# BLOOD TESTOSTERONE CONCENTRATION AND ITS RELATIONSHIP WITH BODY GROWTH AND SEMEN CHARACTERISTICS IN BUNAJI BULLS

BY

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## DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for higher degree.

To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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## CERTIFICATION

This thesis entitled 'BLOOD TESTOSTERONE CONCENTRATION AND ITS RELATIONSHIP WITH BODY GROWTH AND SEMEN CHARACTERISTICS IN BUNAJI BULLS' by Joy Funmilayo Alabi meets the regulations governing the award of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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existed between T and the incidences of cytoplasmic droplets and tail abnormalities. T concentrations were higher in the dry season than in the wet season associated with higher BWT, HGT and SC but poor body condition score (BCS) with the latter being higher in the wet season for all the groups.

This study has determined the T secretion pattern for the Bunaji bulls. T is released in pulses with time. Peaks are more in the morning hours, with reduced numbers of peak in the afternoon.

The study has confirmed the positive relationship between T, body measurements and spermiogram in Bunaji bulls. T is thus important in the body development and reproductive potential of the bull.

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LEGEND

T = Testosterone

BWT = Body weight

HGT = Heartgirth

SC = Scrotal circumference

LSC = Live sperm cell

VOL = semen volume

SC = semen concentration

SM = sperm motility

NS = Normal sperm

FH = Free head

PH = pyriform head

SH = swollen head

DH = Detached head

AA = Abnormal acrosome

PD = Proximal droplet

DD = distal droplet

ABD = Abaxial droplet

RT = Reversed tail

CT = coiled tail

TCT = Tightly coiled tail

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# CHAPTER ONE

## 1.0 INTRODUCTION

In the past more research efforts had been directed at the female than the male even though the male contributes equally to the genetic materials in a herd. Thus information on the performance of Nigeria's indigenous bulls and their contributions to the productivity of the herd, is scanty (Osinowo, 1979; Osinowo *et al.*, 1981; Oyedipe *et al.*, 1981).

It is desirable that information on the reproductive performance of farm animals be provided to assist the farmer in the management of his stock. A sire selected at an early age can have its reproductive and economic potentials maximised. Early genetic testing either for beef or milk production is desirable.

The evaluation of the male for breeding soundness is an important aspect of a reproductive management program. In order to exploit the maximum genetic potential in any livestock enterprise, a thorough screening of the male prior to use is advantageous. Evaluation of the male for breeding soundness is generally based on parameters such as body conformation, scrotal development and semen characteristics, which are influenced by testosterone (T) secretion by the testes. The hypothalamo - pituitary - gonadal axis has a major role in controlling the initiation of reproductive function and growth rate of the intact male (Rawlings *et al.* 1972). It was reported that systemic levels of luteinizing hormone (LH) and T which were low during the first few months of birth in calves, increased at 5 months (Rawlings *et al.*, 1972; Karg *et al.*, 1976 and McCarthy *et al.*, 1979),

then an oscillatory pattern of T secretion was observed at 6.5mths (Secchiari *et al.*, 1976), and which subsequently increased linearly with age (McCarthy *et al.*, 1979; Rekwot *et al.*, 1997). This is said to be persistent until at least 36mths of age (Hafs and McCarthy, 1979) in the *Bos taurus* breed.

General body growth and development are pre-requisites for the initiation of sexual function in males and females. Animals tend to reach puberty at a characteristic time in their growth cycle, but the exact nature of the connection between physical growth and sexual maturation remains elusive. Frisch and Revelle (1970, 1971, 1972) proposed a direct connection between body size and sexual maturation in their critical body weight, a hypothesis which states that menarche is triggered by the attainment of a particular body weight. Subsequently, this idea has been modified to the critical body fat hypothesis which proposes that menarche is triggered by attainment of a particular percentage of body fat (Frisch *et al.*, 1973; Frisch and McArthur, 1974).

Semen production is related to testicular development as was shown by a positive correlation between testicular weight, gonadal and extragonadal sperm reserves and sperm concentration (Amann, 1970). The larger and heavier the testes, the higher the sperm production. Testicular weight provided an accurate estimate of the sperm producing parenchyma in the testis of beef bulls (Coulter and Foote, 1979). Since testicular weight cannot be measured directly in the male and scrotal circumference was found to be significantly correlated to testicular weight in the bull (Coulter and Foote, 1976) the

indirect method of measuring testicular weight through measurement of scrotal circumference has been employed.

Several workers have observed that scrotal circumference is a useful indicator of testicular development and subsequent sperm output especially in young bulls (Hahn *et al.*, 1969; Coulter and Foote, 1976; Elmore *et al.*, 1976).

Osinowo *et al.* (1981) showed that testicular size is highly correlated to seminiferous tubule diameter and gonadal sperm reserve (r=0.81 - 0.85) in Bunaji bulls. They also found that scrotal circumference gave a good indication of testicular development as shown by the correlations between scrotal circumference and gonadal sperm/spermatid reserve (r = 0.69), seminiferous tubule diameter (r = 0.80), age (r = 0.88), body weight (r = 0.88) and testicular weight (r = 0.94).

Information on T secretion as it affects body growth and semen characteristics in indigenous bulls is not available. The most current work (Rekwot *et al.*, 1997) on T secretion dealt with the Friesian-Bunaji crossbreds and did not include the relationship between T, body growth and semen characteristics. Thus it is necessary to establish this relationship for proper evaluation of the indigenous bulls.

This study was therefore designed to:

a) determine the pattern of T secretions in different age groups of Bunaji bulls;

b) establish the relationship between T secretion, body growth and semen characteristics in 12 and 24mths old Bunaji bulls.

# CHAPTER TWO

## 2.0 REVIEW OF LITERATURE

### 2.1 Introduction

Testicular function in bulls is primarily regulated by the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and these, in turn are regulated by the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus (McCarthy *et al.*, 1979; Amann *et al.*, 1986). LH stimulates the interstitial cells of Leydig in the testes to produce testosterone (17B-hydroxyandrost-4-en-3-one) which in turn stimulates the germinal cells and maintains spermatogenesis (Gemzell and Roose, 1966).

Androgens, secreted primarily by the testes, are key regulators of growth rate, food conversion efficiency and carcass leaness (Schanbacher, 1984) and are also associated with the induction of aggresive and sexual behaviour in the male (Johnson and Whalen, 1989; Albert *et al.*, 1990). Also, testosterone helps in the development and maintenance of the accessory sex glands and stimulation of sexual behaviour (White, 1974).

The weight, fructose content and citric acid content of the seminal vesicles reflect the endocrine activity of the testes. Moreover, the diameter of the seminiferous tubules is closely related to Leydig cell function and highly correlated to the weight of seminal vesicles and their fructose and citric acid contents. Within the limits of normal vesicular glands and blood glucose level, seminal fructose is a relatively accurate reflection of Leydig cell function (Faulkner, 1969). Also, insulin-like growth factor I (IGF1) is involved

in the regulation of growth and testicular function (Handelsman *et al.*,1985).

## 2.2 Testosterone profiles for different species

Different T levels have been reported for different breeds and ages of cattle. Mean (Secchiari *et al.*, 1976), basal (McCarthy *et al.*, 1979), and peak T concentrations (Rekwot *et al.*, 1997) increased linearly with age. Some authors (Swanson *et al.*, 1971) reported a mean T of 4.34±0.61 and 6.70±1.10ng/ml for 12 and 12.5mths old Hereford bulls. In a later study, Rawlings *et al.* (1972) reported mean plasma T values of 0.33±0.11, 2.48±1.34, 4.82±2.02, 2.40±1.50, 2.44±1.94, 3.73±1.80 and 0.50±0.16ng/ml for 6, 7, 8, 9, 10, 11 and 12mths old Holstein bulls. A mean T of 0.4 and 0.6ng/ml was reported by Mongkonpunya *et al.* (1975) for 2 and 4mths old bulls. It was found that plasma concentration of T for Friesian bulls gradually increased during the first month of life with an enhanced increase after the fourth month of age (from 64.2ng/100ml at 4months to 118ng/100l at 5.5months), and a substantial rise at 6.5mths reaching 148.3ng/ml (Secchiari *et al.*,1976). In another study, McCarthy *et al.* (1979) reported that at 1-3mths old, peak T ranged from 0.3 to 0.6ng/ml; subsequently at 6, 7, 8, 9 and 10mths old, the frequency (no of peaks per 24hrs), basal and peak T concentrations were 4.5, 6.3, 4.5, 6.3, 0.4, 0.4, 0.5, 0.8 and 1.0ng/ml, and 6.8; 4.2, 3.7, 3.4, 6.5, and 9.3ng/ml; respectively. In the study by Matousek *et al.* (1980) mean T of 0.3±0.16, 1.83±1.14, 5.96±1.07 and 4.20±1.39ng/ml were reported for 20-24, 27-32, 69-104 and 120-210wks old bulls. And a similar study by Amann and Walker (1983) reported a mean T of 1.8, 2.67 and 5.76ng/ml for 18-20, 21-23 and 25-28wks old Holstein bulls.

Renaville *et al.* (1983) reported that T release was low from birth to puberty in the Belgian White Blue Breed-V bulls. The authors did not find an evidence of a secretion rhythm in their 10hr sampling period, and all profiles observed did not follow any pattern. The mean T for the breed at 140 and 180 days were 1.41±0.69 and 2.31±0.43ng/ml, respectively. In the Friesian-Bunaji, the peak T for 7, 10, 14 and 18mths old bulls on low protein diets were 1.3±0.2, 1.3±0.3, 2.1±0.3 and 2.8±0.0ng/ml, while the corresponding basal T and number of peaks were 0.3±0.2, 0.6±0.2, 0.7±0.3 and 1.0±0.3ng/ml; and 5, 3, 2 and 1 peaks (Rekwot *et al.*, 1997). Moreover, the peak T values for bulls on high protein diets were 2.3±0.2, 3.3±0.6, 4.1±0.4 and 4.6±0.6ng/ml and the corresponding basal T and number of peaks for the 7, 10, 14 and 18mths old bulls were 0.7±0.2, 0.9±0.3, 1.5±0.3 and 1.5±0.5ng/ml; and 2, 1, 1 and 2 peaks.

In the peripubertal bulls, a mean T concentration of 1.41±1.1 and 5.4±3.5ng/ml was reported for prepubertal and pubertal bulls, respectively (McCarthy *et al.*, 1979). The authors observed that the number of episodic peaks and mean T were fourfold greater in pubertal than in prepubertal bulls. Other authors (Fields *et al.*, 1982) in a similar study, reported a mean T of 3.96±1.03ng/ml for pubertal(15.9±0.4mths) Brahman bulls. They noted that the mean T was highest (6.94±1.64ng/ml) at 13.9mths and lowest (0.71±0.22ng/ml) at 10.2mths in the Brahman bulls. Moreover, they reported the highest mean T at 14.8mths (10.36±1.31ng/ml) and lowest (2.70±0.57ng/ml) at 9.2mths old in the Angus bulls.

In the Buffalo bull calves, age had highly significant effect ($P<0.01$) on T concentration (Hemeida *et al.*, 1985). Mean T was very low at birth(18.0±2.9pg/ml), rose to 26.0±40.6pg/ml at 8mths and further rise(376.2±139.7pg/ml) at 17-19mths old, with only 1 to 2peaks. Other authors (Chantaraprateep *et al.*, 1981) had earlier reported 1 to 10 peaks in the same buffalo bulls, while Agarwal *et al.* (1983) reported a total of 3 peaks in 24hrs with an average T concentration varying from 0.3 to 3.50ng/ml.

In a study involving two breeds of bucks, Patel *et al.* (1992) found that the peak, basal and mean T concentrations and number of peaks /24hrs were 13.29±0.01, 0.85±0.02 and 5.14±0.15ng/ml and 8 peaks for Surti bucks, whereas for the Marwari bucks, corresponding values were 13.6±0.03, 0.63±0.01 4.96±0.12ng/ml and 7 peaks, respectively, for bucks less than 1yr old.

In the boar, mean and basal T concentrations were 8.8 and 4.5ng/ml at 100d old; and 7.1 and 3.6ng/ml at 150d old (Bonneau *et al.*, 1987). Absolute amplitude (within peak highest hormone concentration) and relative amplitude (absolute amplitude minus baseline at the same time) of T peaks significantly declined with age. T peaks were 12.8/24hrs in 100d old and 7.4 in 150d old. At 125 and 175 d old, the number of peaks were 11.8 and 10.2, respectively.

