 | -0.07 | -0.12 |
| SVL | | | | | 1 | 0.53* | 0.17 | 0.36* | -0.09 | 0.20 | 0.04 | 0.27* | -0.35 | 0.01 | 0.04 | -0.03 |
| SVD | | | | | | 1 | 0.31 | 0.41* | -0.06 | 0.03 | 0.02 | 0.40* | -0.23 | 0.12 | 0.11 | -0.02 |
| EL | | | | | | | 1 | 0.29* | 0.02 | -0.11 | -0.21 | 0.32* | 0.11 | 0.00 | -0.21 | -0.14 |
| EV | | | | | | | | 1 | -0.01 | 0.00 | 0.04 | 0.32* | -0.10 | 0.01 | -0.11 | -0.18 |
| D | | | | | | | | | 1 | -0.08 | -0.07 | -0.04 | 0.05 | -0.05 | 0.04 | -0.03 |
| M | | | | | | | | | | 1 | -0.02 | -0.26 | -0.31 | -0.04 | 0.33* | 0.17 |
| RT | | | | | | | | | | | 1 | 0.03 | -0.05 | -0.09 | 0.02 | -0.04 |
| $T_4$ | | | | | | | | | | | | 1 | -0.14 | 0.16 | -0.07 | -0.11 |
| PRIM | | | | | | | | | | | | | 1 | -0.02 | 0.01 | -0.02 |
| SEC | | | | | | | | | | | | | | 1 | -0.03 | -0.06 |
| % LIVE | | | | | | | | | | | | | | | 1 | 0.59* |
| LORAT | | | | | | | | | | | | | | | | 1 |

* *Significant ($p < 0.05$)*

## 4.3 Results of the all-possible regression analyses.

The results of all-possible regression analyses performed to determine the "best" subset of the explanatory (predictor) variables for SC and testosterone concentration are summarised in Tables 12 and 13 respectively. When the SC was regressed against age, breed, body weight and testosterone concentration, 10 "best" subsets of regression models were obtained (Table 12). However, based on both the highest adjusted $R^2$ and the lowest Mallows Cp, the "best" subset overall was composed of breed and body weight. These two variables explained 69.1 % (Adjusted $R^2$ = 0.691) of the total variation in the SC's .

Although the subset having age and weight had the lowest Mallows Cp of 3.3 and the highest adjusted $R^2$ of 0.696 (Table 12), it was not selected as the "best" subset overall because age and weight were highly correlated ($r$ = 0.77, $p$ = 0.0001). This was done to avoid the effects of multi-collinearity on the sum of squares (Neter *et al.*, 1985).

Table 13 shows that 10 "best" subsets of regression models were fitted for testosterone concentration. Using the same criteria of the lowest Mallows Cp and highest adjusted $R^2$, the "best" subset overall model selected was made of age and breed. These two variables only explained 21.8 % (adjusted $R^2$ = 0.218) of the total variation in testosterone concentration.

Table 12. Summary of the "best" subset regression models for the scrotal circumferences measured for the bulls at C.A.I.S.

| Mallows Cp | ADJUSTED R-SQUARED | R SQUARED | RESIDUAL SS | MODEL VARIABLES |
|---|---|---|---|---|
| 198.2 | 0.0000 | 0.0000 | 3679.86 | INTERCEPT |
| 4.5 | 0.6849 | 0.6885 | 1146.25 | C |
| 5.0 | 0.4695 | 0.4756 | 1929.57 | A |
| 161.5 | 0.1259 | 0.1359 | 3179.72 | D |
| 3.3 | 0.6959 | 0.6999 | 1104.22 | A C |
| 3.7 | 0.6914 | 0.6985 | 1109.66 | B C* |
| 5.3 | 0.6856 | 0.6929 | 1130.25 | C D |
| 3.6 | 0.6952 | 0.7057 | 1082.87 | A B C |
| 4.2 | 0.6931 | 0.7037 | 1090.31 | B C D |
| 4.9 | 0.6905 | 0.7011 | 1099.78 | A C D |
| 5.0 | 0.6938 | 0.7079 | 1074.84 | A B C D |

Unforced independent variables: (A) Age, (B) Breed, (C) Weight, D) Testosterone concentration.

* Best subset overall.

Table 13. Summary of the "best" subset regression models for testosterone concentration measured in bulls at C.A.I.S.

| Mallows Cp | ADJUSTED R-SQUARED | R-SQUARED | RESIDUAL SS | MODEL VARIABLES |
|---|---|---|---|---|
| 23.5 | 0.0000 | 0.0000 | 1232.88 | INTERCEPT |
| 1.7 | 0.2085 | 0.2176 | 964.554 | A |
| 10.5 | 0.1272 | 0.1373 | 1063.65 | D |
| 10.6 | 0.1259 | 0.1359 | 1065.31 | C |
| 1.6 | 0.2184 | 0.2364 | 941.429 | A B * |
| 3.2 | 0.2035 | 0.2218 | 959.378 | A C |
| 3.5 | 0.2004 | 0.2188 | 963.113 | A D |
| 3.3 | 0.2125 | 0.2396 | 937.423 | A B C |
| 3.6 | 0.2091 | 0.2364 | 941.428 | A B D |
| 5.2 | 0.1942 | 0.2220 | 959.234 | A C D |
| 5.0 | 0.2055 | 0.2411 | 934.443 | A B C D |

Unforced independent variables: (A) Age, (B) Breed, (C) Scrotal circumference, (D) Weight.

* Best subset overall.

## 4.4 Scatter plots.

Bivariate scatter plots were constructed for the variables which were considered for their possible correlationships as suggested from the literature. The scatter plots were performed for age against scrotal circumference and testosterone concentration, breed-specific ages against testosterone, scrotal circumference against body weight, seminal vesicle length and testosterone concentration (Figures 6-10).

The scatter plot of the overall scrotal circumference versus age (Figure 6) shows that SC increased exponentially up to about 60 months (5 years) of age after which it linearly increased up to about 110 months (9.2 years) then began to decline. The fitted line suggests a curvilinear relationship between age and SC.

The scatter plots of testosterone concentration against age are shown in Figures 7a-e. Figure 7a shows the overall (all breeds) testosterone concentration plotted against age. This figure indicates that the majority of the bulls had testosterone concentrations of below 10 ng/ml. This plot also depicts a cluster of points occurring below the age of 60 months. After this, the points diverge equidistantly away from the fitted line. That is, some points indicate high testosterone concentrations (> 10 ng/ml) and others low testosterone concentrations (< 5 ng/ml) above 60 months of age.

The scatter plots for breed-specific testosterone concentrations against breed-specific ages are shown in Figures 7b-e. In all of them the fitted lines indicate that up to 40 months of age there are exponential increases of testosterone in all the breeds. After that age, the patterns of testosterone concentration increase following the same pattern observed in Figure 7a.

The scatter plot of SC against body weights is shown in Figure 8. This figure shows that these two variables have strong and positive linear co-relationships ($r$ = 0.83, p = 0.0001). Only a few bulls had SC of less than 25 cm.

Figure 9 shows the scatter plot of SVL against SC. The plot shows a linear co-relationship between the two variables is not strong ($r = 0.55$) as most points cluster between SC of 30 cm and 45 cm.

The plot depicting the co-relationship between SC and testosterone concentration is shown in Figure 10. The figure shows that the testosterone concentrations increased with increase in SC although the gradient was low. This plot shows that although the linear co-relationship between the two variables is significant ($p = 0.004$), it is nevertheless low ($r = 0.4$) (Table 11).