Mean 24hr plasma T levels were similar in young and aged male rhesus Macaques as were the pulse amplitudes of T as reported by Kaler *et al.* (1986). However, the pulsatile pattern of T was different between young and aged males.

Sisk and Desjardins (1986) reported that T was secreted in discrete pulses with an average pulse frequency and amplitude of 0.62±0.04 pulses/hr and 16.96±2.5ng/l, in the intact male European ferrets.

Serum T levels increased markedly at 35-60 days of age (puberty) in the intact rat (Ojeda *et al.*, 1980; Matsumoto *et al.*, 1986) from 1.20ng/ml to 3.25±0.91ng/ml .

## 2.3 Seasonal variation of testosterone

Many studies have shown that seasonal variation in T concentration was associated with changes in the photoperiod (Coulter and Foote, 1976; Sanford *et al.*, 1977; Muduuli *et al.*, 1979; Hamilton *et al.*, 1984; Irby *et al.*, 1984; Ritar, 1991; Gonzalez *et al.*, 1992; Patel *et al.*, 1992; Walkden-Brown *et al.*, 1994; Perez and Mateos, 1995; ) in all the species studied.

In young normal horses (2 to 20yrs old) mean T values ranged from 0.091 to 0.302ng/ml during the non-breeding season and 0.108 to 0.452ng/ml during the breeding season (Hamilton *et al.*, 1984).

In the ram, T levels were found to be highest from March through August (spring and summer) (Gonzalez *et al.*, 1992). In an earlier study, Sanford *et al.* (1977) reported a mean T that increased four to six fold between early August and early November, then declined slightly by mid December. This seasonal trend is in agreement with other studies (Katongole *et al.*, 1974, Sanford *et al.*, 1974).

Diurnal and monthly variation within season has been reported. In the buck, mean T were lowest in the afternoon and increased to a maximum around midnight in May. From September through January the levels were lowest after sunset and highest in the afternoon. In March, the levels were lowest before dawn and highest after dawn. Basal T was highest (6.99ng/ml) in March and lowest (0.44ng/ml) in September. Mean T was highest (10.25ng/ml) in March and lowest (1.05ng/ml) in July. Peak T was highest(13.40ng/ml) in March and lowest (2.36ng/ml) in November. The total number of peaks ranged from 4.50 in September to 7.67 in March for Angora bucks (Ritar, 1991). In the pygmy bucks, Muduuli *et al.* (1979) reported values for mean, basal, peak T concentrations and number of peaks ranging from 1.5±0.2 in June to 13.0±2.2ng/ml in October, 0.6±0.01 in June to 9.2±2.1ng/ml in October, 5.3 ±1.0 in June to 18.7±2.6ng/ml in October and 1.5±0.3 (June) to 4.5±0.3 (October) peaks per 12hrs. Recently, Perez and Mateos (1995) reported that secretion patterns had increases in mean, basal, peak T concentrations and number of peaks during the summer and fall in Verata and Malaguena bucks. For Verata bucks, mean T levels in spring, summer, autumn and winter were 1.69±0.13, 3.94±0.27, 4.07±0.29 and 0.55±0.08ng/ml while that of Malaguena were 1.37±0.11, 4.24±0.23, 3.89±0.27 and 1.67±0.23ng/ml. Moreover, at ascedent photoperiod (December to June) , Verata and Malaguena bucks had 1.39±0.11 and 1.56±0.11ng/ml while at descendent photoperiod(June to December) it was 4.01±0.20 and 4.14±0.19ng/ml, respectively.

In the bush rat, Irby *et al.* (1984) reported that mean T exhibited a rise in

November, attaining a peak during December-January and declining during January-October.

## 2.4 Semen characteristics and sperm morphology

Ejaculate volume and semen concentration increased in Holstein bulls from puberty to 2 years of age (Almquist and Amann, 1962). Angus and Hereford bulls showed highly significant increases in ejaculate volume and weekly total spermatozoa output as the bulls aged from one to two years (Almquist and Cunningham, 1967). A study by Ivankov (1975) showed that bulls aged 3 - 7years had better semen quality than younger or older bulls. Semen production in Charolais (Almquist *et al.*, 1976) and Zebu bulls between puberty and 2 years of age was studied by Almquist *et al.* (1976), Osinowo (1979) and Oyedipe *et al.* (1981). They reported significant increases in semen volume, semen concentration, sperm motility and total spermatozoa output with age.

Mean semen volume, motility and concentration of $5.3 \pm 1.1$ml, $47.0 \pm 4.2\%$ and $171 \pm 64$/mlx$10^6$ had been reported for Brahman bulls at puberty ($15.9 \pm 0.4$mths) (Fields *et al.*, 1982). They observed that per cent motility and concentration increased with age in both Brahman and Angus bulls. Mean mass motility (%) ranged from $8 \pm 5$ to $62 \pm 6$ for 12 and 18.5mths old Brahman bulls; and $28 \pm 10$ to $76 \pm 4$ for 9.2 and 19.5mths old Angus bulls. The sperm concentrations for the two breeds were $10 \pm 0$ to$198 \pm 50$/mlx$10^6$ for the 12 and 18.5mths old Brahman and $30 \pm 7$ to $616 \pm 140$/mlx$10^6$ for 11.1 and 19.5mths old Angus. In the South Devon bull, Sekoni *et al.* (1982) reported a mean semen volume and concentration of $7.85 \pm 3.16$ml and 388.57/mlx$10^6$ for semen collected by electro-ejaculator; and $4.3 \pm 3.04$ml, 445.71/mlx$10^6$ for semen collected by artificial vagina; while % motility

for semen collected by AV was 48.33±24.01.

In the Hereford and Simmental bulls (Johnson *et al.*, 1995), the per cent normal sperm, sperm motility and semen concentration/ml$x10^6$ for Simmental were 494.8±24.9, 75.1±2.8 and 46.0±3.4 at 55.2±0.3wks old; and 303±47.6, 71±2.8 and 48.4±3.2 for Hereford at 57.7±0.5wks old. In the Holstein-Friesian (Gabor *et al.*, 1995) per cent live sperm and motility were 46.9±13.4 and 76.6±12.1 after repeated GnRH challenge. Berndtson and Igboeli (1989) reported per cent normal sperm of 80.8±1.4 with a range of 61.5 to 90.5. First ejaculate had motility (%) of 58.4±1.3 with a range of 39.2 to 71.7 and a second ejaculate with motility of 68.2±1.1 and a range of 50 to 82.5, respectively. The authors noticed seasonality in morphologic defects. Poorest quality semen was collected during the winter and spring (between January and March). Rekwot *et al.* (1987) reported seasonality in the semen characteristics of Friesian-Bunaji bulls in Nigeria. In the dry season, mean volume, %motility, concentration\ml$x10^6$, % abnormalities and % dead sperm were 3.42±0.68, 78.65±2.28, 0.97±0.11, 14.045±1.79 and 17.66±0.90, respectively, while corresponding values in the rainy season were 5.80±0.41, 80.57±2.40, 1.742±0.11, 6.456±0.66 and 15.391±1.63.

The normal sperm cell consists of a head, neck, middle-piece, principal piece and end piece. All specimens of semen contain a proportion of abnormal cells. These can be classified into primary abnormalities which originate during the development of the spermatozoa in the testicular tubules and secondary abnormalities which are due to changes taking place during storage in the epididymis or beyond (Blom, 1950). There may also be

tertiary morphological changes due to handling techniques, e.g. cold or heat shock, osmotic effects, toxicity of stains or changes in pH.

Several deviations from the normal morphology are regarded as abnormalities (Aamdal, 1964). Among them are giant or dwarf heads, double heads, headless or tailless sperm, mutilated heads, bent and coiled tails and protoplasmic heads in the mid-piece. These abnormalities are rare in normal ejaculates. When they occur in large numbers, they impair the fertility of the afflicted male (Glover, 1955; Melrose and Laing, 1970). Zemjanis (1970) reported a maximum of 10 - 20% proximal droplets, 5% detached acrosome, 10% bent tails for semen to be used for breeding. He also reported that the occurence of pathological spermatozoa was associated with disturbances in spermatogenesis, and inflammatory changes in the testis or epididymis.

Increased numbers of proximal droplets of spermatoza indicated either degenerative (disease or old age) or regenerative processes of the spermatogenic epithelium but might in rare cases reflect an epididymal dysfunction (Lagerlof, 1936). In the bull the most striking morphological change taking place in the sperm during their passage through the epididymis is the migration of the cytoplasmic droplet from a proximal to a distal position on the midpiece (Hancock, 1955).

Rao (1971) reported an average proportion of 26% and 6%of head defects in infertile and fertile bulls; and 2%, 0.8% and 3.5% of cytoplasmic droplets, midpiece and sperm tail defects respectively in fertile bulls. The author recommended that age of the

bull should be taken into consideration in sperm morphological studies. For example, the proportion of defective heads in the ejaculate was 4.8% in young bulls (not more than 3 years), 6% in 3 - 9 year old bulls, and 11% in healthy old bulls (more than 9 years). Proximal cytoplasmic droplets were 0.8%, 1.9% and 4.8% in the three age groups respectively (Rao, 1971). Fields *et al.* (1982) reported a total abnormalities of 15±3.3% for pubertal(15.9±0.4mths old) Brahman bulls. A total abnormalities of 12.71±3.50% for electro-ejaculator and 13.15±0.99% for AV, respectively (Sekoni *et al.*, 1982). The mean % values for defects such as deformed heads, detached heads, midpiece defects, proximal droplets, distal droplets, bent tails, coiled tail and total sperm defects were 1.47±0.22, 1.08±0.15, 1.40±0.24, 1.96±0.28, 1.57±0.32, 1.30±0.18, 1.38±0.20 and 10.16±0.63, respectively (Rekwot *et al.*,1987). Evans *et al.* (1995) reported that as bulls matured, there was an increase in the percent of normal and live sperm cells, cell motility and no of cells per ejaculate; and the incidence of abnormal morphologic features of sperm cells decreased. The greatest decreases were in the incidences of proximal droplets and knobbed acrosome.

In the Murciano-Granadima bucks (Roca *et al.*, 1992) distal droplets and acrosomal damage were the predominant abnormalities in the bucks studied. The mean % values for each morphologic defect were: 0.19±0.01, (deformed heads) 0.58±0.02 (midpiece defects), 1.32±0.04(tail defects), 0.31±0.01 (proximal droplets), 4.86±0.12 (distal droplets) and 0.67±0.02 (detached heads).

## 2.5 Bodyweight, scrotal circumference and heartgirth

Studies have shown that bodyweight (BWT) and scrotal circumference (SC) increased with age (Oyedipe *et al.*, 1981; Osinowo *et al.*, 1981; Coulter and Kozub, 1984; Rekwot *et al.*, 1997; ). Fields *et al.* (1982) reported a mean BWT and SC of 432±16kg and 34.4±1.2cm for 15.9±0.4mths (pubertal) Brahman bulls. Moreover, mean BWT and SC ranged from 231±10 to 470±15kg (8.3 to 17.6mths old) and 25.6±1.0 to 36.0±0.8cm (12.0 to 18.5mths old) Brahman bulls; and 242±8 to 475±11kg (9.2-18.5mths old) and 25.3±0.6 to 34.6±0.7cm (9.2 to 19.5mths old) Angus bulls. In a similar study, Johnson *et al.* (1995) reported mean BWT and SC of 581.0±6.4kg and 36.6±0.3cm for Simmental bulls of 55.2±0.3wks old; and 530.4±5.7kg and 33.8±0.3cm for Hereford bulls of 57.7±0.5wks old. An earlier study (Secchiari *et al.*, 1976) reported mean BWTs of 158.6, 196.7, and 232.3kg for 4, 5.5 and 6.5mths old, respectively for Friesian bulls. Coulter and Kozub (1984) reported a mean BWT and SC value of 426 and 628kg, and 34.0 and 37.4cm for Hereford bulls of 1 and 2yrs old on high levels of dietary energy; and 374 and 529kg and 33.9 and 37.3cm for the same animals at the same age but on medium levels of dietary energy. The authors reported values of 424 and 594kg, and 36.9 and 40cm for Angus bulls 1 and 2yrs old on high levels of dietary energy; and 394 and 526kg, and 35.7 and 39.1cm for the same animals at the same age but on medium levels of dietary energy. Swanson *et al.* (1971) reported a mean BWT of 355±6kg for Hereford bulls of 12.5mths old. Coulter and Foote (1977) reported that Holstein bulls grew rapidly and almost linearly up to 24mths of age. SC increased with age, following a pattern similar to BWT. They found variation in SC among bulls with the same BWT; and

variation in BWT and SC among animals of the same age. In the Senepol bulls (Wildeus, 1993), it was reported that SC changes followed a curvilinear relationship ($P < 0.001$) with both BWT and age. At 12, 18, and 24mths, and 6-7yrs, the mean SC were 27.98, 30.97, 33.62 and 42.43cm, respectively. The mean BWT and SC for pubertal (14 to 15mths) Senepol bulls were 326kg and 30.1cm while those for postpubertal (18-30mths) and mature (36-96mths) were 495kg and 33.5cm, 757kg and 40.9cm. Oyedipe *et al.* (1981) reported a mean BWT, HGT and SC for pubertal (66.79±1.01wks old) Bunaji bulls to be 154.3±8.7kg, 133.0±2.2cm and 21.8±1.7cm, respectively. They also reported 157.0±11kg, 134.0±3.6cm and 22.5±1.8cm for 73.4±2.16wks old Sokoto Gudali bulls and 155.0±31.1kg, 125.0±12.7cm and 19.1±3.2cm for 62.43±10.7wks old Friesian-Bunaji.