**Figure 6.** Scatter plot for the relationship between age and scrotal circumference in all bulls at C.A.I.S.

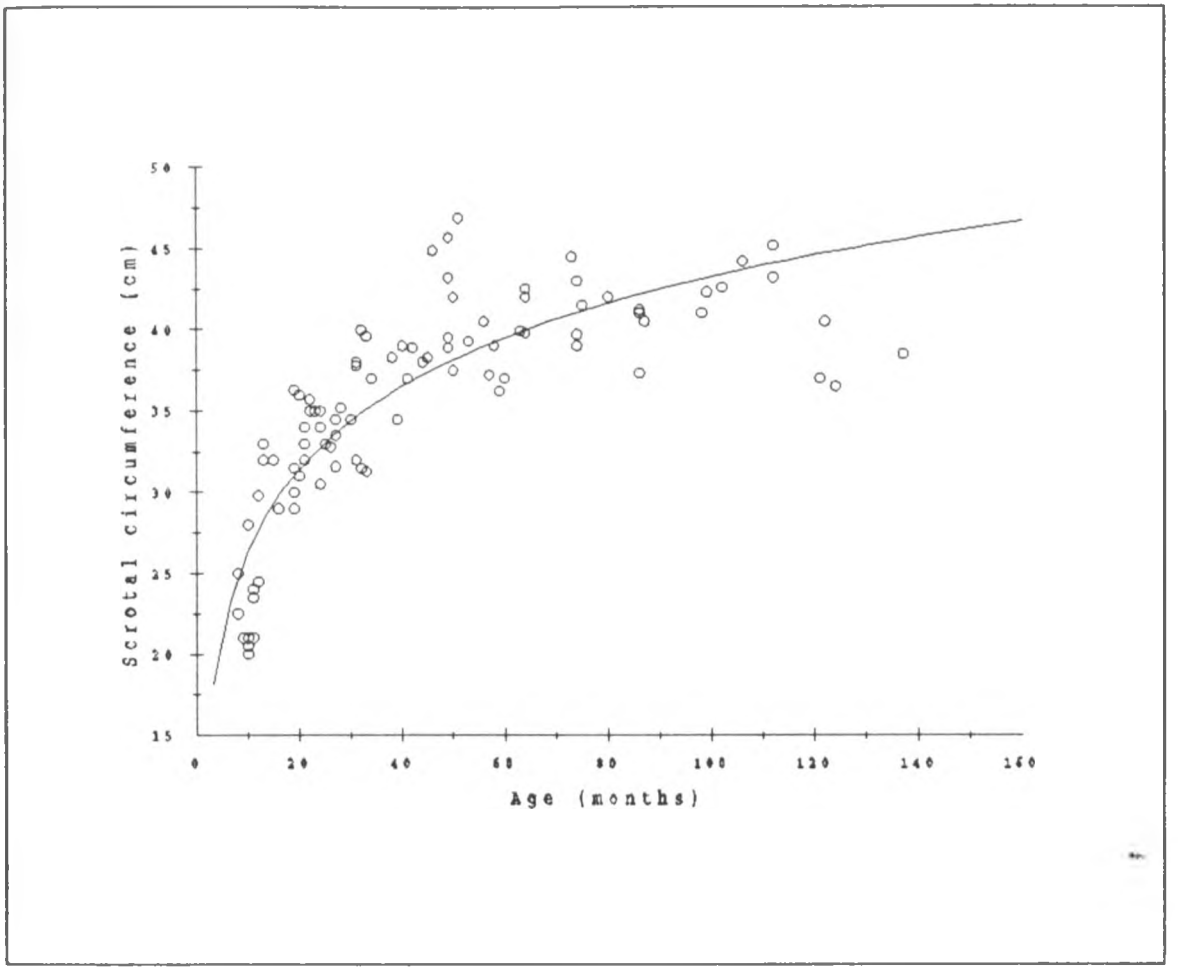


**Figure 7a.** Scatter plot for the relationship between testosterone concentration and age in all bulls at the C.A.I.S.

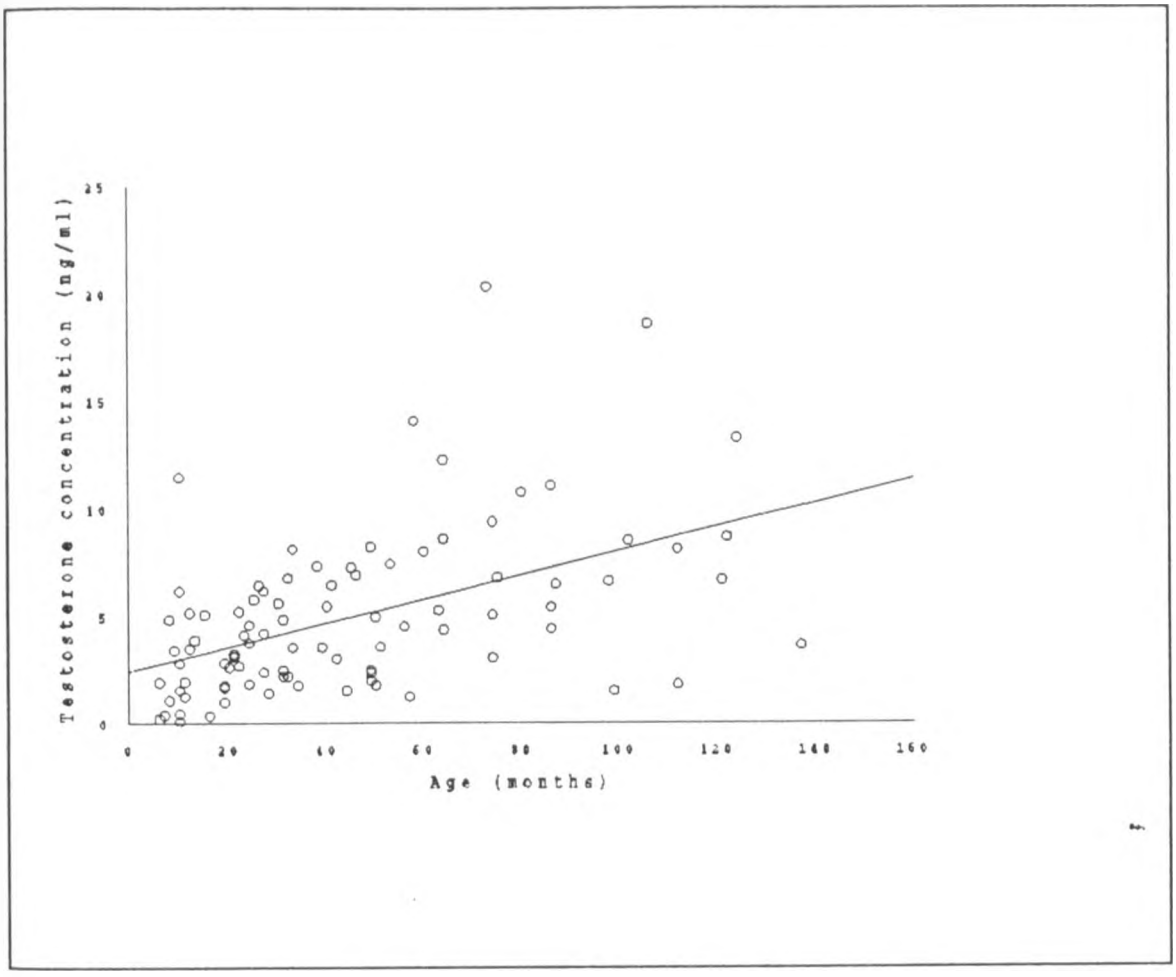


**Figure 7b.** Scatter plot for the relationship between testosterone concentration and age in Ayrshire bulls.

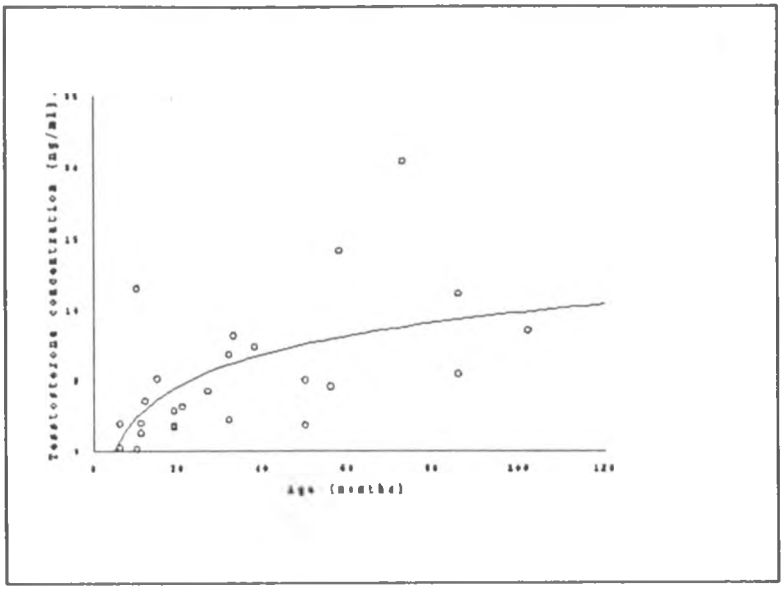


**Figure 7c.** Scatter plot for the relationship between testosterone concentration and age in Friesian bulls.

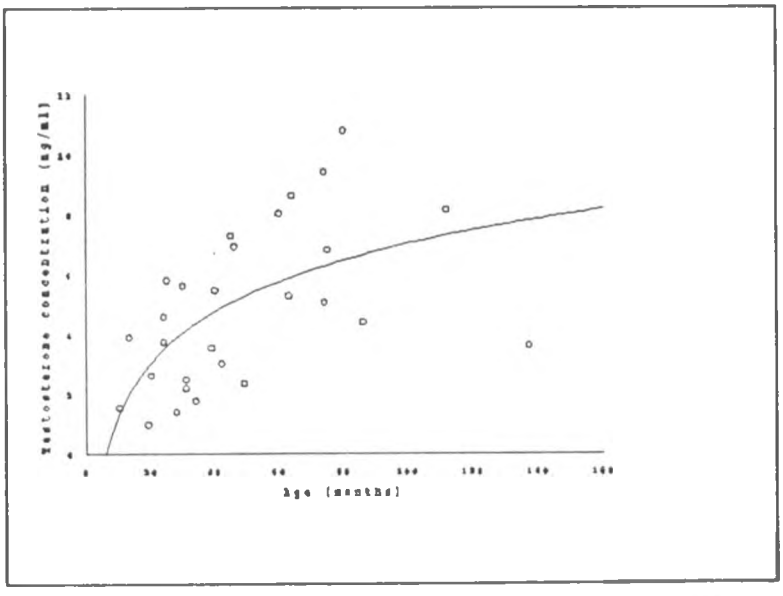


**Figure 7d.** Scatter plot for the relationship between testosterone concentration and age in Guernsey bulls.

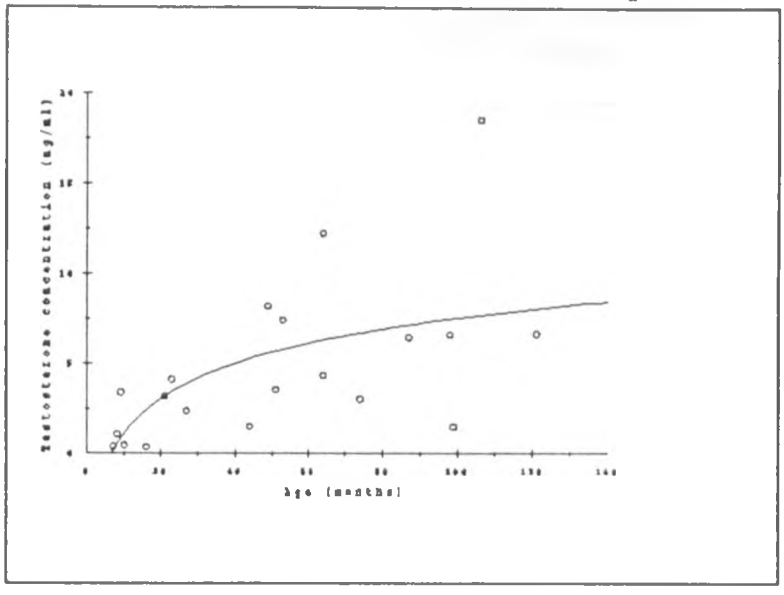



**Figure 7e.** Scatter plot for the relationship between testosterone concentration and age in Jersey bulls.

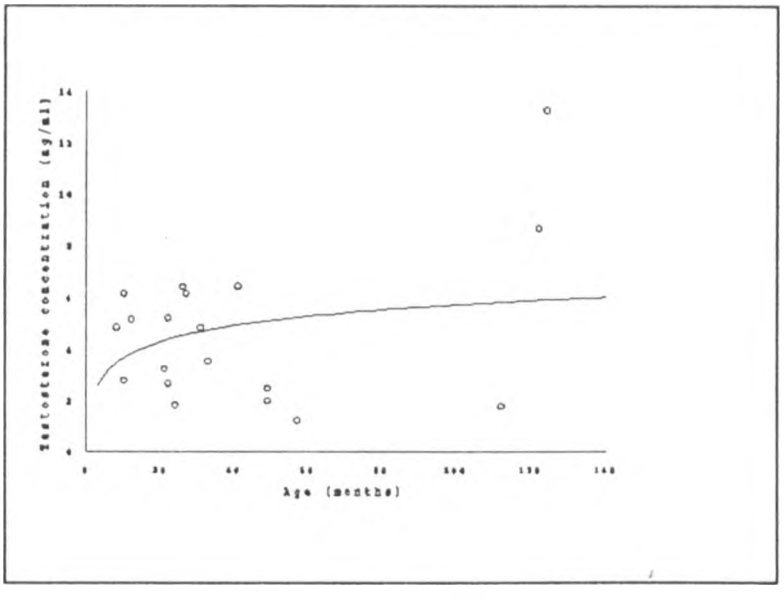


**Figure 8.** Scatter plot for the relationship between scrotal circumference and body weight.

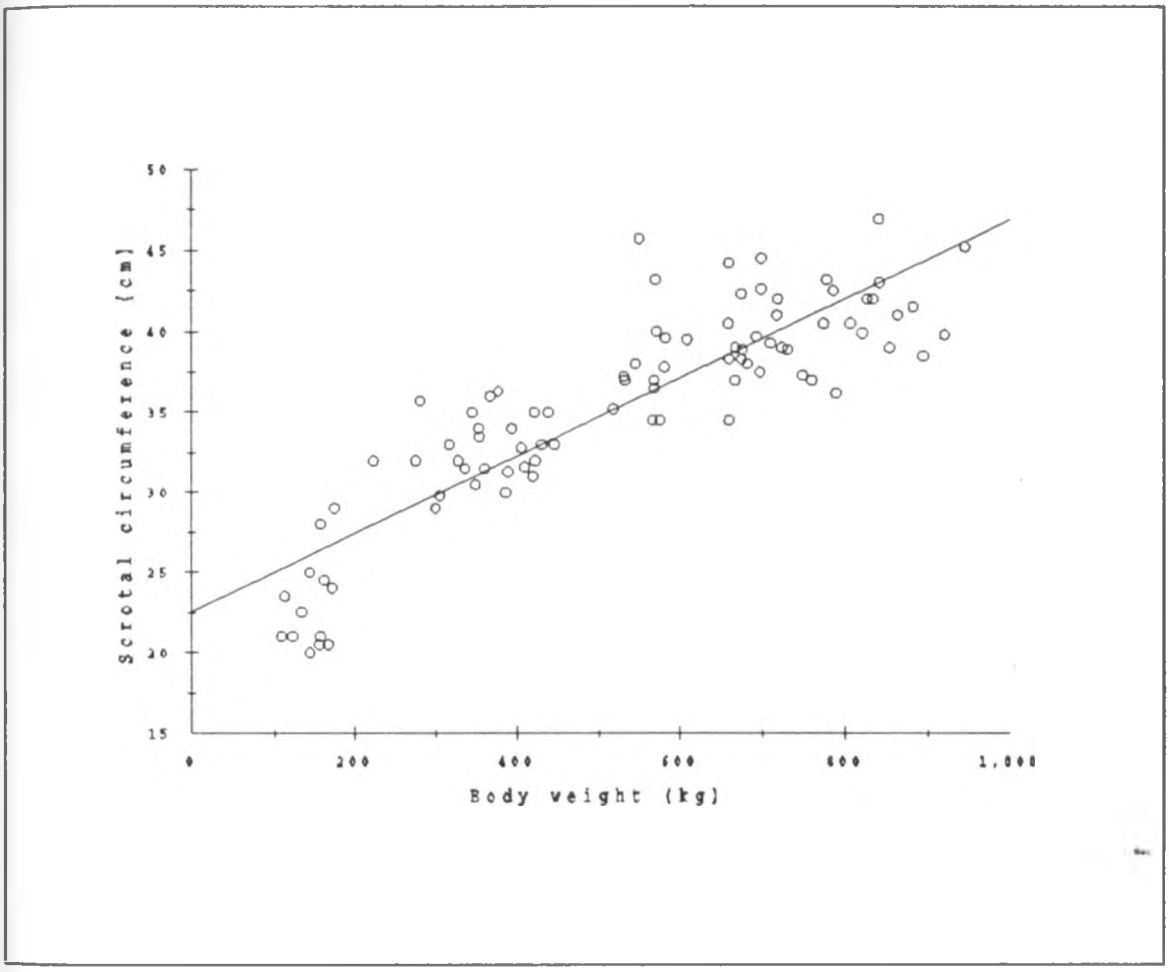


**Figure 9.** Scatter plot for the relationship between seminal vesicle length and scrotal circumference.

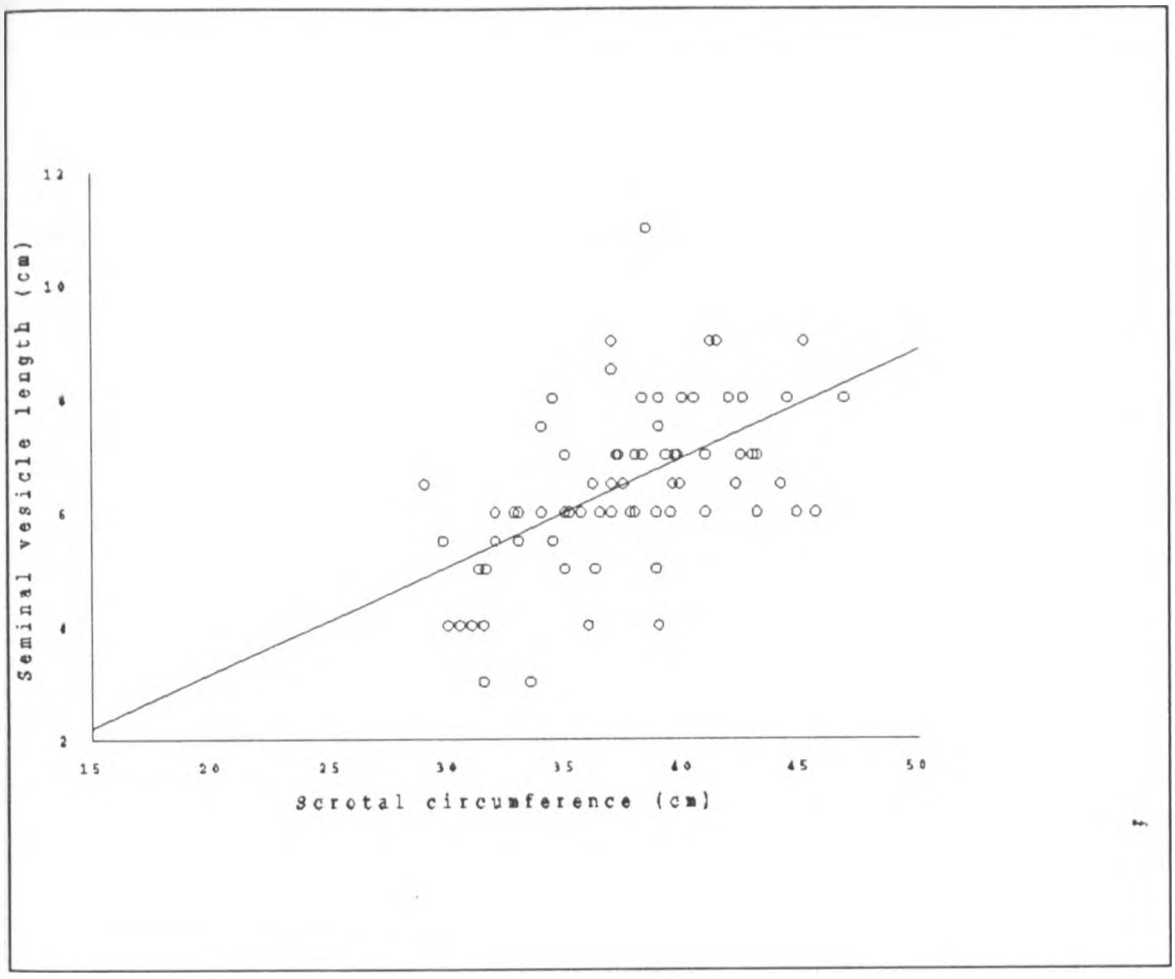
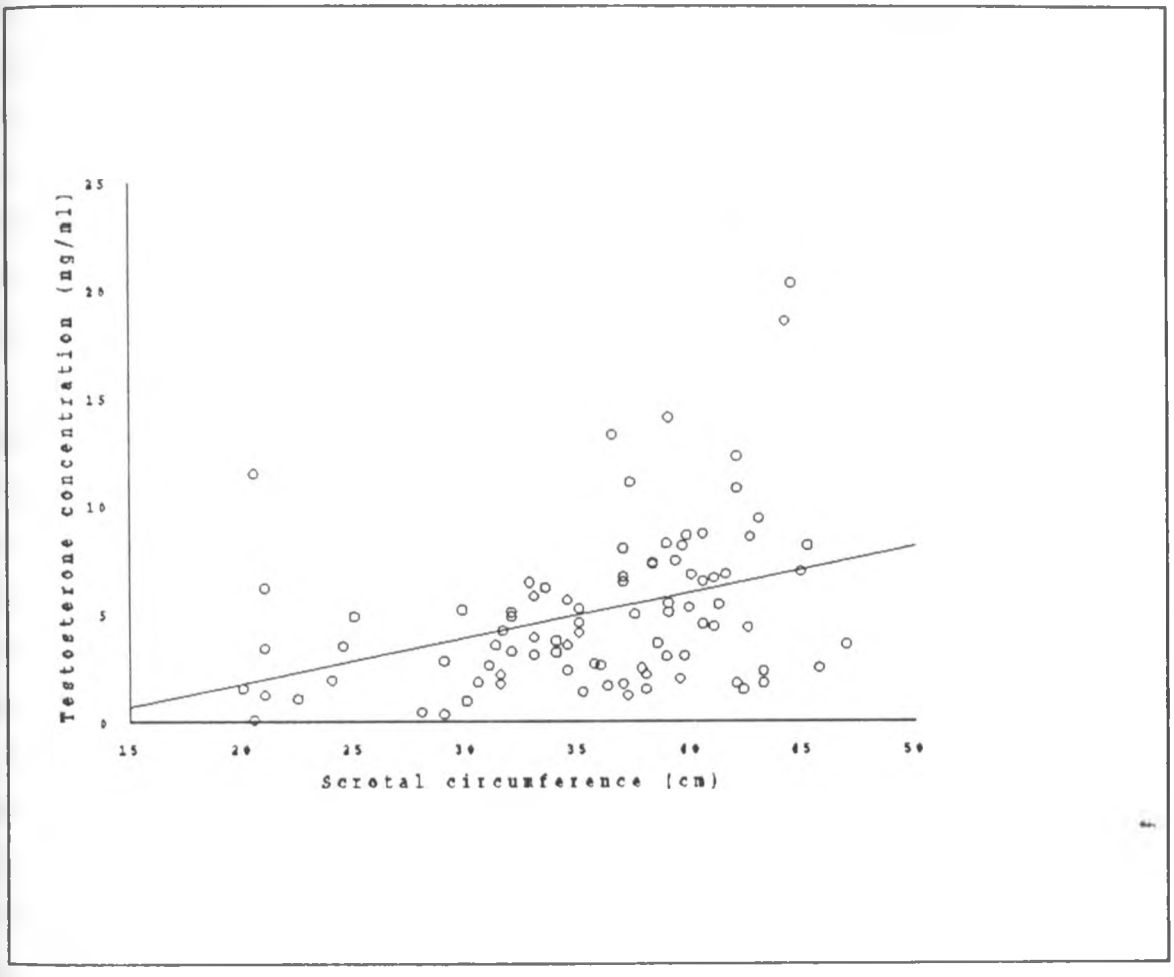


**Figure 10.** Scatter plot for the relationship between testosterone concentration and scrotal circumference.



# CHAPTER FIVE

## 5.0 DISCUSSION AND CONCLUSIONS.

The male reproductive system is comprised of testes, scrotum, a network of ducts, accessory sex glands and the penis (Figure 1) as well as blood and nerve supply (Johnson *et al.*, 1970; Ashdown, 1987). Investigators have evaluated the intricate functional inter-relationships among these various primary and secondary male sexual organs, their secretions and other extraneous factors. However, most of these studies have been carried out in the temperate regions with only scanty information about such work in the tropics (Hahn *et al.*, 1969; Kumi-Diaka *et al.*, 1981; Chrichton *et al.*, 1985; Tegegene *et al.*, 1992). Furthermore, these research activities have focused only at one or two-factor levels.

This study examined the multi-relationships between ages, body weights, scrotal size, sizes of seminal vesicles, length of the cauda epididymis, testosterone levels, reaction time and semen quantity and quality of bulls at the C.A.I.S at Kabete in Kenya. In this cross sectional study of a convenient sample of bulls, a high significant and positive correlation coefficient ($r = 0.77$) between age and body weight was obtained. This was not an unexpected finding since it is well established that as bulls age, body weights increase (Coulter and Foote, 1977). The mean age for Ayrshires was lowest and the highest was observed in Guernseys. This observation was probably due to the high number of bulls less than eight years old in the Ayrshire breed ($n = 8$) and the lowest ($n = 2$) in Guernsey breed. Examination of the correlation matrix (Table 11) showed that the magnitudes and significance of the coefficients between either age or body weight and other variables were quite similar. This is expected since age and body weight were highly correlated. As a result of this, age and body weight will be used inter-changeably in the subsequent discussion. This is because most of the published reports which will be cited to compare the results obtained in this study have used either one or the other.

The scrotal circumferences observed in this study ranged from 20 to 46.9 cm. This range compared well with those circumferences reported for Holstein-Friesian bulls (Hahn *et al.*, 1969; Coulter and Foote, 1977) and for Simmental, Aberdeen Angus; Charolais, Hereford, Short-horn and Limousin bulls (Coulter *et al.*, 1987). The wide range of scrotal circumferences observed in this study was probably due to the wide range of the ages of the bulls (0.5 to 11.4 years). Scrotal circumferences were highly correlated with age ($r = 0.7$) and body weight ($r = 0.83$). This agrees with the observations of Coulter and Foote (1977) and Tegegne *et al.* (1992) in bulls and Braun *et al.*, 1980; Ott and Memon, 1980 in rams and bucks but is higher than those reported by Kupferschmied *et al.* (1974) and Carter *et al.* (1980) of 0.28 and 0.67, respectively in bulls. This could be due to the fact that the bulls used in this study comprised of both young and adults. The results obtained by Carter *et al.* (1980) are based on bulls that were 400 days old hence, probably, the lower correlations. This implies that as bulls gain weight, the SC increases. The above argument is further supported by the findings in Figure 8 which shows a very strong linear relationship between SC and body weight. Coulter and Foote (1977) reported a significant partial correlation of 0.58 ($p < 0.01$) after holding age constant.

A curvilinear relationship between age and SC (Figure 6) was observed at early ages of the bulls. The rapid increase in SC between 0.5 to 3 years is probably due to production of high levels of androgens at these ages (Lindner, 1969). From 5 years, the SC slowly increased up to about 8 years of age after which it stabilised. Again this may be due to stabilisation of testosterone levels and a slow down in the process of growth in adult animals. The reduction in SC at old age may be due to fibrosis and atrophy of the testes (Iljinskaja, 1966). Similar observations between SC and age have been reported in goats (Bongso *et al.*, 1982).