## 2.6 Relationship between testosterone, bodyweight, scrotal circumference, semen parameters and age

Willett and Ohms (1955) reported positve correlations between SC and spermatozoa production values which ranged from 0.30 to 0.46, for bulls in regular service. This increased to 0.68 when a smaller number of bulls were exhausted 8 times, followed by a single ejaculation 1, 3, 5 or 7 days later, and then by an additional rest period of 7 days. In another study, the correlation between SC and sperm production was 0.43 and 0.32 for bulls in routine service, but with 4 exhaustion at weekly intervals, the correlation was 0.92 for 16 young bulls and 0.53 for 6 old bulls (Willett and Ohms, 1957). In Charolais bulls, SC at 1 and 1.5 years of age was significantly correlated (0.44 and 0.52) with six-times

weekly sperm output at 2 years of age, and (0.62 and 0.75) with six times weekly sperm output at 3 years of age (Almquist *et al.*, 1976).

There is a high correlation between SC, age and liveweight, and between testicular size and BWT. Correlation declined from 0.85 before 6 years to 0.12 after 6 years (Hahn *et al.*, 1969). Testicular size is closely correlated to spermatozoa ouput in bulls.The relationship is especially high in young bulls and with frequent ejaculations.

Swanson *et al.* (1971) reported a correlation of 0.04 and 0.05 between testosterone and epididymal sperm and testosterone and gonadal sperm. Serum and testicular levels of T were highly correlated ($r = 0.63$, $P<0.01$). In another study (Secchiari *et al.*, 1976), a correlation of 0.81, 0.81 and 0.99 was reported between testosterone and age, testosterone and bodyweight and age and bodyweight, respectively.

In Holstein bulls, Coulter and Foote (1977) reported correlations of 0.96 for bodyweight and age and 0.81 for bodyweight and scrotal circumference. The correlation between age and bodyweight, age and heartgirth, and age and scrotal circumference were 0.81, 0.77, and 0.84 for Bunaji bulls (Oyedipe *et al.*, 1981). For Sokoto Gudali, the correlation between age and bodyweight, age and scrotal circumference and age and heartgirth were 0.95, 0.81 and 0.48 while that of Friesian crossbred was 0.72, 0.69 and 0.59, respectively. The correlations between testosterone and bodyweight, scrotal circumference, motility, concentration, volume and total abnormalities were 0.39, 0.31,

0.36, 0.22, 0.21 and -0.25 in Brahman bulls (Fields *et al.*, 1982). Wildeus (1993) reported a correlation of 0.86 and 0.82 between age and scrotal circumference and age and bodyweight. Bodyweight was said to be a better indicator of SC ($r^2$=0.856) than age ($r^2$=0.816) as the bulls matured. According to Gabor *et al.* (1995) correlations between

| S/N | X | Y | Regression | Breed | Reference |
|---|---|---|---|---|---|
| 1. | Age,mth | BWT,kg | $Y=-65.18+42.14x-0.39x^2$ | Holstein bulls | Coulter and Foote,1977 |
| 2. | SC,cm | BWT,kg | $Y=22.53+0.32x-0.000013x^2$ | Holstein bulls | Coulter and Foote,1977 |
| 3. | BWT,kg | Age,wks | Y=11.54+2.28X | Bunaji | Oyedipe *et al.* (1981) |
| 4. | BWT,kg | Age,wks | Y=24.63+1.92X | Friesian X | Oyedipe *et al.* (1981) |
| 5. | BWT,kg | Age,wks | Y=33.8+2.80X | Sokoto Gudali | Oyedipe *et al.* (1981) |
| 6. | HGT,cm | Age,wks | Y=81.53+0.79X | Bunaji | Oyedipe *et al.* (1981) |
| 7. | HGT,cm | Age,wks | Y=73.9+0.87X | Friesian X | Oyedipe *et al.* (1981) |
| 8. | HGT,cm | Age,wks | Y=57.96+1.09X | Sokoto Gudali | Oyedipe *et al.* (1981) |
| 9. | SC,cm | Age,wks | Y=4.25+28X | Bunaji | Oyedipe *et al.* (1981) |
| 10. | SC,cm | Age,wks | Y=6.10+23X | Friesian X | Oyedipe *et al.* (1981) |
| 11. | SC,cm | Age,wks | Y=-2.11+35X | Sokoto Gudali | Oyedipe *et al.* (1981) |
| 12. | Age | BWT,kg | $Y=11.55-1.77X+0.32x^2$ | Malabari (bucks) | Bilaspuri and Singh (1992). |
| 13 | Age | SC,cm | $Y=5.85+4.24X-0.176x^2$ | Malabari (bucks) | Bilaspuri and Singh (1992). |

sperm quality parameters were live vs motile spermatozoa (r=0.60, P<0.001), sperm velocity vs live spermatozoa (r=0.43, P<0.01), sperm velocity vs motile sperm (r=0.38,

$P<0.01$) and the total number of sperm cells per ejaculate vs motile sperm ($r=0.35$, $P<0.05$), respectively in the Holstein-Friesian bulls.

In the Malabari goat kids Bilaspuri and Singh (1992) reported correlation between bodyweight and scrotal circumference with age as 0.969 and 0.876 ($P<0.001$).

## 2.7 Relationship between age and testicular development

Several studies (Hahn *et al.*, 1969; Coulter and Foote, 1976; Elmore *et al.*, 1976; Lunstra *et al.*, 1978 and Osinowo *et al.*, 1981) have shown a curvilinear relationship between age and testicular development in bulls. Holstein dairy bulls were found to reach their maximum scrotal circumference in 5 - 6 years (Hahn *et al.*, 1969). Further testicular development in bulls was found to be slow after puberty (Almquist *et al.*, 1976). Testicular size increased steadily in young and declined in old bulls (Coulter and Foote, 1979). The authors found that the consistency of Holstein testes was firmest in young bulls and decreased gradually until 42 to 54 months of age. Thereafter, the firmness increased slightly and remained relatively constant until testicular degeneration occured. The trend was the same for Angus bulls except that they had softer testes than the Holsteins at 12 to 24 months of age.

A general increase in testicular weight and mean cross-sectional area of the seminiferous tubules with increase in age was reported in the ram (Watson *et al.*, 1956).

## 2.8 Relationship between age and semen production

Macmillan and Hafs (1968) reported that extragonadal sperm numbers increased

many folds in the epididymides, ductus deferentia and ampullae from 6 to 12 months of age. The same authors found sperm concentration and total gonadal sperm to be zero from 0 to 4 months but later increased steeply at 9mths, followed by a decrease at 10months before another sharp increase at 11 to 12months. A curvilinear relationship between age and semen production in bulls has been reported by a number of authors (Hahn *et al.*, 1969; Fagerlin *et al.*, 1971).

Killian and Amann (1972) found that by 20 weeks after puberty, or at 14 to 15 months of age, sperm production per gram of testis parenchyma reached the value of 54.8 x $10^6$/g for mature Holstein bulls.

Osinowo *et al.* (1981) observed that spermatogenesis started by 13 months of age in Bunaji bulls. However, they only found consistently large gonadal and epididymal sperm reserves in bulls aged 17 months and above. Gonadal sperm/spermatid reserve increased from 1.507$\pm$0.779 billion in 13 - 16 months old bulls to 11.98$\pm$4.598 billion at 22 - 27 months. The corresponding epididymal sperm reserves increased from 0.06$\pm$0.051 billion to 5.798$\pm$1.641 billion.

High percentage of sperm abnormalities have been reported in aged bulls as a result of senile degeneration (Kumi-Diaka *et al.*, 1981).

## 2.9 Relationship between bodyweight and testicular development

Sexual maturity has been found to be more closely related to BWT than chronological age (Terrill, 1974). Testicular traits in animals have been found to be more influenced by

BWT than any other factor (Coulter and Foote, 1979; Osinowo, 1979; Osinowo *et al.*, 1981).

In Merino rams, testicular weight varied with age but was more closely related to BWT. Waltson *et al.* (1956) observed that increase in BWT of Merino ram from 23 to 27kg was associated with increase in both testicular weight and the cross-sectional area of the seminiferous tubules.

Pant and Mukherjee (1973) reported that the BWT and not age influenced the dimensional characteristics of buffalo bull spermatozoa. In heavier buffaloes the spermatozoan head, breadth and head area were more and the percentage of live spermatozoa was less than in lighter buffaloes. Mammalian males of breedable age with heavier BWT may have less number of live spermatozoa in semen samples and consequently lower fertility than males of lighter BWT.

## 2.10 Effects of seasonal variation on male reproduction

Many workers have found seasonal variations in the reproductive performance of bulls (Coulter and Foote, 1976), bucks (Muduuli *et al.*, 1979,) and rams (Sanford *et al.*, 1977). Photoperiod has been the major seasonal factor influencing reproductive physiology in the ram as observed by Pelletier and Ortavant (1964; 1975). Decreases in daylength stimulated both the synthesis and release of LH and FSH, resulting in enhanced spermatogenesis and T production by the testis (Ortavant, 1956; Lincoln, 1976; Lincoln and Davidson, 1977).

In moderate climates, the effect of season on semen quality and fertility was not pronounced. However, report of study by Salisbury (1967) showed the effects of season on frozen semen. Lodge and Salisbury (1970), also observed seasonal effects on the quality and fertility of bull semen. Bulls subjected to high environmental temperatures had reduced semen quality.

Effect of season on the dimensional characteristics of live spermatozoa was studied in Hariana bulls (Mukherjee and Singh, 1966), Tharparkar bulls (Mishra *et al.*,1969) and Murrah buffaloes (Pant and Mukherjee, 1972). It was observed that during the cold seasons, the head of spermatozoa became longer and narrower with a broader midpiece than obtained in the hotter part of the year. These variations were considered to be due to changes in cellular metabolism.

Though the tropical breeds of cattle mate throughout the year, their fertility rate varies with season. For instance, high incidence of secondary abnormalities and increased protoplasmic droplets have been observed in the dry seson compared to rainy season (Hultnas, 1959; Sekoni, 1979; Sekoni and Gustaffson, 1980; Kumi-Diaka *et al.*, 1981). Elsewhere in the temperate region, study of seasonal effects on bull semen showed that quality of semen was superior in spring and inferior in autumn compared to other seasons (Mukherjee and Bhattacharya ,1952; Singh, 1960; Singh and Prabhu, 1963; Tomer *et al.*, 1964, 1965 and Sinha and Prassad, 1966).

Significant seasonal, monthly and weekly variations in percentage damaged

acrosomes have also been reported with later fall and winter conditions being more detrimental (Salisbury *et al.*, 1978).

Seasonality has been observed in the episodic release of LH and T Lincoln (1976) and Pelletier *et al.* (1982) noted a surge in levels of T and LH during the breeding season in the ram. There is also evidence to suggest that the number of leydig cells per testis undergoes seasonal changes (Kerr *et al.*, 1980).

Coulter *et al.* (1975) observed that there was no seasonal effect on growing bulls; however, there was a decrease in scrotal circumference from early to late summer (Coulter and Foote, 1979).

# CHAPTER THREE

## 3.0 AGE-RELATED BLOOD TESTOSTERONE SECRETION IN BUNAJI BULLS

### 3.1 INTRODUCTION

The testis has the distinction of being the first organ clearly recognized to have endocrine functions. The endocrine status of the testes is controlled by neuro-endocrine mechanisms involving gonadotropin releasing hormone, luteinizing hormone and follicle stimulating hormone.

Gonadotropin secretion rose at 10 to 20 weeks of age in the exotic breed of bull calves (Rawlings *et al.*, 1978; McCarthy *et al.*, 1979; Amann *et al.*, 1986; Wise *et al.*, 1987; Evans *et al.*, 1995). Serum testosterone (T) concentration is low in the postnatal bull calf, but starts to rise from 15 to 20wk (Rawlings *et al.*, 1972, 1978; Secchiari *et al.*, 1976; Lunstra *et al.*, 1978; Evans *et al.*, 1993). At the onset of puberty (first production of fertile sperm) the concentration rises again with numerous pulsatile peaks as a function of time (Karg *et al.*, 1976; Secchiari *et al.*, 1976; Thibier, 1976; Lunstra *et al.*, 1978; Bedair and Thibier, 1979; Renaville *et al.*, 1980).

A number of studies involving serial measurements of T have been conducted in exotic breeds of cattle (Swanson *et al.*, 1971; Rawlings *et al.*, 1972; McCarthy *et al.*, 1979; Amann and Walker, 1983; Renaville *et al.*, 1983) and goats (Perez and Mateos, 1995). These studies indicate a pulsatile secretory pattern with varying basal and peak levels and number of peaks. In a similar study using Friesian-Bunaji cross bulls, Rekwot *et al.*, (1997) reported higher basal and peak T concentrations in bulls fed a high protein diet than

those on a low protein diet. They observed that peak T concentrations ranged from 1.1ng/ml at 7mths to 5.3ng/ml at 18mths. These peaks occured mainly in the morning hours and ranged from one to five in number.

The importance of T in the spermatogenic activities and thus reproductive capability of the male animal cannot be overemphasized. Yet, there is no information on T secretion in the Bunaji bull which is the most predominant breed of cattle in Nigeria. This study was therefore designed to characterize the T secretion pattern and determine its changes with age in three age groups of Bunaji bulls.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Location

The study was carried out at the National Animal Production Research Institute, Shika, Zaria. It is located between latitudes 11° and 12°N at an altitude of 640m above sea level. Mean annual rainfall is about 1100mm (May to October). The daily temperature ranges from 21°C to 36°C, with mean relative humidities of 21% and 72% during the harmattan and rainy seasons, respectively.

### 3.2.2 Animals

Nine Bunaji bulls alloted on the basis of age to three groups, A, B and C of three animals, were used. The ages of the animals at the beginning of the study were 6 (GpA), 12 (GpB) and 24 (GpC) months.The mean bodyweights of the animals were 83.3, 146.67

and 266.67kg for groups A, B and C, respectively at the onset of the study.

### 3.2.3 Management of animals

The animals grazed mixed grass pasture of Digitaria, Bracharia and Panicum for 8hrs every day (from 8.00am to 4.00pm). During the dry season the animals had access to crop residues. Water and mineral licks were supplied *ad lib.* The animals were kept in an open paddock overnight. They were screened for blood and helminth parasites and dewormed with Sambezole before introduction to the study. They were also dipped in acaricide twice a week during the rainy season and once a week during the dry season to control ectoparasites.

### 3.2.4 Blood collection

Blood was collected into 5ml heparinized vacutainer tubes (sodium heparin was the anticoagulant) via jugular venipuncture, centrifuged for 3min at 3000g, plasma harvested and stored at -20°C until assayed for testosterone. Samples were obtained from the animals every 30min from 7.30am -4pm for 7h weekly for 4wks, then monthly for 3mths. A total of 882 plasma samples were analyzed. The study was carried out between August and December.

### 3.2.5 Radioimmunoassay (RIA) for Testosterone

The Coat- A- Count total testosterone RIA kit was used. This is a solid phase RIA technique based on testosterone antibody immobilized on the wall of a polypropylene tube. $^{125}$I-labelled testosterone competed for a fixed time with testosterone in the sample for antibody sites. Human serum based standards having testosterone concentrations ranging from 0, 0.7, 3.5, 14.0, 28, and 55 nmol/l were used (Diagnostic Products, Corporation,

1996; 1997). The standards provided were validated to cover the physiological testosterone concentration range prevalent in the plasma of most domesticated livestock species. The antisera were highly specific for testosterone.

### 3.2.6 Assay procedure

Four plain (uncoated) 12x75mm polypropylene total counts (T) and non specific binding (NSB) tubes, twelve total testosterone Ab-coated tubes, A (maximum binding) through F representing the various standard concentrations and all sample tubes were labelled in duplicate. 50ml of the zero standard was then pipetted into the A and NSB tubes, while 50ml of each of the remaining standards, control and plasma samples were pipetted into the appropriate tubes. This was followed by the addition of 1.0ml of $^{125}I$ total testosterone to every tube. The tubes were vortexed and incubated for 3h at 37°C. All the tubes except the total count tubes were then decanted thoroughly to separate bound from free testosterone. The radioactivity in each tube was determined by counting in a gamma counter (NE 1612), for 1 minute. A standard curve was produced from the standard samples and this was used to estimate the testosterone concentrations in the samples. The sensitivity of the assay was 0.14nmol/l while the intra and inter assay coefficients of variation were 8.5% and 12.5%, respectively.

### 3.2.7 Definition of hormone profile

Mean basal level was defined as the mean of the 7 lowest values observed during the 7h sampling period. Peaks were defined as each single value or series of values 2-fold above

mean basal concentration (Renaville *et al.*, 1983).

### 3.3 **Statistical analysis**

Analysis of variance (GLM) was used to compare the groups while the differences between the least square means of different age groups were determined by pairwise difference (PDIFF) method (SAS, 1989).

## 3.4 RESULTS

The mean plasma T concentrations for the different groups of animals are presented in Table 3.1.

Group C animals had the highest mean T concentrations followed by group B animals, indicating that mean T increased significantly ($P<0.001$) with age. The basal and peak T concentrations showed a similar trend between the groups except for the basal concentrations that group A animals had higher concentrations than group B animals.

The T secretory profile for the different groups of animals is presented in Fig. 3.1 Group C animals had the highest number of peaks (11), followed by group B animals (8). Animals in group A had no peaks (Table 3.1). The older the animal, the higher the peak concentration and number of peaks. In group B, the first four peaks occured at 30mins intervals followed by a pulse of 1h before the next three peaks which occured also at 30mins apart. The last peak occured 2.5h after the initial three peaks. In group C, the first six peaks occured at 30mins intervals followed by a pause of 1h before the remaining five peaks occured 30mins apart.

The general T secretory profile for all the animals is shown in Fig. 3.2. The profile showed a decline in the concentration and number of peaks of T with time during a 7h sampling period. The basal and peak concentrations and number of peaks for the profile were $2.77\pm8.17$, $5.69\pm4.5$nmol/L and 1, respectively.

Individual animal variations in T concentrations and profile as well as number of peaks were observed (Table 3.2; Figs. 3.3, 3.4 and 3.5).

The relationship between the age of the animals (X) and T concentrations (Y) was exponential with a prediction equation of $Y=0.272e^{0.024Age(wk)}$, with R-square value of 0.33 (Fig. 3.6). This implies that an of Xnmol/L increase in T concentration will give rise to a hypothetical increase of $10^y$ weeks increase in age.

The mean T concentration for all the animals was higher during the dry than rainy season although the difference was not statistically significant (Table 3.3).

Groups C and A animals had higher T concentrations during the dry ($P<0.05$) than rainy season, but the concentrations were higher in the rainy than dry season for the group B animals.

Monthly variation was observed in the basal, peak and total T concentrations for the different groups. Total T concentration was highest for the group A animals in the 2nd month and lowest in the first month. The group B animals had the highest T concentration in the 4th month and lowest in the 2nd month, while the group C animals had the highest T concentration in the 2nd month and lowest in the 3rd month (Table 3.4).

## 3.5 DISCUSSION

This study showed that T concentration increased exponentially with age. However, some authors (Rawlings *et al.*, 1972; Secchiari *et al.*, 1976 and Lunstra *et al.*, 1978) had reported a linear relationship. The increase with age of mean basal and peak T concentrations agrees with the report of Nolan *et al.* (1990). Generally, increase in plasma T concentration with age could be attributed to higher testicular sensitivity to LH and increase in the number of LH receptor cells on the testes as pubertal development proceeds (Catt, 1977; Schanbacher, 1979).

The mean T concentrations reported in this study are different from those reported for the Angus, Brahman and *Bos taurus* breeds (Fields *et al.*, 1982; Rawlings *et al.*, 1972). A mean T of 0.74ng/ml had been reported by Schanbacher, (1979) for 6mnths old bull calves, whereas this study obtained 0.87nmol/L (0.27ng/ml) for the same age in Bunaji bulls. This difference might be due to breed.

The seasonal changes in T concentration as observed in this study agrees with other reports (Ritar, 1991; Perez and Mateos, 1995). Seasonal variations have been associated with the sinusoidal changes of daylength or/and air temperature variations (Coulter and Foote, 1976). Bulls at different ages interact differently with the seasons. In this study, younger bulls tended to be more affected as the differences in T concentration observed are significant ($P<0.05$) , and this also holds true for the oldest group. The secretion patterns had increases in mean, basal and peak T concentrations and number of peaks during the dry

season, and this is in agreement with previous findings (Perez and Mateos, 1995) in the buck.

The average number of 8 and 11 peaks observed from the secretory profile for the groups B and C animals (Fig. 3.1) and 1 peak observed from the general hormone secretory profile (Fig. 3.2) disagree with the mean of 2 to 4 peaks reported for bulls by Katangole *et al.* (1971) and Rekwot *et al.* (1997). This may be due to the different age groups of animals used in the different studies. However, in breeds such as the swamp buffalo bulls 1 to 10 peaks have been reported (Chantaraprateep *et al.*, 1981), while other authors (Perera *et al.*, 1979, Agarwal *et al.*, 1983) reported 3 to 4 peaks in buffalo bulls. The exact significance of the number of peaks is not clear but they are said to be probably related to the sexual behaviour of the animal (Patel *et al.*, 1992).

T concentration differed significantly among animals within age, and relative to the mean, basal and peak, T was more variable in younger than in older bulls.This finding agrees with the report by Mongkonpunya *et al.* (1975).

The r values recorded in this study for T and age are higher than those of Secchiari *et al.*, (1976). The present study employed a logarithmic regression while the other authors used a stepwise regression analysis; and breed probably accounted for the differences.

It is concluded from the hormone profile that T secretion increased with age and occured in pulses with more peaks in the morning than afternoon hours. This implies that these animals might have higher libido in the morning than afternoon.

**Table 3.1: Mean basal, peak and overall plasma testosterone concentration (nmol) and number of peaks for the different groups of Bunaji bulls.**

| Group | Basal | Peak | Overall | Number of peaks |
|---|---|---|---|---|
| A | $1.47 \pm 5.62^{a}$ | $1.49 \pm 1.54^{c}$ | $0.87 \pm 0.41^{b}$ | 0 |
| B | $0.55 \pm 1.46^{b}$ | $2.89 \pm 3.11^{b}$ | $1.54 \pm 1.18^{b}$ | 8 |
| C | $1.62 \pm 14.16^{a}$ | $11.37 \pm 10.11^{a}$ | $5.93 \pm 4.32^{a}$ | 11 |

Means within the same column carrying different superscripts are significantly different ($P < 0.001$).