The strong correlation between age and SC up to about 5 years of age and a poor correlation thereafter (Figure 6), agrees with the observations of Hahn *et al.* (1969) who reported correlations between age and SC of 0.85 before 6 years of age and 0.12 after that age. The good correlation observed between SC and

body weight (Figure 8) was consistent with earlier reports in Holstein bulls (Amann, 1970; Coulter *et al.*, 1975; Coulter and Foote, 1977). Overall 69 % (Adjusted $R^2$ = 0.6849) of the variation in SC was accounted for by body weight. Age alone could only explain 47 % (Adjusted $R^2$ = 0.4695) and breed only 13 % (Adjusted $R^2$ = 0.1299) of the among-breed variation in the SC's. The unexplained variability may be accounted for by other factors other than the body weight and breed, for instance levels of energy intake and other feed supplements (Van Demark and Mauger, 1964; Wilsey *et al.*, 1971; Morrow *et al.*, 1981).

Using best subset regression models (Table 12 ), weight alone accounted for 69 % of the variation in SC. For models with two variables, the model containing breed and body weight was the best, based on the value of Mallows Cp and the adjusted $R^2$. These findings are similar to those of Coulter and Foote (1977) who reported an overall 64 % of the variation in SC which was accounted for by body weight.

A significant and positive correlation of 0.54 was obtained between SC and seminal vesicle length (Table 11), indicating that large seminal vesicles are associated with larger scrotal circumferences. These results are in agreement with those of Berry *et al.* (1983) who reported a significant positive correlation between SC and seminal vesicle length in yearling beef bulls. The SC has an indirect effect on the size of the seminal vesicles via the stimulatory effects of testosterone on the growth of accessory sex glands (Cox, 1984).

The positive correlation between SC and testosterone concentration (r= 0.38) is comparable to those reported by Chenoweth *et al.* (1977), Lunstra *et al.* (1978) and Pruitt and Corah (1986). These results, however, differ from those of Sitarz *et al.* (1977) who reported no co-relationship between the two variables in young Angus bulls. Scrotal circumference should have an influence on levels of testosterone since the larger the SC the greater would be the spermatogenic epithelium which is necessary for formation of spermatozoa and the greater the number of Leydig cells which produce testosterone.

The correlation coefficients observed in this study between SC and spermatozoa motility, primary abnormalities and secondary abnormalities were

negative and not significant. These results are in agreement with those of Nwakalor and Ezinma (1989) who reported non significant and negative correlations between motility, primary abnormalities and secondary abnormalities. This suggests that the size of the testis plays no role in determining the motility, and spermatozoa morphology.

Testosterone levels reported in this study are within the ranges reported by Katongole *et al.* (1971) and Sanwal *et al.* (1974). However, the correlation coefficients between testosterone and age ($r = 0.49$) as well as body weight ($r = 0.47$) are lower than those reported by Secchiari *et al.* (1976). These researchers obtained correlation coefficients of 0.81 and 0.81 respectively. They therefore concluded that age and body weight have similar effects on testosterone concentration.

Rawlings *et al.* (1972) found large variations in androgen secretion among bulls within the same age group. They also reported that testosterone concentration increases erratically from birth to 11 months of age and then drops abruptly at 12 months. This pattern was not observed in this study probably because the majority of the bulls used were above 12 months of age. However, the increase in testosterone concentration with age observed in this study confirms earlier reports (Wildeus *et al.*, 1984; Tegegne *et al.*, 1992). This increase with age is due to increased stimulation of the leydig cells by gonadotropins from the pituitary gland (Cox, 1984)

The mean reaction times for the various breeds found in this study are lower than those reported for other breeds. Fraser (1960) in his work with dairy bulls reported an average reaction time of 12.5 min with 50 % of the bulls serving within 2 min. Similarly in this study more than 90 % of the bulls served the AV within 2 min. The breed-specific mean reaction times observed in this study were low probably due to the long term training of the bulls. Bhatia (1960) reported that in Jersey breed, the reaction time ranges between 30 and 60 sec. This compares favourably to 67 sec observed in the same breed in this study. However, it was lower than those reported for other breeds for example, Hariana (8.63 min), Sindhi (5.5 min), Gir (208.48 sec), by Bhatia (1960).