Mean $\pm$ SE

**Table 3.2: Mean basal, peak and overall testosterone concentration (nmol/l) and number of peaks for the individual animals in each group**

| Group | Animal number | Basal | Peak | Overall | Number of peaks |
|---|---|---|---|---|---|
| A | 1082 | 0.01±0.02 | 1.60±3.25 | 0.90±2.71 | 10 |
| A | 1274 | 0.06±0.11 | 1.48±2.29 | 0.86±2.00 | 14 |
| A | 1280 | 0.14±0.26 | 1.40±2.45 | 0.74±1.81 | 10 |
| B | 1278 | 0.47±1.89 | 2.81±5.28 | 1.45±3.87 | 6 |
| B | 1601 | 1.12±4.28 | 4.24±7.17 | 2.25±5.37 | 5 |
| B | 1658 | 0.29±0.24 | 1.55±2.25 | 0.90±1.70 | 8 |
| C | 0114 | 3.51±4.74 | 24.8±20.92 | 13.60±18.34 | 11 |
| C | 0115 | 1.68±5.96 | 3.47±3.33 | 1.93±2.76 | 3 |
| C | 0116 | 2.36±6.00 | 3.91±3.48 | 2.25±2.93 | 1 |

Mean±SE

Table 3.3: Mean testosterone concentration (nmol/l) for the groups in the wet and dry seasons.

| Season | Groups A | B | C | All groups |
|---|---|---|---|---|
| Wet(August-Oct) | $0.71 \pm 0.41^{b}$ | $1.69 \pm 1.25^{a}$ | $5.46 \pm 4.19^{b}$ | $2.62 \pm 1.58^{a}$ |
| Dry(Nov-Dec) | $1.09 \pm 0.41^{a}$ | $1.33 \pm 1.14^{a}$ | $6.55 \pm 4.70^{a}$ | $2.99 \pm 1.78^{a}$ |

Means within the same column carrying different superscripts are significantly different ($P < 0.05$).

Mean$\pm$SE

**Table 3.4: Monthly mean basal, peak and overall testosterone concentrations (nmol/l) for different groups of Bunaji bulls for plasma samples collected over four months**

| | Groups | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | | | B | | | C | | |
| Mth | Basal | Peak | Overall | Basal | Peak | Overal | Basal | Peak | Overall |
| 1 | 0.09±0.12$^{c}$ | 1.22±1.18$^{c}$ | 0.66±0.90$^{c}$ | 0.34±0.28$^{b}$ | 3.21±3.20$^{b}$ | 1.78±2.43$^{b}$ | 1.48±1.33$^{c}$ | 0.29±9.56$^{c}$ | 5.91±7.30$^{c}$ |
| 2 | 2.57±3.59$^{a}$ | 3.14±2.60$^{b}$ | 1.59±0.35$^{b}$ | 0.12±0.04$^{b}$ | 1.07±0.61$^{c}$ | 0.60±0.16$^{c}$ | 2.98±3.24$^{a}$ | 15.31±11.27$^{a}$ | 9.14±7.49$^{a}$ |
| 3 | 0.04±0.08$^{c}$ | 1.47±1.47$^{a}$ | 0.76±0.35$^{b}$ | 0.23±0.11$^{b}$ | 0.68±0.41$^{c}$ | 0.68±0.13$^{b}$ | 0.95±0.52$^{c}$ | 7.13±4.34$^{c}$ | 3.67±2.17$^{c}$ |
| 4 | 0.01±0.03$^{c}$ | 0.79±0.71$^{c}$ | 0.91±0.49$^{c}$ | 0.32±0.14$^{b}$ | 5.10±4.64$^{b}$ | 2.71±1.93$^{b}$ | 1.54±1.79$^{c}$ | 11.44±7.81$^{c}$ | 6.83±4.49$^{c}$ |

Means within the same column carrying different supersripts are significantly different ($P<0.05$)

Means±SE

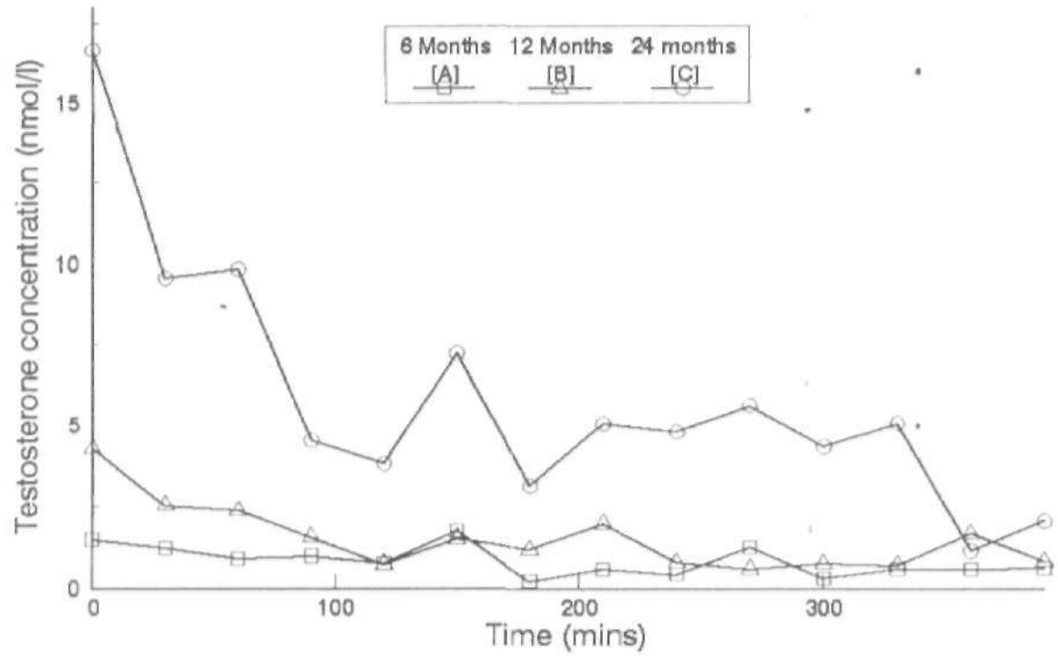



Figure 3.1: Testosterone profiles for different age groups of Bunaji bulls.

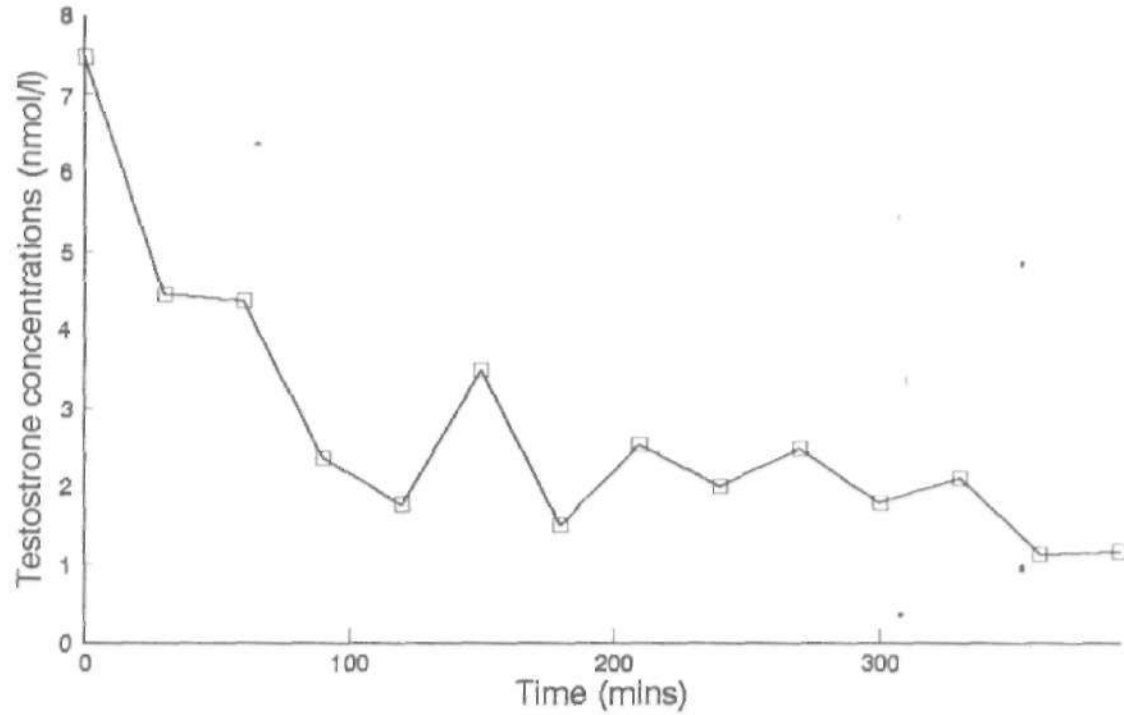



Figure 3.2: General hormone secretory profile for Bunaji bulls.

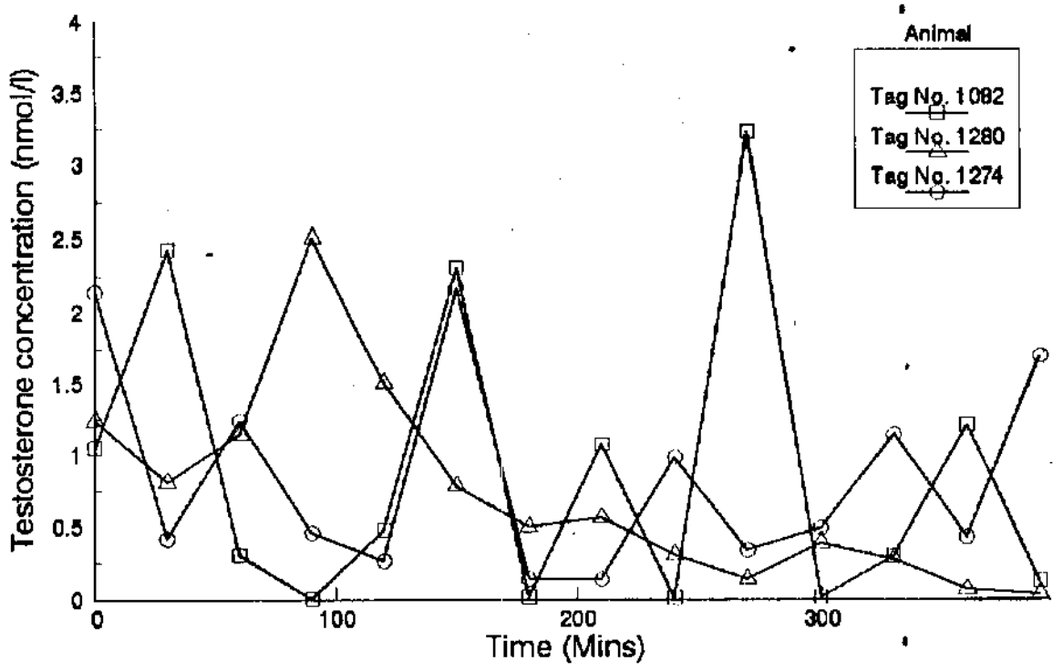



Figure 3.3: Testosterone profiles for the three animals in group A.

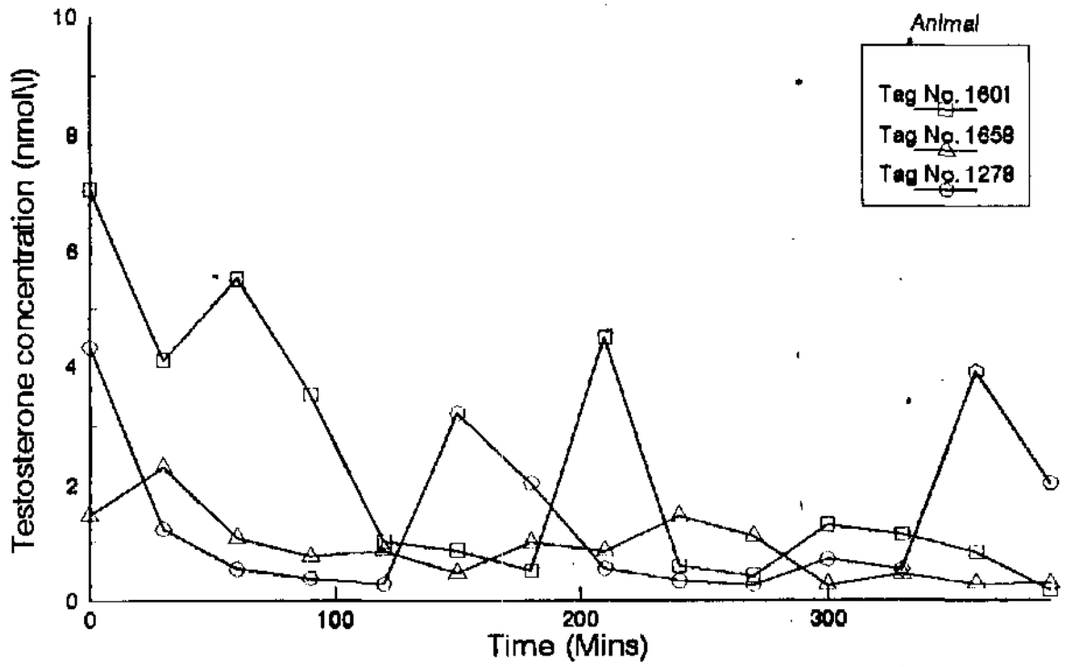



Figure 3.4: Testosterone profiles for the three animals in group B.