Abeyratne (1976) measured the reaction times of Jersey, Friesian, Ayrshire, Short-horn and Red Sindhi A.I. sires in Sri Lanka and found them to be 75.2, 162.8, 144.2, 158.9 and 555.5 seconds respectively. These values are higher than the ones observed in similar breeds in this study. Bhrttacharya and Prabhu (1955) reported the average reaction time for Sahiwals to be 267.83 sec. Sinha (1964) reported average reaction time in Tharpakar, Hariana and Taylor as 10.08, 4.71, and 11.31 min. respectively. These values are comparatively higher than the ones observed in this study.

Season of the year affects reaction time in bulls. Nath *et al.* (1980) studied the reaction time of Holsteins in India and observed an average of 125.1 seconds with the longest (166.5 seconds) reaction time occurring in Summer and the shortest (89.2 seconds) in Winter. In contrast, Ali et al. (1981) reported no effect of season on mounting behaviour in bulls but there were significant differences among bulls with respect to reaction time. They obtained an average of 114.6 seconds for the first ejaculate and 81 seconds for the second ejaculate. Sharma *et al.* (1982) found that the hot season in India led to increases in reaction times of Jersey bulls but other traits like semen volume, concentration and motility were not affected. This study did not examine the seasonal effects on reaction time since it was cross-sectionally designed.

No differences were observed in mean reaction times between breeds in the current study. In contrast to this, breed differences in reaction times have been reported by Galina and Arthur (1991). Chenoweth and Osborne (1975) in Australia and Chrichton and Leishman (1985) in South Africa compared *Bos taurus* and *Bos indicus* breeds for their serving capacity and found that whereas *Bos taurus* breeds accomplished 1.29-2.96 services, Zebu bulls only averaged 0-1.07 services in the same period of time. Marked within-breed variations in reaction times were observed in this study which could be attributed to the genetic make up in individual bulls (Donham *et al.*, 1931).

There was no statistical significance in the differences in the mean reaction times between breeds in this study, however, Friesians and Ayrshires had relatively shorter reaction times than Guernseys and Jerseys. This confirms

earlier reports by Smith, (1951) and Bonadonna (1956) who reported that Friesians under A.I. conditions react more quickly to stimulation than other breeds probably due to genetic reasons.

The correlations between reaction time and all the other parameters were low and not significant (Table 11). Since testosterone is responsible for libido (Chenoweth, 1981; Cox, 1987) one would have expected this to have a high correlation with reaction time. However, a low and non significant correlation ($r = 0.03$, $p = 0.86$) was observed in this study. Reaction times were different even in bulls with the same levels of testosterone. These differences between bulls may be due to differences in somatic responsiveness to the threshold levels of testosterone as discussed below.

Blockey and Galloway (1978) have reported that differences in serving capacity between bulls are not due to differences in plasma testosterone levels but to their somatic responsiveness to threshold levels of that testosterone. In a study involving 113 yearling beef bulls, single LH and testosterone values showed low positive or negative correlations with assessments of sex drive (Chenoweth *et al.*, 1979). Borg *et al.* (1991) carried out experiments to correlate mating behaviour in bulls with endocrine secretion in the presence of cows in estrus, flehmen responses, elapsed time to mount and elapsed time to service were negatively correlated with testosterone concentration ($r = -0.50$ to $-0.79$) whereas high positive correlations were observed between testosterone concentration, penis extension and mounting ($r = 0.69$ to $0.81$, $p < 0.01$). This suggests that although testosterone concentration may not affect reaction time to service, some behavioral aspects of the copulatory exercise are affected. Lunstra *et al.* (1989) identified the flehmen response (curling of the upper lip) in bulls as the only behaviour observed during mating activity that was correlated with testosterone secretion. Previous evidence had shown that a threshold level of testosterone was required to activate male reproductive behaviour (Blockey and Galloway, 1978; D'Occhio and Brooks, 1982). Prediction of sexual performance of the bull based on testosterone profiles concurrent with female exposure has not been successful (Price *et al.*, 1986). Similarly studies

done in rams have shown that testosterone levels in peripheral blood do not reflect their effectiveness in inducing early onset of estrus in ewes (Tervit and peterson, 1978).

In this study, reaction time was used as a proxy measure for libido and no relationship was observed between reaction time and SC ($r = -0.01$) as well as testosterone concentration ($r = 0.03$). Post *et al.* (1987) and Byerley *et al.* (1990) found no differences in age, height, and SC between high or low libido bulls. Moreover, Boyd *et al.* (1988) reported that bulls with low testosterone concentrations also exhibited high libido. This indicates that low testosterone concentrations do not conclusively reflect low libido in all cases.

Berry *et al.* (1983) reported breed differences with respect to the length of seminal vesicles in beef (Hereford and Angus) bulls. In this study, there were similar tendencies though not significant. Guernseys had the longest seminal vesicles followed by Friesians, Ayrshires and Jerseys. This pattern follows that of the body weights which indicates that seminal vesicle size depends on the weight of the bull. Furthermore, the correlation coefficient between seminal vesicle length and body weight of 0.64 is an indication that the two variables are highly correlated.

Among the variables investigated in this study in relation to ejaculate volume, the highest correlations were found between the volume and seminal vesicle diameter, seminal vesicle length and body weight in that order. This in physiological terms means that the size of the seminal vesicles plays a big role in determining the ejaculate volume. Guernseys and Friesians with slightly longer seminal vesicles and *cauda epididymal* lengths were associated with slightly higher ejaculate volumes than Ayrshires and Jerseys with shorter seminal vesicles. Testicles with longer *cauda epididymis* are capable of storing more spermatozoa than those with shorter ones. These results confirm earlier reports by Roberts (1986) that the greatest ejaculate volume is contributed by the seminal vesicles.

The ejaculate volumes observed in this study are lower than those reported earlier by Njogu (1989), probably because only first ejaculates were recorded.

The marked variations between and within breeds suggest that differences in ejaculate volumes depend on other variable factors such as SC and size of the seminal vesicles.

Although Jersey bulls were restrained for a longer time than bulls from the other breeds before semen collection, this did not yield larger ejaculate volumes. These results were similar to those reported earlier by Njogu (1989) who observed smaller ejaculate volumes in Jersey bulls despite prolonged restraining time, but they contradict the reports of Hafs *et al.* (1962) who concluded that large ejaculate volumes were associated with prolonged restraining time between breeds.

The positive correlation coefficient between age and ejaculate volume reported in this study was based on results from all the four breeds. Ejaculate volume has been reported to increase with age and may decrease at old age due to atrophy or fibrosis of the testicles (Amann *et al.*, 1974; Everett *et al.*, 1978; Njogu, 1989).

The most common of the head abnormalities were free heads, however the obtained proportion was within the recommended range for normal bulls (Rao, 1971). The separation of the head and tail occurs within the *caput epididymis* (Hancock, 1955) and this condition has been reported to be hereditary in Guernsey bulls (Jones, 1962). Amann and Almquist (1962) studied the morphology of epididymal spermatozoa in bulls and suggested that tailless spermatozoa may be preceded by spermatozoa with bent and broken tails.

The mean percent proportions of head abnormalities in all the breeds (0.24, 0.22, 0.35 and 0.27) for Ayrshires, Friesians, Guernseys and Jerseys respectively were lower than that reported by Satyanarayana *et al.* (1982) in Brown Swiss x Ongole cross-breds of 8.69. These values were within the range for normal fertile bulls (Rao, 1971).

The percent proportions of midpiece abnormalities for Guernseys and Jerseys were lower than those reported by Rao and Kottaya (1974) and in cross-bred bulls (Rao, 1948) but comparable to those reported by Satyanarayana et al. (1982) in cross-bred and pure-bred bulls. These differences may be due to variation in the

genotype of bulls and environmental factors like season of the year when the studies were done. The values for the proximal cytoplasmic droplets are also in the range (mean 0.6 %, range 0-2.4%) given for normal bulls (Lagerlöf, 1934).

The tail abnormalities of the spermatozoa were mostly coiled tails and were frequent in semen samples from Jersey bulls. This confirms earlier reports by Saxena and Tripathi (1983) who reported that coiled tails were the most common abnormality in ejaculates of Jersey bulls. Bending or coiling of spermatozoa tails may be a response to adverse conditions such as osmotic stress, inhibition of metabolic activity or cationic imbalances (Jones, 1975).

Saxena and Tripathi (1981) found seasonal differences in sperm morphology in Holsteins, as evidenced by a higher percentage of abnormal heads and middle pieces in Summer months. Kumi-Diaka *et al.* (1981) studied semen production during a period of twelve months in exotic and indigenous breeds in Nigeria. They found no significant seasonal variations in sperm concentration and the percentage of live spermatozoa or sperm abnormalities in indigenous breeds. The percentage of primary and secondary abnormalities observed were not correlated with anatomical factors such as SC, testicular length, epididymal length and size of the seminal vesicles. The present study did not consider season, but thermal stress has been reported to be a predominant cause of tailless spermatozoa arising from errors which may occur in the region of the implantation socket before spermiation (Vogler *et al.*, 1993). Spermatozoal abnormalities were very low in all the ejaculates from the bulls probably because of long-term selection and hence the morphology was not affected by the sizes of the various anatomical organs studied. Since the bulls used in this study connstitute the best bulls in term of fertility, it is not suprising that their semen quality was within normal limits. This further supports the observation that there were no significant differences in most of the semen traits examined.

Breed differences in spermatozoa concentrations for various dairy breeds have been reported. Erb *et al.* (1955) reported values of $1.497 \times 10^9$ for Jerseys, $1.38 \times 10^9$ for Guernseys and $1.208 \times 10^9$ for Friesians per ml. In this study, the spermatozoa concentration in ejaculates from Jersey bulls was

significantly higher than for the other breeds. These results are similar to those of Njogu (1989). Similar to results of an earlier study (Njogu, 1989), no significant differences ($p > 0.05$) were observed between the mean spermatozoa concentrations of ejaculates from Ayrshire and Guernsey bulls.

The results for the spermatozoa concentrations for all the breeds in this study were lower than those reported by Njogu (1989). This could be due to the fact that this study was carried out at the time when nutritional levels were low. Low feed intake has been associated with low spermatozoa concentration through reduced testosterone and gonadotropic hormone production (Roberts, 1986). Spermatozoa concentration was not related to either testicular, epididymal or seminal vesicle measurements.

The means of the spermatozoa motility observed in this study were not significantly different among breeds. However, Ayrshires which had the lowest motility had the highest percentage of coiled tails suggesting that this could have contributed to the lowest progressive motility in this breed. Contrary to reports by Gipson *et al.* (1985), spermatozoa motility in this study was not associated with scrotal circumference as shown by the very low and non significant ($r = - 0.04$) correlation coefficient. This contradiction may be due to the fact that the data for SC in this study were not categorised into classes within the breeds, while Gipson *et al.* (1985) categorised the SC into various classes. A negative correlation was found between spermatozoa motility and age, which supports earlier reports of no significant differences between young and adult bulls with respect to spermatozoa motility (Everett and Bean, 1982). Similar results of no direct relationship between spermatozoa motility and SC have been reported in rams (Mickelsen *et al.*, 1981).

The correlation coefficient between the live/dead ratio and progressive motility was positive and significant but low ($r = 0.33$). Progressive motility is also influenced by other factors for instance spermatozoa morphology. Although no differences were found between means for live/dead ratio and percentage of live spermatozoa among the breeds, the results show that the Jersey

breed with the highest live/dead ratio had also the highest percentage of live spermatozoa.

## Conclusions.

Based on the results from this study, the following conclusions were made:

1. No differences existed between the mean scrotal circumferences of Ayrshire, Friesian, Guernsey and Jersey bulls.
2. Age had more influence on the rate of increase in scrotal circumference than body weight.
3. Ejaculate volume was highly correlated with scrotal circumference, testicular length, epididymal length and size of the seminal vesicles hence the above measurements could be useful in selecting bulls to be used for breeding.
4. Spermatozoa concentration, motility and morphology were not affected by the size of the testicles, and seminal vesicles.
5. There were no breed differences in mean testosterone concentrations and mean reaction times of Ayrshire, Friesian, Guernsey and Jersey bulls.
6. Reaction time was not correlated with the level of testosterone in bulls and hence would be a poor measure for libido.
7. Overall, semen quantity was affected by the sizes of the anatomical organs studied whereas quality was not.
8. Most estimates of semen quality were within limits reported for normal bulls.

## REFERENCES

Aalseth, E.P. and Sacke, R.G. (1986). Vital staining and acrosomal evaluation of bovine sperm. *Gamete. Res.* **15**: 73-81.

Abeyratne, A.S. (1976). Libido of A.I. sires at Central Artificial Insemination Station, Kundsale. *Ceylon Vet. J.* **24**: 39-41.

Al-Hakim, M.K., Graham, E.F. and Schmel, M.L. (1970). Free amino acids, amino compounds in bovine seminal plasma. *J. Dairy Sci.* **53**: 84-88.

Ali, S.B.A., Al-Hakim, M.K., Singh, B.P. (1981). Studies on sexual behaviour of Karadi bulls. *Indian J. Anim. Health.* **20**: 9-13.

Almquist, J.O. (1968). Dairy cattle. In: The Artificial Insemination of Farm Animals. Perry, E.J. (Ed). Fourth edition. Rutgers University Press, New Jersey. Chapter 6, pp 94-141.

Almquist, J.O. and Amann, R.P. (1961). Reproductive capacity of dairy bulls. II Gonadal and extragonadal sperm reserves as determined by direct counts and depletion trials; dimensions and weight of genitalia. *J. Dairy. Sci.* **44**: 1668-1678.

Almquist, J.O. and Cunningham, D. (1967). Reproductive capacity of beef bulls. Postpubertal changes in semen production at different ejaculation frequencies. *J. Anim. Sci.* **29**: 30-33.

Almquist, J.O., Branas, R.J. and Barber, K.A. (1976). Postpubertal changes in semen production of Charolais bulls ejaculated at high frequency and the relationship between testicular measurements and sperm output. *J. Anim. Sci.* **42**: 670-676.

Amann, R.P. (1970). Sperm production rates. In: The Testis. Johnson, A.D., Gomes, W.R. and Van Demark, N.L. (Eds). Vol. 1 Academic Press, New York, pp 433-440.

Amann, R.P. and Almquist, J.O. (1962). Reproductive capacity of dairy bulls. VII. Morphology of epididymal sperm. *J. Dairy Sci.* **45**: 1516-1526.

Amann, R.P. and Almquist, J.O. (1976). Bull management to maximise sperm output. *Proc. 6th N.A.A.B. Tech. Conf. on Anim. Reprod. and A.I.* Milwaukee, Wisconsin.

Amann, R.P., Kavanaugah, J.F., Griel, L.C. and Vogimayr, J.K. (1974). Sperm production of Holstein bulls determined by testicular spermatid reserves, after cannulation of *rete testis or vas deferens* and by daily ejaculation. *J. Dairy Sci.* **57**: 93-99.

Anderson, J. (1941). Further investigation of the semen quality of the bull. *Vet. Rec.* **53**: 197-205.

Anderson, J. (1942). The hydrogen ion concentration of the semen of the bull. *J. Agric. Sci.* **32**: 298-307.

Anderson, J. (1948). Improvement of cattle in East Africa by artificial insemination. *Proc. 1st Int. Congr. Physiol. Path. Anim. Reprod. and Artif. Insem.* Milano, Italy. pp 14-15.

Arthur, G.H., Noakes, D.E. and Pearson, H. (1986). Normal sexual apparatus. In: Veterinary Reproduction and Obstetrics, Balliere Tindall, London. pp 509-524.

Ashdown, R.R. (1987). Anatomy of male reproduction. In: Reproduction in Farm Animals. Hafez, E.S.E. (Ed). Fifth edition. Lea and Febiger, Philadelphia. pp 17-34.

Baggett, B., Engel, L., Savard, K. and Durfman, R.I. (1956). Biological aromatisation of steroids. *J. Biol. Chem.* **234**: 268-272.

Bane, A. (1954). Studies on monozygous cattle twins. XV. Sexual functions in bulls in relation to heredity, rearing intensity and climatic conditions. *Acta. agric. Scand.* **4**: 95-105.

Bansal, M.R. and Davies, A.G. (1986). Effect of testosterone oenanthate on spermatogenesis and serum testosterone concentrations in adult mice. *J. Reprod. Fert.* **78**: 219-224.

Barber, K.A. and Almquist, J.O. (1978). Growth and feed efficiency and their relationship to pubertal traits of Charolais bulls. *J. Anim. Sci.* **40**: 288-301.

Barth, A.D. and Oko, R.J. (1989). Abnormal morphology of bovine spermatozoa. Iowa University Press, Ames, IA, pp 130-271.

Bartoov, B., Kalay, D. and Mayevsky, A. (1981). Sperm motility analyzer (SMA). A practical tool of motility and cell concentration determination in Artificial Insemination centres. *Theriogenology*, **15**: 173-182.

Beach, F.A. and Fowler, H. (1959). Individual differences in the response of male rats to androgen. *J. Comp. Physiol. Psychol.* **52**: 50-52.

Bedford, J.M. and Hoskins, D.D. (1990). The mammalian spermatozoon: morphology, biochemistry and physiology. In: Marshalls' Physiology of Reproduction. Lamming, G.E. (Ed). Fourth Edition, Vol. 12. Reproduction in the male. Church Hill Livingstone, Edinburgh, London. pp 379-568.

Berndtson, W.E., Desjardins, C. and Ewing, L.L. (1974). Inhibition and maintenance of spermatogenesis in rats implanted with polydimethyl siloxane capsules containing various androgens. *J. Endocr.* **62**: 125-135.

Berndtson, W.E., Igboeli, G. and Pickett, B.W. (1987). Relationship of absolute numbers of sertoli cells to testicular size and spermatogenesis in young beef bulls. *J. Anim. Sci.* **64**: 241-246.

Berry, J.G., Brinks, J.S. and Russel, W.C. (1983). Relationship of seminal vesicle size and measures of libido in yearling beef bulls. *Theriogenology*, **19**: 279-284.

Beseth, L. (1962). Biological testing of the toxicity of rubber used in artificial vaginas with boar semen. *Nord. Vet. Med.* **14**: 689-701.

Bhargva, P.M., Bishop, M.W.H. and Work, T.S. (1959). The chemical composition of bull semen with special reference to nucleic acids, free nucleotides and free amino acids. *Biochem. J.* **73**: 242.

Bhatia, H.M. (1960). Animal breeding. Coordination of animal husbandry research in India. Indian Council of Agricultural Research, New Delhi.

Bhosrekar, M. and Nagpaul, P.K. (1972). Studies on the effect of exercise on the reaction time and semen production. *Indian J. Dairy Sci.* **25**: 194-195.

Bhrttacharya, P. and Prabhu, S.S. (1955). Field application of artificial insemination in cattle. *Indian. J. Vet. Sci.* **25**: 277.

Bindon, B.M., Hewettson, R.W. and Post, T.B. (1976). Plasma LH and testosterone in Zebu cross bred bulls after exposure to an oestrus cow and injection of GnRH. *Theriogenology*, **5**: 45-53.

Bishop, M.W.H., Campbell, R.C., Hancock, J.L. and Walton, H. (1954). Semen characteristics and fertility in the bull. *J. Agric. Sci.* **44**: 227-248.

Blockey, M.A. deB (1976). Serving capacity as a measure of serving efficiency of bulls during pasture mating. *Theriogenology*, **6**: 393-401.

Blockey, M.A. deB and Galloway, D.B. (1978). Hormonal control of serving capacity in bulls. *Theriogenology*, **9**: 143-151.

Blom, E. (1966). A new sterilising and hereditary defect (the "Dag-defect") located in the bull sperm tail. *Nature* (London) **209**: 739-740.

Blom, E. and Birch-Andersen, A. (1968). The ultrastructure of the pseudo-droplet defect in the bull sperm. *6th. Internat. Cong. Anim. Reprod. Artif. Insem.* Paris 1: 117-119.

Bloom, D. and Fawcett, D.W. (1975). A Textbook of Histology. W.B. Saunders, Philadelphia.

Bonadonna, T. (1956). On some biological factors that may affect the quality of semen. *Proc. 3rd Int. Cong. Anim. Reprod.* (Cambridge) plenary paper pp 105-112.

Bone, J.F. (1982). The reproductive system. In: Animal Anatomy and Physiology. Second edition. Reston Publishing Company, Inc. Reston Varginia, pp 364-377.

Bongso, T.A., Jainudeen, M.R. and Siti Zahrah, A. (1982). Relationship of scrotal circumference to age, body weight and onset of spermatogenesis in goats. *Theriogenology*, **18**: 513-524.

Borg, K.E., Esbaneshade, K.L. and Johnson, B.H. (1991). Cortisol, growth hormone and testosterone concentrations during mating behaviour in the bull and boar. *J. Anim. Sci.* **69**: 3230-3240.

Boucher, J.H., Foote, R.H. and Kirk, R.W. (1958). The evaluation of semen quality in the dog and the effects of frequency of ejaculation upon semen quality, libido and depletion of sperm reserves. *Cornell Vet.* **48**: 67-86.

Boyd, G.W., Lunstra, D.D., Corah, L.R. and Schanbacher, B.D. (1988). Serum concentrations of luteinizing hormone, testosterone and thyroid hormones in low and high serving capacity crossbred yearling beef bulls. *Proc. Western Sect. Amer. Society of Anim. Sci.* **39**: 297-301.

Boyd, L.J. and Van Demark, N.L. (1957). Spermatogenic capacity of the male bovine. 1. A measurement technique. *J. Dairy Sci.* **40**: 689.

Braun, W.F., Thompson, J.M. and Ross, C.V. (1980). Ram scrotal circumference measurements. *Theriogenology*, **13**: 221-229.

Byerley, D.J., Bertrand, J.K., Berardinelli, J.G. and Kiser, T.E. (1990). Testosterone and luteinizing hormone response to GnRH in yearling bulls of different libido. *Theriogenology*, **34**: 1041-1049.

Campbell, R.C., Dott, H.M. and Glover, T.D. (1956). Nigrosin-eosin as a stain for differentiating live and dead spermatozoa. *J. Agric. Sci.* Cambridge, **48**: 1-8.

Campbell, R.C., Hancock, J.L. and Shaw, I.G. (1960). Cytological characteristics and fertilising capacity of bull spermatozoa. *J. Agric. Sci.* **55**: 91-99.

Carrol, E.J., Ball, L. and James, A.S. (1963). Breeding soundness in bulls. A summary of 10,940 examinations. *J. Am. Vet. Med. Assoc.* **142**: 1105-1111.

Carter, A.P., Wood, P.D. and Wright, P.A. (1980). Association between scrotal circumference, live weight and sperm output in cattle. *J. Reprod. Fert.* **59**: 477-481.

Chard, T. (1987). An Introduction to Radioimmunoassay and related techniques. Work, T.S. and Work, E. (Eds). Nort-Holland publishing Co. Amsterdam, Netherlands.

Chenoweth, P.J. (1981). Libido and mating behaviour in bulls, boars and rams. A review. *Theriogenology* **16**: 155-178.

Chenoweth, P.J., Abbit, B., McInerney, M.J. and Brinks, J.S. (1977). Libido, serving capacity and breeding soundness in bulls. 28th Annu. Beef cattle Improve. Rep. Sale Data, Colorado State University Exp. Farm San Juan Res Centre. 966 pp 13.

Chenoweth, P.J., Brinks, J.S. and Nett, J.M. (1979). A comparison of three methods of assessing sex-drive in yearling beef bulls and relationships with testosterone and LH levels. *Theriogenology*, **12**: 223-233.

Chenoweth, P.J. and Osborne, H.G. (1975). Breed differences in the reproductive function of young beef bulls in Central Queensland. *Aust. Vet. J.* **51**: 405-408.

Chrichton, J.S. and Leishman, A.W. (1985). Libido evaluation of virgin beef bulls. *S. Afr. J. Anim. Sci.* **15**: 2-24.

Cole, H.H. and Cupps, P.J. (1977). Reproduction in Domestic Animals. Third Edition. New York. Academic Press Publishers.

Coulter, G.H. (1978). Effect of plane of nutrition and breed on total testicular lipids in young beef bulls. *Proc. 70th Ann. Meet. Amer. Soc. Anim. Sci. (Abstr).* pp 353.

Coulter, G.H. and Foote, R.H. (1976). Relationship of testicular weight to age and scrotal circumference of Holstein bulls. *J. Dairy Sci.* **59**: 730-732.

Coulter, G.H. and Foote, R.H. (1977). Relationship of body weight to testicular size and consistency in growing Holstein bulls. *J. Anim. Sci.* **44**: 1076-1079.

Coulter, G.H. and Foote, R.H. (1979). Bovine testicular measurements as indicators of reproductive performance traits in cattle. A Review. *Theriogenology*, **11**: 297-311.

Coulter, G.H., Larson, L.L., and Foote, R.H. (1975). Effect of age on testicular growth and consistency in Holstein bulls. *J. Anim. Sci.* **41**: 1383-1389.

Coulter, G.H., Mapletoft, R.J., Kozub, G.C. and Cates, W.F. (1987). Scrotal circumference of two-year-old bulls of several beef breeds. *Theriogenology*, **27**: 485-491.

Cox, J. (1984). Aspects of male endocrinology. *In Practice*, **6**: 137-141.

Crabo, B. (1965). Studies on the composition of epididymal content in bulls and boars. *Acta. Vet. Scand.* **6**: (Suppl. 5) 94.

Cragle, R.G., Salisbury, G.W. and Muntz, J.H. (1958). Distribution of bulk and trace minerals in bull reproductive tract fluids and semen. *J. Dairy Sci.* **41**: 1273-1277.

Cupps, P.T. and Briggs, J.R. (1965). Changes in the epididymis associated with changes in the spermatozoa. *J. Dairy. Sci.* **48**: 1241-1244.

Davies, D.V., Mann, T. and Rowson, L.E.A. (1957). Effect of nutrition on the onset of male sex hormone activity and sperm formation in monozygous bull calves. *Proc. Roy. Soc.* (London) **147**: 332.

D'Occhio, M.J. and Brooks, D.E. (1982). Threshold of plasma testosterone required for normal mating activity in male sheep. *Horm. Behav.* **16**: 383 (*Abstr*).

Donham, C.R., Simms, B.T. and Shaw, J.N. (1931). Fertility studies in bulls. *J. Am. Vet. Med. Assoc.* **78**: 665-667.

Dott, H.M. (1975). The estimation of the proportion of motile bull spermatozoa in various diluents and a comparison with the proportion eosinophilic. *J. Reprod. Fert.* **45**: 47-55.