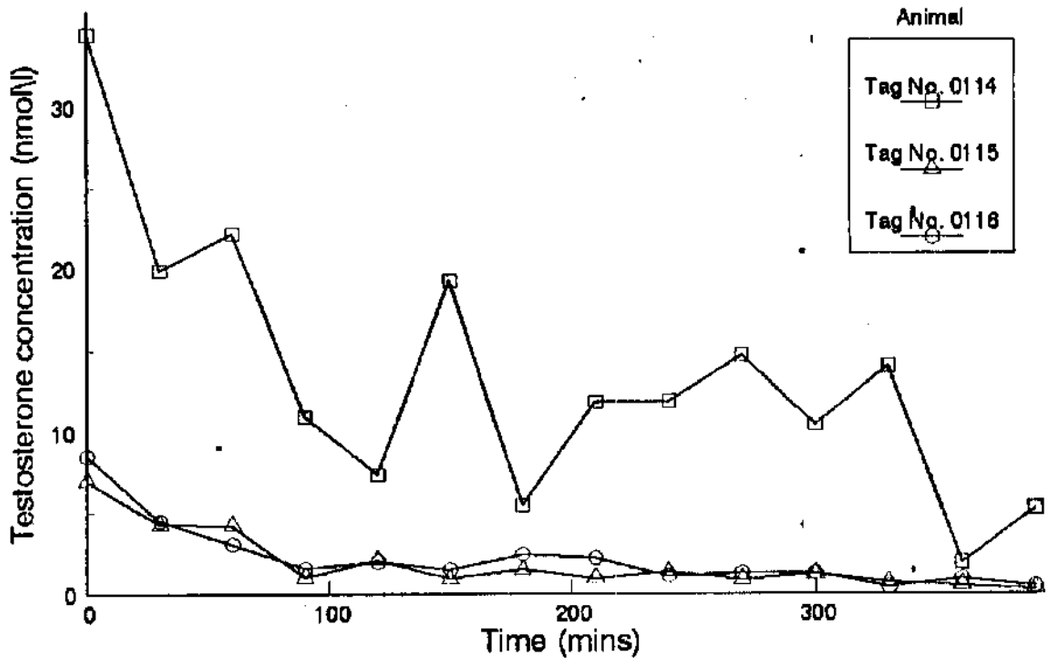



Figure 3.5: Testosterone profiles for the three animals in group C.

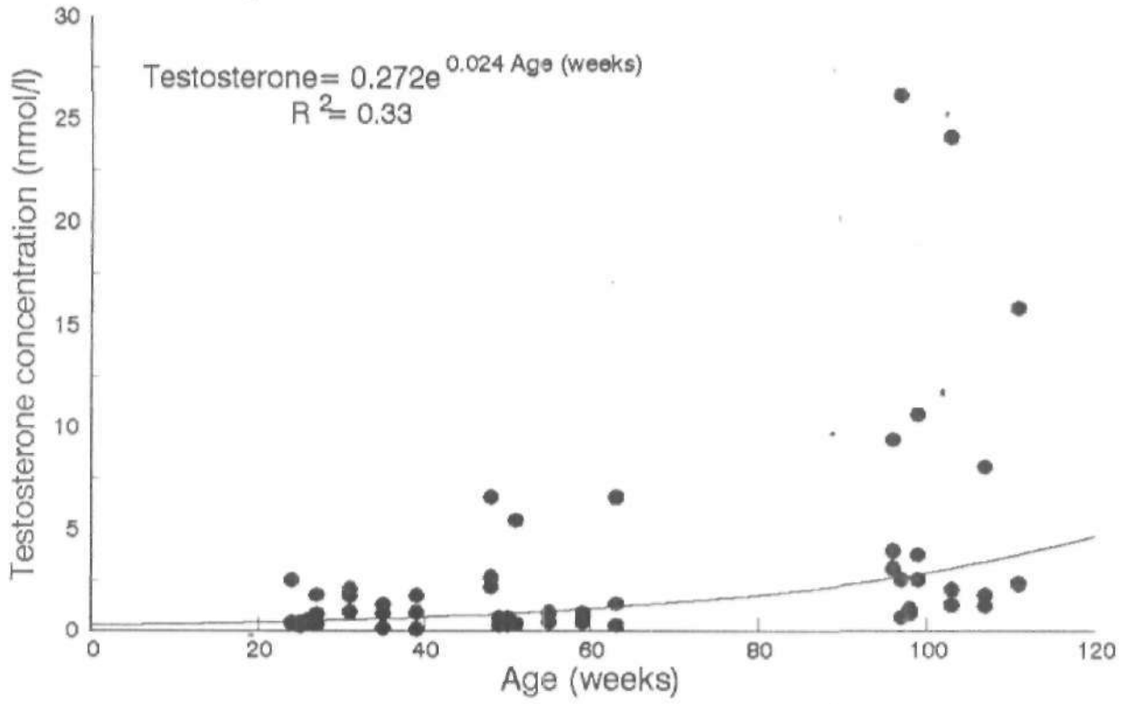



Figure 3.6: Relationship between age in weeks of Bunaji bulls with testosterone concentration.

# CHAPTER FOUR

## 4.0 RELATIONSHIP BETWEEN PLASMA TESTOSTERONE, BODY MEASUREMENTS AND SEMEN CHARACTERISTICS IN BUNAJI BULLS.

### 4.1 INTRODUCTION

The reproductive performance of livestock is a major component of their overall productivity. In growing bulls, 64% of the variation in scrotal circumference (SC) was found to be due to changes in body weight (BWT) (Coulter and Foote, 1977). Body development was highly correlated to testicular size, which in turn was closely correlated to spermatozoa output in bulls (Coulter and Foote, 1975). Osinowo *et al.* (1981) reported that testicular size was highly correlated to seminiferous tubule diameter and gonadal sperm reserve. Correlations between T and BWT, SC, sperm motility, concentration, volume and total spermatozoa abnormalities were 0.39, 0.31, 0.36, 0.22, 0.21 and -0.25 in the Angus and Brahman bulls of postpubertal age (Fields *et al.*, 1982).

Information on the semen characteristics of Nigerian bulls (Osinowo *et al.*, 1981; Oyedipe *et al.*,1981) and their crossbreds (Rekwot *et al.*, 1987; 1997) is scanty. Also there is no report on the association between T secretion, body and reproductive organ development and semen characteristics in the indigenous breeds of cattle. Yet these information are important in bull selection for breeding and artificial insemination.

This study was therefore designed to establish the relationship between testosterone secretion, body growth and semen characteristics in growing Bunaji bulls.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Location

The location, animals and their grouping, as well as their management are as reported in Chapter Three.

### 4.2.2 Body and scrotal measurements

The animals were packed inside a crush to restrain them for the measurements. In order to ensure consistency all measurements were done by the same person. A weigh band was put round the girth as close to the forelegs as possible and pulled tight to measure the heartgirth (HGT) from which the bodyweight (BWT) was determined. A measuring tape was put round the scrotum at the widest point to measure the SC.

Body condition score (BCS) was carried out as described by Pullan (1978). All measurements were taken weekly for four weeks and then monthly for three months.

Blood sampling for T determination was carried along with body and scrotal measurements. The details of the blood sampling are as described in Chapter Three.

### 4.2.3 Semen collection and evaluation

Semen could not be collected from animals in group A (because of their ages) until the 4th month when one ejaculated. Animals in this group were thus excluded from the semen study.

The method of semen collection was by rectal massage using a gloved hand. The gloved hand was lubricated in soap solution and inserted in the rectum to stimulate the seminal vesicles. A collection vial consisting of a funnel attached to a handle leading to a graduated test tube was held under the animal (in front of the prepuce) to collect the ejaculates. Immediately after collection, the colour and volume were recorded. Thereafter, a drop of the undiluted semen was put on a slide (the slides were labelled with animal number and date of collection) covered with a coverslip and mounted on a field microscope to determine the mass motility of the spermatozoa.

For more detailed laboratory study of the ejaculates, one drop of semen was added to four drops of eosin-nigrosin stain. It was mixed with a pipette and allowed to stand for 3 minutes. Two smears were prepared by placing a small drop of semen on the end of each slide. A second slide held at an angle of $45^0$ was used to draw out the drops along the slide.This was air-dried and stored for live:dead and morphology studies. Smears were examined under the microscope, counting 100 spermatozoa in random fields on the slide. Spermatozoa in which the head took up the stain were classed as dead. The count was expressed as percentage live. Four hundred spermatozoa were counted and the percentage of abnormal sperm based on the morphology of the midpiece and tail, proximal or distal cytoplasmic droplets, decapitated sperm and bent and coiled tails, was recorded. A phase contrast microscope was used at magnification of x400. At collection, 0.1ml semen was added to 4.9ml buffered formal saline in a Bijou bottle (the bottles were labelled immediately with the animal number and date of collection) and kept for determining the

sperm concentration using a haemocytometer. Both chambers of the haemocytometer were carefully filled with diluted semen that was already pipetted into the haemocytometer tubes, and allowed to stand for four minutes to allow the spermatozoa to settle. The counting chamber lines were located using a magnification of x320. Five large squares each containing sixteen small squares were counted. Only sperm heads touching the top or right hand lines, were counted. Both chambers were counted and the mean determined.

### 4.3 Statistical analysis

Data on body measurements, semen parameters and T concentration were subjected to an analysis of variance (SAS, 1989). Sources of variation were age (mnth), animal effect, season, time and week of collection. Data for semen parameters and T concentration were analyzed using general linear model (GLM) (SAS, 1989). Differences between the body measurements, semen parameters and T concentration were determined by pairwise difference (PDIFF) methods. Pearson's correlation and regression analysis were used to determine the degree of association between the parameters; and prediction equations were obtained using regression analysis (SAS, 1989).

## 4.4 RESULTS

The results of all body measurements, semen characteristics and T secretion are presented in Table 4.1

Group C animals had the highest BWT followed by B and A. The highest BWT was attained in the 4th month for all groups of animals. While BWT changes during the 4th month of study were statistically different ($P<0.05$) for group A animals it was not so ($P>0.05$) in the other groups. A substantial increase in T concentrations was followed by a substantial increase in BWT for all the groups. Similarly a drop in T concentration was associated with a drop in BWT.

Bodyweight increased linearly with age (Fig. 4.1). There was a highly significant ($P<0.001$) difference in BWT between the groups. T concentration increased with increased BWT (Fig. 4.2).

There was a highly significant difference ($P<0.001$) in HGT between the groups with group C animals having the highest HGT. Within the groups, group A animals attained the highest HGT in the 4th month. The trend was the same for group B but C had the highest in the 2nd month. The difference in HGT changes within the groups were not statistically significant ($P>0.05$). HGT increased with age (Fig. 4.3). Increase in T concentration was also associated with increase in HGT (Fig. 4.4).

There was a highly significant difference in SC ($P<0.05$) between groups. Group C animals consistently had the largest SC, followed by B and A in that order. Within the

groups, the largest SC was attained in the 3rd month in the three groups of animals. The observed differences were not statistically significant ($P>0.05$). SC increased with age (Fig.4.5). T concentration increased with increased SC (Fig.4.6).

The regression analysis showed that there is an exponential relationship between T and BWT, HGT or SC with the following regression equations: $T=0.185e^{0.01BWT}$ r square $=0.34$, $T=0.013e^{0.035HGT}$, r square $=0.31$ and $T=0.095e^{0.115SC}$, r square $=0.35$ (Figs. 4.7, 4.8, 4.9). For every Xnmol/l increase in T concentration, there is an n% increase in BWT, HGT or SC.