Dott, H.M. and Foster, G.C.A. (1975). Preservation of differential staining of spermatozoa by formal citrate. *J. Reprod. Fert.* **45**: 57-60.

Dott, H.M. and Foster, G.C.A. (1979). The estimation of sperm motility in semen on a membrane slide by measuring the area change frequency with an image analyzer computer. *J. Reprod. Fert.* **55**: 161-166.

Duane, L.G. and Hafez, E.S.E. (1987). Spermatozoa and seminal plasma. In Reproduction in Farm Animals. Hafez, E.S.E. (Ed). Fourth edition, Lea and Febiger, Philadelphia, pp 189-209.

Dufau, M.L., Winters, C.A., Hatlori, M., Aquilano, D., Baranao, J.L.S., Nozu, K., Baukau, A. and Catt, K.J. (1984). Hormonal regulation of androgen production by the Leydig cells. *J. Steroid Biochem.* **20**: 161-173.

Dym, M. and Fawcett, D.W. (1970). The blood testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol. Reprod.* **3**: 308.

Dziuk, P.J., Graham, E.F. and Petersen, W.E. (1954). The technique of electroejaculation and its use in dairy bulls. *J. Dairy Sci.* **37**: 1035.

Eik-Nes, K.B. (1975). Biosynthesis and secretion of testicular steroids. In: Handbook of Physiology, Volume 5, Male Reproductive System. Hamilton, D.W. and Greep, R.O. (Eds). Am. Physiol. Soc. Washington, D.C. pp 95-115.

Elmore, R.G., Bierschwal, C.J., Martin, C.E. and Youngquist, R.S. (1975). A summary of 1127 breeding soundness examinations in beef bulls. *Theriogenology*, **3**: 209-218.

Elmore, R.G., Bierschwal, C.J. and Youngquist, R.S. (1976). Scrotal circumference measurements in 764 beef bulls. *Theriogenology*, **6**: 485-494.

Erb, R.E., Flerchinger, F.H., Ehlers, M.H. and Gassner, F.X. (1955). Metabolism of bull semen. II. Fructolysis relationships with sperm concentration and fertility. *J. Dairy Sci.* **39**: 326-338.

Everett, R.W., Bean, B. and Foote, R.H. (1978). Sources of variation of semen output. *J. Dairy Sci.* **61**: 90 (Abstr).

Everett, R.W. and Bean, B. (1982). Environmental influence on semen output. *J. Dairy Sci.* **65**: 1303-1310.

Ewing, L.L., Stratton, L.G. and Desjardins, C. (1973). Effects of polydimethyl siloxane implants upon semen production, libido and accessory sex organ function in rabbits. *J. Reprod. Fert.* **35**: 245-253.

FAO (1977). Animal production and health paper, Animal breeding: selected articles from the *World Animal Review* pp 1-6.

Faulkner, L.C., Hopwood, M.L., Masken, J.F., Kingman, H.E. and Stoddard, H.L. (1967). Scrotal frost bite in bulls. *J. Am. Vet. Med. Assoc.* **151**: 602-605.

Faulkner, L.C. and Pineda, M.H. (1975). Artificial insemination. In: Veterinary Endocrinology and Reproduction. McDonald, M.E. (Ed.) Second edition. Lea and Febiger, Philadelphia, pp 304-341.

Fields, M.J., Burns, W.C. and Warnick, A.C. (1979). Age, season and breed effects on testicular volume and semen traits in young beef bulls. *J. Anim. Sci.* **48**: 1299-1304.

Finn, C.A. (1979). Oxford Review of Reproductive Biology. Vol 1. Clarenden Press, Oxford Publishers, pp 283-290.

Flerchinger, F.H. and Erb, R.E. (1955). Metabolism of bull semen. Inorganic and total phosphorus relationships. *J. Dairy Sci.* **38**: 1028-1036.

Flick, D.L. and Merilan, C.P. (1984). Toxicity evaluation with bovine spermatozoa. *J. Anim. Sci.* **59**: (Suppl. 1) 310 (Abstr).

Flick, D.L. and Merilan, C.P. (1988). Toxicity of artificial vagina liners for bovine spermatozoa. *Theriogenology*, **29**: 1207-1213.

Flipse, R.J. and Almquist, J.O. (1961). Effect of total digestible nutrient intake from birth to four years of age on growth and reproductive development and performance of dairy bulls. *J. Dairy Sci.* **44**: 905-914.

Foote, R.H. (1964). Sperm losses from semen collection to insemination. *Int. Congr. Anim. Reprod. Artif. Insem.* **4**: 416-420.

Foote, R.H. (1972). How to measure sperm cell concentration by turbidity (optical density). *Proc. Fourth Tech. Conf. Anim. Reprod. and A.I.* N.A.A.B.

Foote, R.H. (1978). Factors influencing the quality and quantity of semen harvested from bulls, rams, boars and stallions. *J. Anim. Sci.* **47**: (Suppl. 2) 1-11.

Foote, R.H. and Heath, A. (1963). Effect of sperm losses in semen collection equipment on estimated sperm output by bulls. *J. Dairy Sci* **46**: 242-244.

Foote, R.H., Munkenbeck, N. and Greene, W.A. (1976). Testosterone and libido in Holstein bulls of various ages. *J. Dairy Sci.* **59**: 2011-2013.

Foster, J., Almquist, J.O. and Martig, R.C. (1970). Reproductive capacity in beef bulls. IV. Changes in sexual behaviour and semen characteristics among successive ejaculations. *J. Anim. Sci.* **30**: 245-252.

Foulkes, J.A. and McDonald, B.J. (1979). The relationship between ATP content and motility of bovine spermatozoa. *Theriogenology*, **11**: 313-320.

Fraser, A.F. (1960). The influence of psychological and other factors on reaction time in the bull. *Cornell Vet.* **50**: 126-132.

Frandson, R.D. (1986). Anatomy of the male reproductive system. In: Anatomy and Physiology of Farm animals. Fourth edition. Lea and Febiger, Philadelphia. pp 439-451.

Galina, C.S. and Arthur, G.H. (1991). Review of cattle reproduction in the tropics. Part 6, The male. (*Anim. Breeding Abstr.*) **59**: 404-412.

Ganjam, V.K. and Amann, R.P. (1976). Steroids in fluids and sperm entering and leaving bovine epididymis, epididymal tissue and accessory sex gland secretions. *Endocrinol*, **99**: 1618-1630.

Garbers, D.L., Wakabayashi, T. and Reed, P.W. (1970). Enzyme profile of the cytoplasmic droplet from the bovine epididymal spermatozoa. *Biol. Reprod.* **3**: 327-337.

Garner, D.L. and Hafez, E.S.E. (1987). Spermatozoa and seminal plasma. In: Reproduction in Farm Animals. Hafez, E.S.E. (Ed). 5th Edition. Lea and Febiger, Philadelphia, pp 189-209.

Gipson, T.A., Vogt, W. and Massey, J.W. (1985). Association of scrotal circumference with semen traits in young beef bulls. *Theriogenology*, **24**: 217-225.

Glover, T.D. (1956). The effect of scrotal insulation and the influence of the breeding season upon fructose concentration in the semen of the ram. *J. Endocr.* **13**: 235.

Glover, T.D. (1961). Disintegrated spermatozoa from the epididymis. *Nature* (London) **190**: 185-186.

Glover, F.A. (1968). A new physical method of measuring the motility of bull spermatozoa. *IVth. Int. Cong. Anim. Reprod. and A.I.* VOL II, Paris. pp 1045-107

Gray, J. (1958). The movement of spermatozoa of the bull. *J. Expt. Biol.* **35**: 96-108.

Grunt, J.A. and Young, W.C. (1952). Differential reactivity of individuals and the response of the male guinea pig to testosterone propionate. *Endocrinology*, **51**: 237-248.

Gustafsson, B., Crabo, B. and Rao, A.R. (1972). Two cases of bovine epididymal dysfunction. *Cornell Vet.* **62**: 393-402.

Hackett, A.J. and Macpherson, J.W. (1965). Some staining procedures for spermatozoa. A review. *Canadian Vet. J.* **6**: 55-61.

Hafez, E.S.E. (1959). Reproductive capacity of farm animals in relation to climate and nutrition. *J. Am. Vet. Med. Assoc.* **135**: 606-614.

Hafez, E.S.E. (1960). Analysis of ejaculatory reflexes and sex drive in the bull. *Cornell Vet.* **50**: 384-411.

Hafez, E.S.E. (1987). Artificial insemination. In: Reproduction in Farm Animals. Hafez, E.S.E. (Ed). 5th Edition. Lea and Febiger, Philadelphia. pp 481-506.

Hafez, E.S.E. and Thibault, C.G. (1975). The biology of spermatozoa, transport, survival and fertilising ability. Karger-Bansel, S. Munchen, pp 1-35.

Hafs, H.D. (1978). Contaminants in semen collection and processing. *Proceedings of the 7th Technical conference on Artificial Insemination and Reproduction.* National Association of Animal Breeders, 14-15, April, Madison, W I. pp 13-15.

Hafs, H.D., Kinsley, R.C. and Desjardins, C. (1962). Sperm output of dairy bulls with varying degrees of sexual preparation. *J. Dairy Sci.* **45**: 788-793.

Hahn, J., Foote, R.H. and Cranch, E.T. (1969). Tonometer to measure testicular consistency of bulls to predict semen quality. *J. Anim. Sci.* **29**: 483-489.

Hahn, J., Foote, R.H. and Seidel, G.E. (1969). Testicular growth and related sperm output in dairy bulls. *J. Anim. Sci.* **29**: 41-47.

Hale, E.B. (1966). Visual stimuli and reproductive behaviour in bulls. *J. Anim. Sci.* suppl. **25**: 36-48.

Hale, E.B. and Almquist, J.O. (1960). Relation of sexual behaviour to germ cell output in farm animals. *J. Dairy Sci.* **43**: 788-793.

Hancock, J.L. (1951). A staining technique for the study of temperature shock in semen. *Nature* (London) **167**: 323-324.

Hancock, J.L. (1955). The disintegration of bull spermatozoa. *Vet. Rec.* **67**: 825-826.

Hannson, V., Weddington, S.C., McLean, W.S., Smith, A.A., Nayfeh, S.N., French, S.F. and Ritzen, E.M. (1975). Regulation of seminiferous tubular function by FSH and Androgens. *J. Reprod. Fert.* **44**: 363-375.

Herrick, J.B. (1958.) An aid to collection of semen by electroejaculation, Chloropromazine Hydrochloride. *Vet. Med.* **53**: 473-474.

Hill, H.J., Scott, F.S., Homann, N. and Gassner, F.Y. (1956). Electroejaculation in the bull. *J. Am. Vet. Med. Assoc.* **128**: 375-380.

Holy, L. and Barba, F. (1972). Desarrolls Vallores testimetricos de los Toros de las Razas Brown Swiss, Holstein, en el clima subtropical. *Rota. Cuba. Clienc Vet.* **3**: 31 (Abstr).

Hooker, C.W. (1944). The postnatal history and function of interstitial cells of the testis of the bull. *Am. J. Anat.* **74**: 1-37.

Hoopwood, M.L. and Gassner, F.X. (1962). The free amino acids of bovine semen. *Fert. Steril.* **13**: 290.

Hopkins, S.M. and Evans, L.E. (1989). Artificial insemination. In: Veterinary Endocrinology and Reproduction. McDonald, L.E. and Pineda, M.H. (Eds). Fourth edition. Lea and Febiger, Philadelphia, pp 355-388.

Howard, T.H. and Pace, M.M. (1988). Seminal evaluation and artificial insemination. In: Fertility and Infertility in Veterinary Practice. Laing, J.A., Brinley Morgan, W.J. and Wagner, W.C. (Eds). Fourth edition. Balliere Tindall, London. pp 39-51.

Hulet, C.V., Blackwell, R.L. and Ercanbrack, S.K. (1964). Observations on sexually inhibited rams. *J. Anim. Sci.* **23**: 1095-1097.

Hulet, C.V., Foote, W.C. and Blackwell, R.L. (1964). Effect of natural and electrical ejaculation on predicting fertility in the ram. *J. Anim. Sci.* **23**: 418-424.

Hültnas, C.A. (1959). Studies on variation in mating behaviour of young bulls of the Swedish Red and White breed and on causes of this variation. *Acta. agric. Scand.* suppl. **6**: 1-8.

Igboeli, G. and Rakha, A.M. (1971). Ejaculation and ejaculate characteristics of Angoni (Short-horn Zebu) bulls. *J. Reprod. Fert.* **26**: 183-187.

Iljinskaja, T.P. (1966). Changes in the testes of bulls of different ages. *Veterinariya* **43**: 67, (In *Russian). Anim. Breed. Abstr.* **35**: 429).

Islam, A.B.M.M. and Land, R.B. (1977). Seasonal variation in testes diameter and sperm output of rams of different prolificacy. *Anim Prod.* **25**: 311-317.

Johnson, A.D, Gomes, W.R. and Van Demark, N.L. (1970). The Testis. Volume 1. Development, Anatomy and Physiology. Editors (same as authors). Academic Press Publishers, New York.

Johnson, B.H. and Ewing, L.L. (1971). Follicle stimulating hormone and the regulation of testosterone secretion in the rabbit testis. *Science*, N.Y. **173**: 635-637.

Jones, R.C. (1975). Fertility and Infertility in mammals in relation to sperm structure. In: The Biology of the Male Gamete. Duckett, J.G. and Racey, P.A. Academic Press, London. pp 343-365.

Jones, W.A. (1962). Abnormal morphology of the spermatozoa in Guernsey bulls. *Br. Vet. J.* **118**: 257-261.

Karagiannidis, A. (1976). The distribution of calcium in bovine spermatozoa and seminal plasma in relation to cold shock. *J. Reprod. Fert.* **46**: 83-90.

Karg, H., Gimerez, T., Hartl, M., Hoffman, B., Schallenberger, E. and Schams, D. (1976). Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in peripheral plasma of bulls: levels from birth through puberty and short term variations. *Zentrlb. Vet. Med. A.* **23**: 793-803.

Katongole, C.B., Naftolin, F. and Short, R.V. (1971). Relationship between blood levels of luteinizing hormone and testosterone in bulls, and the effects of sexual stimulation. *J. Endocr.* **50**: 457-466.

Katz, D.F. and Dott, H.M. (1975). Methods of measuring swimming speed of spermatozoa. *J. Reprod. Fert.* **45**: 263-272.

Katz, L.S. and McDonald, T.J. (1992). Sexual behaviour of farm animals. *Theriogenology*, **38**: 239-253.

Kent, P. and Niswender, G.D. (1979). Radioiodinated steroid hormones. General principles. In: Methods of Radioimmunoassay. Jaffe, B.M. and Behrman, H.R. (Eds). Second Edition. Academic Press, New York. pp 727-741.

Killian, G.J. and Amann, R.P. (1972). Reproductive capacity in dairy bulls. IX. Changes in reproductive organ weights and semen characteristics of Holstein bulls during the first thirty weeks after puberty. *J. Dairy Sci.* **55**: 1631-1635.

Koefoed-Johnsen, H.H. and Pederson, H. (1971). Further observations on the Dag effect of the tail of the bull spermatozoa. *J. Reprod. Fert.* **26**: 77-84.

Kumi-Diaka, J., Nagaratnam, V. and Rwuaan, J.S. (1981). Seasonal and age related changes in semen quality and testicular morphology of bulls in a tropical environment. *Vet. Rec.* **108**: 13-15.

Kupferschmied, H., Gaillard, C. und Kozàk, A. (1974). Biometrische Untersuchungen über den skrotalum fang beim Simmertaler Fleckvieh Schweiz. *Arch: Tierheikal*, **116**: 263.

Lacroix, A. and Pelletier, J. (1979). Short-term variations in plasma LH and testosterone in bull calves from birth to 1 year of age. *J. Reprod. Fert.* **55**: 81-85.

Lacroix, A., Garnier, D.H. and Pelletier, J. (1977). Temporal fluctuations of plasma LH and testosterone in Charolais bull calves during the first year of life. *Annls. Biol. Anim. Biochim. Biophys.* **17**: 1013-1019.

Lagerlöf, N. (1934). Morphologische Untersuchungen Über Veranderungen im spermbild und in den Hoden bei Bullen mit Verminderter oder aufgehobener Ferfilität. *Acta. Path. microbilo. Scand. suppl. 19.* Thesis, Stoclholm.

Lagerlöf, N. (1936). Sterility in bulls. *Vet. Rec.* **41**: 1159-1170.

Land, R.B. and Carr, W.R. (1975). Testis growth and plasma LH concentration following hemicastration and its relation with female prolificacy in sheep. *J. Reprod. Fert.* **45**: 495-501.

Lasley, J.F. (1951). Sperm motility as a measure of semen quality. *J. Anim. Sci.* **10**: 211-218.

Lasley, J.F. and Bogart, R. (1944). A comparative study of epididymal and ejaculated spermatozoa of the boar. *J. Anim. Sci.* **3**: 360-370.

Lasley, J.F., Easley, G.T. and McKenzie, F.F. (1942). A staining method for the differentiation of live and dead spermatozoa. *Anat. Rec.* **82**: 167-174.

Lindner, H.R. (1969). The androgenic secretion of the testis in domestic ungulates. In: The Gonads. McKerns, K.W. (Ed). Appleton-Century-Crofts, New York, pp 165.

Lindner, H.R. and Mann, T. (1960). Relationship between the content of androgenic steroids in the testes and the secretory activity of the seminal vesicles in the bull. *J. Endocr.* **21**: 341-360.

Linford, E., Glover, F.A., Bishop, C. and Stewart, D.L. (1976). The relationship between semen evaluation methods and fertility in the bull. *J. Reprod. Fert.* **37**: 239-250.

Lino, B.F. (1971). Cell count correction factors for the quantitative histological analysis of the germinal epithelium of the ram. *Anat. Rec.* **170**: 413.

Liptrap, R.M. and Raeside, J.I. (1978). Increase in plasma testosterone after injection of adrenocorticotrophin into the boar. *J. Endocr.* **66**: 123-131.

Ludwig, D.J. (1950). The effect of androgens on spermatogenesis. *Endocrinology*, **46**: 453-481.

Lunstra, D.D., Boyd, G.W. and Corah, L.R. (1989). Effects of natural mating stimuli on serum luteinizing hormone, testosterone and estradiol-17ß in yearling beef bulls. *J. Anim. Sci.* **67**: 3277-3288.

Lunstra, D.D., Ford, J.J. and Echternkamp, S.E. (1978). Puberty in beef bulls: Hormone concentrations, growth, testicular development, sperm production and sexual aggressiveness in bulls of different breeds. *J. Anim.* Sci. **46**: 1054-1062.

Mann, T. (1964). The Biochemistry of Semen and of the Male Reproductive tract. John Wiley and sons, New York, pp 160.

Mann, T. (1964a). Studies on the metabolism of semen. 1 General aspects, occurrence and distribution of cytochromes, certain enzymes and coenzymes. *Biochem. J.* **39**: 451-458.

Mann, T. (1964b). Studies on the metabolism of semen. 2 Glycolysis in spermatozoa. *Biochem. J.* **39**: 458-465.

Mann, T. and Lutwak-Mann, C. (1981). Male Reproductive Function and Semen. New York, Springer-Verlag.

Mann, T. and Rowson, L.E.A. (1960). Appraisal of androgenic and gonadotrophic activity in male twin-calves by chemical analysis of semen and seminal vesicles. *J. Endocr.* **20**: *IV Proceedings of the society for endocrinology.*

Mann, T., Rowson, L.E.A. and Hay, M. (1960). Evaluation of androgenic and gonadotrophic activity in male twin calves by analysis of seminal vesicles and semen. *J. Endocr.* **21**: 361-372.

Mann, T.J. (1974). Secretory function of the prostate, seminal vesicle and other male accessory organs of reproduction. *J. Reprod. Fert.* **37**: 179-187.

Mann, T,J. (1975). In Hand book of Physiology. Hamilton, D.W. and Greep, R.O. (Eds). Sect. 7. Vol. V. American Physiological Soc, Washington, D.C. p 461.

Manton, V.J.A. (1956). The fertility of bovine semen collected by electroejaculation. *Vet. Rec.* **68**: 1015.

Maree, C., Casey, N.H. and Jacobi, I.E. (1989). The non significant effect of feeding level, growth rate and age and libido of young Afrikaner bulls. *J. S. Afr. Vet. Assoc.* **60**: 143 (Abstr).

Mayer, D.T., Squires, C.D., Bogart, R. and Olouffa, M.M. (1951). The technique for characterising mammalian spermatozoa as dead or living by differential staining. *J. Anim. Sci.* **10**: 226-231.

McMillan, K.L. and Hafs, H.D. (1967). Semen output of rabbits ejaculated after varying sexual preparation. *Proc. Soc. Exp. Biol. Med.* **125**: 1278-1281.

McMillan, K.L. and Hafs, H.D. (1968a). Gonadal and extra gonadal sperm numbers during reproductive development of Holstein bulls. *J. Anim. Sci.* **27**: 697.

McMillan, K.L. and Hafs, H.D. (1968b). Pituitary and hypothalmic changes associated with reproductive development of Holstein bulls. *J. Anim. Sci.* **27**: 1614-1620.

McMillan, K.L., Hafs, H.D., Desjardins, C. and Kirton, K.T. (1966). Some semen characteristics of dairy bulls ejaculated with artificial vaginas at varying temperatures. *J. Dairy Sci.* **49**: 1132-1133.

Meacham, T.N., Cunha, T.J., Warnick, A.C., Hentges, J.F. and Hargrove, D.D. (1963). Influence of low protein rations on growth and semen characteristics of young beef bulls. *J. Anim. Sci.* **22**: 115-120.

Means, A.R. (1977). Mechanisms of action of follicle stimulating hormone (FSH). In: The Testis. Johnson, A.D. and Gomes, W.R. (Eds). Vol. IV. Academic Press, New York, pp 163-188.

Memon, M.A., Bretzlaff, K.N. and Ott, R.S. (1986). Comparison of semen collection techniques in goats. *Theriogenology*, **26**: 823-828.

Mercier, E., Bratton, R.W. and Salisbury, G.W. (1949). Semen production and fertility in dairy bulls as related to frequency of ejaculation. *Cornell Vet.* **39**: 32-38.

Mickelsen, D.W. and Paisley, L.G. (1979). Scrotal circumference: Its relationship to breeding soundness evaluation scores for yearling bulls. *Vet. Med. Small. Anim. Clin.* **74**: 1767-1769.

Mickelsen, D.W., Paisley, L.G. and Dahmen, J.J. (1981). The effect of scrotal circumference, sperm motility and morphology in the ram on conception rates and lambing percentage in the ewe. *Theriogenology*, **16**: 53-59.

Mita, M., Price, J.M. and Hall, P.F. (1982). Stimulation by follicle stimulating hormone of the synthesis of lactate by sertoli cells from the rat testis. *Endocrinology*, **110**: 1535-1541.

Moger, W.H. and Murphy, P.R. (1982). Reevaluation of the effect of follicle stimulating hormone on the steroidogenic capacity of the testis: the effects of neuraminidase treated FSH preparations. *Biol. Reprod.* **26**: 422-428.

Morris, D.L., Tyner, C.L., Morris, P.G., Forgason, R.L., Forgason, J.L. and Williams, J.S. (1989). Correlation of scrotal circumference and age in American Brahman bulls. *Theriogenology*, **31**: 488-494.

Morrow, R.E., Elmore, R.G., Brooks, A.L. and Luebker, J.P. (1981). Growth and reproductive development of beef bulls tested on two levels of energy. *J. Anim. Sci.* **53**: suppl 1: 188.

Moule, G.R. and Waites, F.M.H. (1963). Seminal degeneration in the ram and its relation to the temperature of the scrotum. *J. Reprod. Fert.* **5**: 433-446.

Mwansa, P.B. and Makarechian, M. (1991). The effect of postweaning level of dietary energy on sex drive and semen quality of young beef bulls. *Theriogenology*, **35**: 1169-1178.

Nath, S., Mishra, H.R. and Singh, C.S.P. (1980). Note on the breeding behaviour of Holstein-Friesian bulls. *Indian J. Anim. Sci. 50*: 1124-1125.

Nelson, L. (1972). Quantitative evaluation of sperm motility control mechanisms. *Biol. Reprod.* **6**: 319-324.

Neter, N., Wasserman, W. and Kutner, M.H. (1985). Applied Linear Statistical Models. 2nd Edition. Richard, D. Irwin, Inc. Homewood, Illinois. pp 271-281.

Ndama, P.H., Entwistle, K.W. and Lindsay, J.A. (1983). Effect of protected protein supplements on some testicular traits in Brahman cross bulls. *Theriogenology*, **20**: 639-649.

Njogu, M.N. (1989). A study of factors affecting semen quality and quantity in dairy bulls. *MSc. Thesis*, University of Nairobi, Kenya.

Noakes, D.E. (1988). The normal genital organs. In: Fertility and Infertility in Veterinary Practice. Laing, J.A., Brinley Morgan, W.J. and Wagner, W.C. (Eds). Fourth edition. Balliere Tindall, London. pp 1-29.

Nwakalor, L.N. and Ezinma, C.O. (1989). Libido, serving capacity and breeding soundness in Muturu and N'Dama beef bulls. *Theriogenology*, **32**: 901-911.

Nwakalor, L.N., Orji, B.I. and Igboeli, G. (1979). Sexual behaviour in Muturu, N'Dama and Holstein-Friesian bulls in a humid tropical environment. *World Review of Animal Production*. **15**: 35-46.

Olson, H.H. and Petersen, W.E. (1951). Uniformity of semen production and behaviour in monozygous triplet bulls. *J. Dairy. Sci*. **34**: 489-490.

Ortavant, R., Courot, M. and Hochereau de Reviers, M.T. (1977). Spermatogenesis in domestic animals. In: Reproduction in Domestic Animals. Cole, H.H. and Cupps, P.T. (Eds). Third edition. Academic Press, New York. pp 203-224.

Osborne, H.G., Williams, L.G. and Galloway, D.B. (1971). A test for libido and serving ability in beef bulls. *Aust. Vet. J.* **47**: 465-467.

Ott, R.S, Heath, E.H. and Bane, A. (1982). Abnormal spermatozoa, testicular degeneration and variocele in a ram. *Am. J. Vet. Res.* **43**: 241-245.

Ott, R.S. and Memon, M.A. (1980). Breeding soundness examinations of rams and bucks. A review. *Theriogenology*, **13**: 221-229.

Pickett, B.W., Voss, J.L. and Gebouer, M.R. (1973). The effect of teasing on stallion seminal characteristics. *J. Anim. Sci.* **37**: 324.