There was a highly significant difference ($P<0.001$) between group C animals and the remaining two groups. BCS was highest in C followed by B and then A. There was no statistical difference in BCS for months 1 and 4 for the groups. Group C animals had the highest BCS in the first month while it was high for group B animals in the 3rd month. Within group, A had the highest BCS in the first month. There was a significant difference ($P<0.05$) between months 1 and 3 and the remaining months. In group B, the highest BCS was in the 3rd month. There was a significant difference in BCS between the 2nd month and the remaining months ($P<0.05$). In group C, there was a significant difference ($P<0.05$) between the 1st month and the remaining months. BCS increased with age.

T concentration increased with improved BCS.

The ejaculate characteristics showed that the group B animals had generally milky colored semen while that of C animals was creamy. The group C animals had higher semen volume, concentration and % live sperm than the group B animals.

Increased T concentration was associated with milky coloured semen, higher volume, and higher % motility and live sperm .

Table 4.2 shows the sperm morphology for all the animals. Over 60% of the spermatozoa in semen collected were normal. Pyriform heads had the highest per cent of occurence followed by free heads and abnormal acrosome, distal and cytoplasmit droplets were the least, while coiled and reversed tail had acceptable per cent of occurence. Abaxial droplet was also low. The total head abnormalities averaged 15.27%, cytoplasmic droplets was 2.99% and tail abnormalities was 4.83%.

The relationship between sperm morphology and T secretion for all the two groups of bulls is presented in Table 4.3. Total abnormalities was higher for the group B than group C animals. The group C animals had higher per cent of head abnormalities than the group B animals. The incidence of cytoplasmic droplet and tail abnormalities were higher in the group B than group C animals.

Low T concentration was associated with high per cent of total abnormalities, cytoplasmic droplets and tail abnormalities.

Total abnormalities was 25.27% for the group B animals and 20.96% for the group C animals. Occurence of pyriform heads was slightly higher for the group B animals (6.94±1.67%) than for the group C animals (5.28±1.67%). There were more free heads in the semen of the group C animals (4.67±1.44%) than that of the group B animals (2.83±1.44%). On the other hand the semen of group B animals had slightly more abnormal acrosomes (2.56±0.90%) than that of the group C animals (2.11±0.90%).

**Seasonal variations in body measurements, semen parameters and T concentration**

Seasonal variations in body measurements, spermiogram and T secretion for bulls in gps B and C are shown in Table 4.4

Ejaculate color was milky in the wet season for the group B animals, while semen volume was higher for the two groups in the wet season. Sperm motility and live sperm were higher in the dry than wet season for the two groups. Sperm concentration was also higher in the dry than in the wet season for the group B animals while the reverse was the case for the group C animals. The differences in the semen characteristics for the different seasons were significant ($P < 0.05$). T concentration was higher in the wet season for the group B animals and in the dry season for the group C animals.

Sperm morphology for the different seasons are presented in Table 4.5

Total abnormalities was higher in the dry than in the wet season for both groups. The group B animals had higher per cent of head abnormalities in the wet season than the group C animals; moreover, the group B animals had higher per cent of cytoplasmic droplets in the wet season than the group C animals. Tail abnormalities followed a similar pattern.

Table 4.6 shows the seasonal variations for all the parameters measured for all the animals. There was a generally higher BWT, HGT and SC in the dry than in the wet season for the animals in the three groups. However BCSs were better in the wet than the dry season.

Within the group, there was a highly significant difference ($P<0.001$; $P<0.05$) in the BWT and heartgirth of groups A and B animals in the dry season. This is presented in table 4.7. There was no sinificant difference in the heartgirth of the oldest group

T concentration was higher in the dry than in the wet season for groups A and C animals ($P<0.05$), however it was higher in the wet for group B.

The correlation matrix for age (wk), BWT, HGT, SC, BCS and T for all the animals is presented in table 4.8. The probability level for the correlation was at the 1% level.

Table 4.9 shows the correlation coefficients for groups B and C animals for T, body measurements and semen parameters. T was positively correlated to all the parameters except sperm motility, free heads, pyriform head, proximal, distal and abaxial droplets and reversed tail with which it was negatively correlated. The prediction equations establishing the degree of association between these parameters are presented in Table 4.10.

## 4.5 DISCUSSION

The mean BWTs observed in this study for the three groups of animals are close to those reported by Osinowo (1979) and Oyedipe *et al.* (1981) for the same Bunaji breeds but dissimilar to those reported for Friesian-Bunaji cross (Rekwot *et al.*, 1997). The report is at variance with that of Fields *et al.* (1982) who reported a BWT of 335 and 344kg for Brahma and Angus bulls at 12 and 12.9mnths old in contrast to the 172.14kg observed in the present study. The result also differs from that of Wildeus (1993) on Senepol bulls. A BWT range of 300-400kg was reported for bulls aged 18-30mnths. The differences observed between this study and the other could be attributed to breed and environmental differences.

The mean HGTs recorded for the different groups of animals in this study are close to that reported by Oyedipe *et al.* (1981) for the same breed and dissimilar to that of Rekwot *et al.* (1997) on the Friesian-Bunaji cross. The paucity of data on HGT in literature might be due to the high correlation that has been found to exist between BWT and HGT (0.99) suggesting that either of the two can be employed. The present study has confirmed this report.

The mean SCs recorded in this study are close to those reported by Osinowo *et al.* (1981) and Oyedipe *et al.* (1981) for the same breed but different from that of Rekwot *et al.* (1997), and other breeds such as the Brahman and Angus, and Simmental and Hereford bulls (Fields *et al.* 1982, Pruitt *et al.* 1986).

The correlations obtained in this study between age and BWT, age and HGT and age and SC were higher than those reported by Osinowo *et al.* (1981) and Oyedipe *et al.* (1981) at $P<0.001$. The r value for BWT and SC (0.96) was higher than that of age and SC (0.94). These values are higher than that of Wildeus (1993); however, there is an agreement between the findings that BWT was a better indicator of SC than age.

The individual bull variation in SC that were observed suggests that there is a large degree of variability in SC and other reproductive traits (as SC was highly correlated to daily sperm production and extra gonadal sperm reserves (Johnson *et al.*, 1995 and Osinowo *et al.*, 1981)) within the Bunaji breed. These differences might be exploited to increase the reproductive efficiency of the breed by selection.

The increase in T concentration with increase in BWT, HGT and SC is in agreement with that of other authors for the Friesian bulls (Secchiari *et al.*, 1976) and for the Brahman and Angus bulls (Fields *et al.*, 1982). The r values reported in this study for T-BWT are higher than those of Secchiari *et al.* (1976). The differences might be due to the number of observations and the statistical tools used.

Semen quality improved as the animals matured. This agrees with the report of Evans *et al.* (1995). The changes observed in semen characteristics and sperm cell morphology in this study are similar to those reported by Almquist and Cunningham (1967) and Evans *et al.* (1995). The reduction in the incidence of cytoplasmic droplet and abnormal acrosome

with age is in close agreement with that reported by Evans *et al.* (1995). Cytoplasmic droplets removal and other changes to the acrosome occur in the epididymis (Hancock, 1955), whose actions are T dependent; hence a decrease in these abnormalities could be attributed to increasing T concentrations as the bulls matured. Tho total abnormalities recorded in this study are within the acceptable range (Zemjanis, 1970; Rao,1971; Berndtson and Igboeli, 1989). The high percentage of free and detached sperm heads observed in this study may be due to handling of the semen (Roberts, 1971).

The predominant morphologic defect observed in this study was that of pyriform heads in both groups which may be attributable to disturbances of spermatogenesis (Blom, 1950). However, Rao (1971) proposed that some sperm head defects, e.g narrow heads, short broad heads may be considered to be normal morphologic variations since they were not connected with any pathological conditions in the testicles. The incidence of tail abnormalities has been attributed to epididymal malfunction (Swanson *et al.*, 1962). From the r valde observed in the present study T is negatively correlated with tail abnormalities, with the incidence being higher in younger animals with lower T concentrations.

There seems to be a seasonal influence as observed in this study, this however, can not be separated from age influence since the animals grew older as the season progressed. The seasonal variation in semen quality observed in this study had also been reported by other workers. Igboeli and Ralika (1971) reported seasonal changes in ejaculate characteristics of Angoni (Zebu) bulls aged between 3 and 4yrs. They attributed the poor quality ejaculates observed during the hot season not only to the high ambient temperatures

during that season but also to the concommitant poor quality grazing available at that time. In another study, Rekwot *et al.* (1987) observed a decline in semen quality during the dry season and attributed this to high ambient temperature during the hot period and low temperature and severe winds during the harmattan. Kumi-Diaka *et al.* (1981) is at variance with the current report as the authors reported seasonal variation in exotic temperate breeds but not zebu breeds. The differences in observations of these reports might be attributed to breed, and the method and frequency of semen collection. The dry season had better quality semen as observed in this study, which might probably be due to the availability of crop residues at this time of the year. Ejaculate volume, sperm concentration and total sperm have been found (Almquist and Amann, 1962) to increase with increased frequency of collection from biweekly to six times per week with no deleterious effects. Also lack of teasing of bulls prior to semen collection has also been reported to affect the quality and quantity of semen (Amann, 1970).

The parallel increase in T and semen traits observed in this study reflect a close relationship between spermatogenesis and T concentration, and this is in agreement with other studies (Steinberger, 1971; Desjardins *et al.*, 1973) but disagrees with those of Gabor *et al.* (1995). The very low r square value recorded between T and live sperm, semen volume and normal sperm suggests that the spermatogenesis is regulated by a more complex mechanism than T alone. The r square value obtained for SC/%live sperm and SC/%normal sperm in this study suggests that selecting yearling bulls for larger SC provides high selection pressure for sperm production. But SC was not an accurate predictor of

morphology or motility when used to predict the recommended minimal standards of semen quality (Thompson *et al.*, 1992; Thompson and Johnson, 1995).

The study has confirmed the positive relationship between T, body measurement, and spermiograms in Bunaji bulls. T is thus important in the body development and reproductive potential of the bull.

Table 4.1: Mean body measurements, semen characteristics and T secretion for three groups of Bunaji during a four month sampling period

| | Groups | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | | | | | B | | | | | C | | | | |
| | Months | | | | | Months | | | | | Months | | | | |
| Parameters | 1 | 2 | 3 | 4 | Average | 1 | 2 | 3 | 4 | Average | 1 | 2 | 3 | 4 | Average |
| BWT | 97.17 | 125.67 | 122.33 | 128.67 | 109.33 | 161.08 | 187.33 | 182.00 | 191.33 | 172.14 | 278.67 | 280.00 | 273.67 | 281.00 | 278.48 |
| HGT | 101.92 | 112.00 | 111.00 | 113.00 | 106.24 | 121.92 | 129.33 | 128.00 | 130.33 | 125.05 | 151.09 | 152.33 | 150.00 | 151.33 | 151.14 |
| SC | 14.00 | 15.00 | 15.33 | 14.00 | 14.33 | 21.00 | 21.67 | 23.33 | 22.33 | 21.62 | 29.58 | 29.33 | 29.67 | 29.33 | 29.52 |
| BCS | 4.13 | 3.50 | 4.00 | 3.67 | 3.95 | 4.09 | 3.50 | 4.00 | 4.02 | 4.05 | 4.54 | 4.00 | 4.00 | 4.00 | 4.31 |
| Semen characteristics | | | | | | | | | | | | | | | |
| Colour | - | - | - | - | - | milky | milky | creamy | milky | | milky | milky | milky | creamy | |
| Vol,ml | - | - | - | - | - | 2.24 | 1.83 | 1.40 | 1.17 | 1.86 | 3.09 | 1.83 | 3.77 | 2.83 | 2.95 |
| Conc./mlx$10^6$ | - | - | - | - | - | 19.00 | 30.00 | 30.00 | 38.33 | 25.89 | 111.11 | 14.33 | 26.67 | 18.33 | 65.44 |
| Mot.,% | - | - | - | - | - | 12.11 | 43.33 | 36.67 | 46.67 | 27.17 | 21.11 | 16.67 | 51.67 | 10.00 | 23.61 |
| Live,% | - | - | - | - | - | 67.78 | 75.33 | 76.67 | 85.67 | 73.50 | 76.44 | 73.33 | 75.00 | 81.00 | 76.44 |
| T(nmol/l) | 0.66±0.90 | 1.59±0.35 | 0.76±0.35 | 0.91±0.49 | 0.87 | 1.78±2.43 | 0.60±0.16 | 0.68±0.13 | 2.71±1.93 | 1.54 | 5.91±7.30 | 9.14±7.49 | 3.67±2.17 | 6.83±4.49 | 5.93 |