Post, T.B., Christenson, H.R. and Seifert, G.W. (1987). Reproductive performance and productive traits of beef bulls selected for different levels of testosterone response to GnRH. *Theriogenology*, **27**: 317-328.

Post, T.B., Reich, M.M., Bindon, B.M. (1987). Characterisation of LH and testosterone responses to intramuscular injection of GnRH in tropical postpubertal bulls. *Theriogenology*, **27**: 305-315.

Price, E.O., Katz, L.S., Moberg, G.P. and Wallach, S.J.R. (1986). Inability to predict sexual aggressive behaviour by plasma concentrations of testosterone and luteinizing hormone in Hereford bulls. *J. Anim. Sci.* **62**: 613-617.

Pruitt, R.J. and Corah, L.R. (1986). Effect of energy intake after weaning on sexual development of beef bulls. II. Age at first mating, age at puberty, testosterone and scrotal circumference. *J. Anim. Sci.* **63**: 579-585.

Quinn, P.J. and White, I.G. (1968). Distribution of adenosine triphosphate activity in ram and bull spermatozoa. *J. Reprod. Fert.* **15**: 449-452.

Quinn, P.J., White, I.G. and Wirrick, B.R. (1965). Studies on the distribution of the major cations in semen and male accessory secretions. *J. Reprod. Fert.* **10**: 379-388.

Rao, A.R. (1971). Changes in the morphology of sperm during their passage through the genital tract in bulls with normal and impaired spermatogenesis. Ph.D. Thesis. Royal Veterinary College, Stockholm, Sweden.

Rao, C.K. and Hart, G.H. (1948). Morphology of bovine spermatozoa. *Am. J. Vet. Res.* **9**: 117-124.

Rao, K.V. and Kottaya, K. (1974). A note on morphological abnormalities of spermatozoa in cross-bred bulls. *Indian J. Anim. Sci.* **44**: 581-582.

Rathore, A.K. (1970). Acrosomal abnormality in ram spermatozoa due to heat stress. *Br. Vet. J.* **126**: 440-443.

Rawlings, N.C., Hafs, H.D. and Swanson, L.V. (1972). Testicular and blood plasma androgens in Holstein bulls from birth through puberty. *J. Anim.* Sci. **34**: 435-440.

Reeves, J.J. (1987). Endocrinology of reproduction. In: Reproduction in Farm Animals. Hafez, E.S.E. (Ed). 5th edition. Lea and Febiger, Philadelphia. pp 85-106.

Rekwot, P.I., Oyedipe, E.O., Akerejola, O.O., Kumi-Diaka, J. and Umoh, J.E. (1987). The effects of protein intake on the onset of puberty in Bunaji and Friesian x Bunaji crossbred bulls in Nigeria. *Theriogenology*, **28**: 427-434.

Ritzen, E.M. and French, F.S. (1974). Demonstration of Androgen Binding Protein (ABP) in rabbit testis: Secretion in efferent duct fluid and passage into epididymis. *J. Steroid. Biochem.* **20**: 151-154.

Rob, O. (1967). Degeneration of the germinal epithelium in bulls: Histopathology and spermatozoa morphology. *Vet. Med. Praha.* **11**: 437-444. *Abstr* in *Vet. Bull.* **37**: 192.

Roberts, S.J. (1986). Infertility in male animals. In: Veterinary Obstetrics and Genital diseases. Theriogenology. 3rd edition. Woodstock, Vermont Publishers, New York. pp 857-872.

Rollinson, D.H.L. (1951). Studies on the abnormal spermatozoa of bull semen. *Br. Vet. J.* **107**: 203-214.

Rothschild, L. (1949). Measurement of sperm activity before artificial insemination. *Nature* (London) **163**: 358-359.

Salamon, S. (1964). The effect of nutritional regimen on the potential semen production of rams. *Aust. J. Agric. Res.* **15**: 645-646.

Salisbury, G.W., Van Demark, N.L. and Lodge, J.R. (1985). Physiology of Reproduction and Artificial Insemination of cattle. CBS Publishers and Distributors. Delhi, India. Chapt. 13, pp 385-395.

Salisbury, G.W., Willet, E.L. and Seligman, J. (1942). The effect of the method of making semen smears upon the number off morphologically abnormal spermatozoa. *J. Anim. Sci.* **1**: 199-205.

Santamarina, E. and Reece, R.P. (1957). Normal development of the germinal epithelium and seminiferous tubules in the bull. *Am. J. Vet. Res.* **18**: 261-278.

Sanwal, P., Sundby, A. and Edqvist, L.E. (1974). Diurnal variation of peripheral plasma levels of testosterone in bulls measured by a rapid radioimmunoassay procedure. *Acta Veterinariya Scandinavia*, **15**: 90-99.

Satyanarayana, Raju, M. and Ramamohana Rao, A. (1982). Note on the semen characteristics of cross-bred and pure-bred bulls. *Indian J. Anim. Sci.* **53**: 193-194.

Saxena, V.B. and Tripathi, S.S. (1981). Seasonal variation in semen characteristics and preservability in cross-bred bulls. *Indian J. Anim. Res.* **15**: 19-24.

Saxena, V.B. and Tripathi, S.S. (1983). Seasonal variation in abnormalities of spermatozoa of Jersey bulls. *Indian J. Anim. Sci.* **53**: 193-194.

Secchiari, P., Martorana, F., Pelligrini, S. and Luisi, M. (1976). Variation in plasma testosterone in developing Friesian bulls. *J. Anim. Sci.* **42**: 405-409.

Sekoni, V.O., Sanusi, A., Abatan, M.O.I., Oyedipe, E.O., Rekwot, E.I. and Eduvie, L.O. (1992). Loss of libido and terminal sterility in a Friesian bull naturally infected with *Besnoitia besnoiti* in Northern Nigeria. *Theriogenology*, **37**: 533-549.

Setchell, B.P., Hinks, N.T., Voglmayr, J.K. and Scott, T.W. (1967). Amino acids in the ram testicular fluid and semen and their metabolism by spermatozoa. *Biochem. J.* **105**: 1061.

Sexton, T.J., Amann, R.P. and Flipse, R.J. (1971). Free amino acids in the *rete* fluid, *vas deferens* plasma, accessory sex gland fluid and seminal plasma of the conscious bull. *J. Dairy Sci.* **54**: 412-416.

Sharma, R.K., Pareek, P.K. and Vyas, K.K. (1982). Climatic effect of sexual behaviour and semen quality of Jersey bulls in Arid zone of Rajasthan. *Indian J. Anim. Reprod.* **2**: 45.

Sinha, H.S. (1964). A critical study of semen evaluation of three artificial insemination centres in Bihar. M.Sc. (Vet). A.H. Thesis, Magadh University, Bihar.

Sitarz, N.E., Erb, R.E., Martin, T.G. and Singleton, W.L. (1977). Relationship between blood plasma testosterone, weaning treatment, daily gains and certain physical traits of young Angus bulls. *J. Anim. Sci.* **45**: 342-349.

Skinner, J.D., Mann, T. and Rowson, L.E.A. (1968). Androstenedione in relationship to puberty and growth of the male calf. *J. Endocr.* **40**: 261-262.

Slaunwhite, W.R. Jr. and Samuels, L.T. (1956). Progesterone as a precursor of testicular androgen. *J. Biol. Chem.* **220**: 341-351.

Smith, G.F. (1951). Behaviour of bulls at Artificial Insemination centres. *Proc. 13th meet. of Brit. Soc. Anim. Prod.* pp 25-35.

Smith, M.F., Morris, D.L., Amoss, M.S., Parish, N.R., Williams, J.D. and Wiltbank, J.N. (1981). Relationship among fertility, scrotal circumference, seminal quality and libido in Santa Gertrudis bulls. *Theriogenology*, **16**: 379-397.

Smith, O.W. and Hafs, H.D. (1973). Competitive protein binding and Radioimmunoassay for testosterone in bulls and rabbits: Blood serum testosterone after injection of LH or prolactin in rabbits. *Proc. Soc. Exp. Biol. Med.* **142**: 804.

Soderquist, L. and Stalhammar, E.M. (1991). Relationship between ATP content and motility in bovine spermatozoa with reference to the effects of bull and the A.I. centre. *Acta. Vet. Scand.* **32**: 353-359.

Steinberger, E. (1971). Hormonal control of mammalian spermatogenesis. *Physiol. Rev.* **51**: 1-22.

Stephan, E., Lorrman, W. and Dycka, J. (1971). Problems of susceptibility to climate in domestic animals. The effect of experimental heat stress on bulls. First report: Some measurable ejaculate characteristics. *Zuchthygiene*, **6**: 19-38. *In: Animal breeding Abstr.* **39**: 497.

Swanson, E.W. and Boyd, L.J. (1962). Factors affecting coiled-tail spermatozoa in the bull. *Am. J. Vet. Res.* **23**: 300-308.

Täkha, K.M. (1986). Current aspects of Leydig cell function and its regulation. *J. Reprod. Fert.* **78**: 367-380.

Tegegne, A., Dembarga, Y. and Kassa, T. (1992). Gonadal and extragonadal sperm reserves in Boran and Boran x Friesian bulls raised on two planes of nutrition in the highlands of Ethiopia. *Theriogenology*, **37**: 953-961.

Tegegene, A., Entwistle, K.W. and Mukasa-Mugerwa, E. (1992). Nutritional influences on growth and onset of puberty in Boran and Boran x Friesian bulls in Ethiopia. *Theriogenology*, **37**: 1005-1016.

Tervit, H.R. and Peterson, A.J. (1978). Testosterone levels in Dorset and Romney rams and the effectiveness of these breeds in stimulating early onset of estrus in Romney ewes. *Theriogenology*, **9**: 271-273.

Thibier, M. (1975). Peripheral plasma testosterone concentration in bulls around puberty. *J. Reprod. Fert.* **42**: 567-569.

Thilander, G., Settergren, I. and Polen, L. (1985). Abnormalities of testicular origin in the neck region of bull spermatozoa. *Anim. Reprod. Sci.* **8**: 151-157.

Van Demark, N.L., Fritz, G.R. and Mauger, R.E. (1964). Effect of energy intake on reproductive performance of dairy bulls. 11. Semen production and replenishment. *J. Dairy Sci.* **47**: 898-901.

Van Demark, N.L. and Mauger, R.E. (1964). Effect of energy intake on reproductive performance of dairy bulls. 1. Growth, reproductive organs and puberty. *J. Dairy Sci.* **47**: 798.

Vogler, C.J., Saacke, R.G., Bane, J.H., De Jarnette, J.M. and McGilliard, M.L. (1991). Effects of scrotal insulation on viability characteristics of cryopreserved bovine semen. *J. Dairy Sci.* **74**: 3827-3835.

Vogler, C.J., Bame, J.H., DeJarnette, J.M., McGilliard, M.L. and Saacke, R.G. (1993). Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. *Theriogenology*, **40**: 1207-1219.

Watson, P.F. (1990). Artificial Insemination and preservation of semen. In: Marshalls' Physiology of Reproduction. Lamming, G.E. (Ed). Fourth edition. Vol. 2. Reproduction in the male. Churchill Livingstone, Edinburgh. pp 747-869.

Wells, M.E., Awa, A.O. and Fancy, S.S. (1972). Effect of season on acrosome status in the bull. *J. Dairy. Sci.* **55**: 1174-1178.

Wells, M.E., Philpot, W.N., Musgrave, S.D., Jones, E.W. and Brock, W.E. (1966). Effect of method of semen collection and tranquillization on semen quality and bull behaviour. *J. Dairy Sci.* **49**: 500.

Wenkoff, M.S. (1978). A sperm mid-piece defect of epididymal origin in two Hereford bulls. *Theriogenology*, **10**: 275-281.

Wildeus, S., Entwistle, K.W. and Holroyd, R.G. (1984). Patterns of pubertal development in Sahiwal and Brahman cross bulls in tropical Australia. II. LH and testosterone concentrations before and after GnRH. *Theriogenology*, **22**: 375-384.

Wildeus, S. and Hammond, A.C. (1993). Testicular, semen and blood parameters in adapted and non adapted *Bos taurus* bulls in the semi-arid tropics. *Theriogenology*, **40**: 345-355.

Willet, E.L. and Ohms, J.I. (1957). Measurements of testicular size and its relation to production of spermatozoa by bulls. *J. Dairy Sci.* **40**: 1559. (Abstr).

Williams, G. (1965). An abnormality of the spermatozoa of Hereford bulls. *Vet. Rec.* **77**: 1204-1206.

Wilsey, C.O., Pickett, B.W., Carroll, E.J. and Wiltbank, J.N. (1971). Effect of nutrition on growth of beef bulls. *J. Anim. Sci.* **33**: 272 (Abstr).

Wolfe, D.F., Bradley, J.T. and Riddell, M.G. (1993). Characterisation of seminal plasma proteins and sperm proteins in ejaculates from non spermic bulls and bulls with thermally induced testicular degeneration. *Theriogenology*, **40**: 1083-1091.

Wu, S.H. and McKenzie, F. (1955). Microstructure of spermatozoa after denudation as revealed by the electron microscope. *J. Anim. Sci.* **14**: 1151-1166.

Zoder, H.F., Auerdunk, G., Schwartz, E. and Eibl, K. (1969). The influence of a high rearing intensity of febrile diseases on characteristics of mating behaviour and semen quality of young bulls. *Tierartzl. Wschr.* **76**: 351-354.