Table 4.2: Sperm morphology for Bunaji bulls

| Sperm parameter | Total,% | SD |
|---|---|---|
| Normal sperm | 76.91 | 27.48 |
| Free heads | 3.75 | 6.08 |
| Pyriform head | 6.11 | 9.56 |
| Swollen head | 1.39 | 5.18 |
| Detached head | 1.69 | 2.08 |
| Abnormal acrosome | 2.33 | 4.82 |
| Proximal droplet | 1.61 | 6.80 |
| Distal droplet | 0.44 | 1.42 |
| Abaxial droplet | 0.94 | 3.48 |
| Reversed tail | 1.81 | 2.24 |
| Coiled tail | 1.33 | 1.91 |
| Tightly coiled tail | 1.69 | 2.14 |

**Table 4.3: Relationship between sperm morphology and T secretion for Bunaji bulls aged 12 and 24months**

| Sperm morphology,% | GP B | GP C |
|---|---|---|
| Total Abnormalites,% | 25.27[a] | 20.96[b] |
| Normal sperm,% | 74.73±6.10[b] | 79.04±6.10[a] |
| Swollen head,% | 0.56±1.16[b] | 2.22±1.16[a] |
| Free head,% | 2.83±1.44[a] | 4.67±1.44[a] |
| Proximal droplet,% | 2.83±1.56[a] | 0.39±1.56[b] |
| Distal droplet,% | 0.61±0.33[a] | 0.28±0.33[b] |
| Detached head,% | 1.39±0.48[b] | 2.00±0.48[a] |
| Abaxial droplet,% | 1.83±0.56[a] | 0.06±0.56[b] |
| Reversed tail,% | 2.50±0.39[a] | 1.11±0.39[b] |
| Coiled tail,% | 1.50±0.47[a] | 1.17±0.47[a] |
| Tightly coiled tail,% | 1.72±0.51[a] | 1.67±0.51[a] |
| Pyriform head,% | 6.94±1.67[a] | 5.28±1.67[a] |
| Acrosome abnormalities,% | 2.56±0.90[a] | 2.11±0.90[a] |
| Testosterone conc.,nmol/l | 1.5±1.18[b] | 5.93±4.32[a] |

Means within the same row carrying different superscripts are significantly different ($P < 0.05$).

Mean±SE

Table 4.4: **Seasonal variations in body measurements, spermiogram and T secretion for Bunaji bulls aged 12 and 24months**

| | B | | C | |
|---|---|---|---|---|
| Parameters | Dry | Wet | Dry | Wet |
| BWT | 186.89[a] | 161.08[b] | 278.22[a] | 278.67[a] |
| HGT | 129.22[a] | 121.92[b] | 151.22[a] | 151.08[a] |
| SC | 22.44[a] | 21.00[a] | 29.44[a] | 29.58[a] |
| BCS | 4.00[a] | 4.08[a] | 4.00[b] | 4.54[a] |
| Semen characteristics | | | | |
| Colour | Milky | Milky | Milky | Milky |
| Volume,ml | 1.47[b] | 2.24[a] | 2.81[b] | 3.09[a] |
| Conc./mlx10^6 | 32.78[a] | 19.00[b] | 19.78[b] | 111.11[a] |
| Motility,% | 42.22[a] | 12.11[b] | 26.11[a] | 21.11[b] |
| Live,% | 79.22[a] | 67.78[b] | 76.44[a] | 76.44[a] |
| Total abnormalities,% | 29.77[a] | 20.78[b] | 24.65[a] | 17.22[b] |
| T conc.(nmol/l) | 1.33±1.14[b] | 1.69±1.25[a] | 6.55±4.70[a] | 5.46±4.19[b] |

Means in the same row for the same group carrying the same superscript are not significanly different ($P<0.05$).

Mean±SE

Table 4.5: Seasonal variations in sperm morphology of 12 and 24mnths old Bunaji bulls

| | Age (months) | | | |
|---|---|---|---|---|
| | 12 | | 24 | |
| Sperm morphology | Dry | Wet | Dry | Wet |
| Total Abnormalities, % | 29.77[a] | 20.78[b] | 24.65[a] | 17.22[b] |
| Normal sperm, % | 70.23±9.43[b] | 79.22±9.43[a] | 75.35±9.43[b] | 82.78±9.43[a] |
| Swollen head, % | 0.11±1.72[b] | 1.00±1.72[a] | 0.44±1.72[b] | 4.00±1.72[a] |
| Free head, % | 3.89±2.00[a] | 1.78±2.00[b] | 7.00±2.00 | 2.33±2.00[b] |
| Proximal droplet, % | 5.11±2.26[a] | 0.56±2.26[b] | 0.33±2.26[a] | 0.22±2.26[a] |
| Distal droplet, % | 1.00±0.48[a] | 0.22±0.48[b] | 0.33±0.48[a] | 0.22±0.48[a] |
| Detached head, % | 1.44±0.71[a] | 1.33±0.71[a] | 2.44±0.71[a] | 1.56±0.71[b] |
| Abaxial droplet, % | 3.67±1.08[a] | 0.00±1.08[b] | 0.11±1.08[a] | 0.00±1.08[b] |
| Reversed tail, % | 0.89±0.61[b] | 4.11±0.61[a] | 0.67±0.61[b] | 1.56±0.61[a] Coiled |
| tail, % | 1.33±0.66[a] | 1.67±0.66[a] | 1.11±0.66[a] | 1.22±0.66[a] |
| Tightly coiled tail, % | 2.11±0.74[a] | 1.33±0.74[b] | 1.56±0.74[a] | 1.78±0.74[a] |
| Pyriform head, % | 5.89±3.30[a] | 8.00±3.30[a] | 6.44±3.30[a] | 4.11±3.30[b] |
| Abnormal acrosome, % | 4.33±1.53[a] | 0.78±1.53[b] | 4.22±1.53[a] | -0.00±1.53[b] |
| Testosterone, nmol/l | 1.33±1.14[b] | 1.69±1.25[a] | 6.55±4.70[a] | 5.46±4.19[b] |

Means within the same row for the same group carrying the same superscript are not statitistically different (P>0.05).

Mean±SE

Table 4.6: **Seasonal variation in body condition score, testosterone concentration (nmol/l), bodyweight(kg), heartgirth(cm) and scrotal circumference(cm) in Bunaji bulls**

| Seasons | BCS | T | BWT | HGT | SC |
|---|---|---|---|---|---|
| Dry | $3.91^{b}$ | $6.60^{a}$ | $196.89^{a}$ | $130.82^{a}$ | $22.22^{a}$ |
| Wet | $4.25^{a}$ | $4.12^{b}$ | $178.97^{b}$ | $124.97^{b}$ | $21.51^{a}$ |

Means within the same column carrying different superscripts are significantly higher ($P<0.05$).

Table 4.7: Seasonal body measurements and testosterone concentration changes for the different groups

| Season | Gp | BWT | HGT | SC | BCS | T |
|---|---|---|---|---|---|---|
| Dry | A | 125.56±28.50$^{a}$ | 112.00±9.15$^{a}$ | 14.78±1.39$^{a}$ | 3.72±0.36$^{b}$ | 1.09±0.71$^{a}$ |
| Wet | A | 97.17±21.67$^{b}$ | 101.92±8.69$^{b}$ | 14.00±0.74$^{a}$ | 4.13±0.31$^{a}$ | 0.71±0.71$^{b}$ |
| Dry | B | 186.89±17.06$^{a}$ | 129.22±4.27$^{a}$ | 22.44±2.51$^{a}$ | 4.00±0.43$^{a}$ | 1.33±1.98$^{b}$ |
| Wet | B | 161.08±15.32$^{b}$ | 121.92±5.04$^{b}$ | 21.00±2.30$^{b}$ | 4.08±0.3$^{a}$ | 1.69±2.17$^{a}$ |
| Dry | C | 278.22±28.51$^{a}$ | 151.22±5.95$^{a}$ | 29.44±1.59$^{a}$ | 4.00±0.00$^{a}$ | 6.55±8.15$^{a}$ |
| Wet | C | 278.67±25.02$^{a}$ | 151.08±5.57$^{a}$ | 29.58±1.68$^{a}$ | 4.54±0.33$^{a}$ | 5.46±7.27$^{b}$ |

Mean±SE

Table 4.8: Pearson Correlation Coefficients, and level of probability for age(wk), body measurements and testosterone concentration (nmol/l)

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1,Age | - | | | | |
| 2,BWT | 0.95 | - | | | |
| 3,HGT | 0.94 | 0.99 | - | | |
| 4,SC | 0.94 | 0.96 | 0.95 | - | |
| 5,BCS | 0.31 | 0.36 | 0.34 | 0.41 | - |
| 6,T | 0.44 | 0.50 | 0.47 | 0.47 | 0.10 |

R- values greater than 0.30 are significant at 5%.

No. of observations = 63

**Table 4.9: Pearson's correlation coefficients for body measurements, testosterone concentration, semen parameters and age**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1, Age | | | | | | | | | | | | | | | | | | | | | | |
| 2,BWT | 0.95 | | | | | | | | | | | | | | | | | | | | | |
| 3,GT | 0.93 | 0.99 | | | | | | | | | | | | | | | | | | | | |
| 4,SC | 0.94 | 0.96 | 0.95 | | | | | | | | | | | | | | | | | | | |
| 5,BCS | 0.31 | 0.36 | 0.34 | 0.41 | | | | | | | | | | | | | | | | | | |
| 6,TT | 0.44 | 0.50 | 0.47 | 0.47 | 0.10 | | | | | | | | | | | | | | | | | |
| 7,LS | 0.65 | 0.61 | 0.62 | 0.68 | 0.16 | 0.15 | | | | | | | | | | | | | | | | |
| 8,V | 0.59 | 0.52 | 0.53 | 0.57 | 0.06 | 0.27 | 0.65 | | | | | | | | | | | | | | | |
| 9,CON | 0.29 | 0.19 | 0.20 | 0.25 | 0.08 | -0.01 | 0.37 | 0.35 | | | | | | | | | | | | | | |
| 10,MOT | 0.36 | 0.30 | 0.33 | 0.38 | 0.03 | -0.05 | 0.60 | 0.45 | 0.44 | | | | | | | | | | | | | |
| 11,NOR | 0.55 | 0.52 | 0.53 | 0.57 | 0.18 | 0.02 | 0.80 | 0.63 | 0.36 | 0.46 | | | | | | | | | | | | |
| 12,FH | 0.36 | 0.33 | 0.33 | 0.29 | -0.04 | -0.02 | 0.36 | 0.26 | -0.05 | 0.03 | 0.38 | | | | | | | | | | | |
| 13,PH | 0.18 | 0.20 | 0.21 | 0.25 | 0.19 | -0.07 | 0.31 | 0.29 | 0.13 | 0.40 | 0.38 | 0.01 | | | | | | | | | | |
| 14,SH | 0.19 | 0.21 | 0.20 | 0.24 | 0.09 | 0.20 | 0.03 | 0.19 | -0.04 | 0.01 | 0.15 | -0.01 | -0.04 | | | | | | | | | |
| 15,DH | 0.42 | 0.37 | 0.37 | 0.43 | 0.02 | 0.07 | 0.51 | 0.38 | -0.02 | 0.14 | 0.50 | 0.41 | 0.05 | 0.10 | | | | | | | | |
| 16,AA | 0.20 | 0.25 | 0.25 | 0.19 | -0.28 | 0.26 | 0.30 | 0.14 | -0.03 | 0.16 | 0.22 | 0.28 | -0.15 | 0.00 | 0.16 | | | | | | | |
| 17,PD | -0.01 | 0.06 | 0.07 | 0.06 | -0.19 | -0.06 | 0.17 | 0.03 | 0.02 | 0.15 | -0.01 | -0.02 | -0.06 | -0.03 | 0.03 | 0.23 | | | | | | |
| 18,DD | 0.06 | 0.12 | 0.13 | 0.12 | -0.21 | -0.08 | 0.19 | 0.11 | 0.00 | 0.13 | 0.03 | 0.23 | -0.09 | -0.02 | 0.14 | 0.32 | 0.91 | | | | | |
| 19,ABD | -0.03 | 0.04 | 0.05 | 0.04 | -0.27 | -0.07 | 0.19 | 0.04 | 0.02 | 0.24 | 0.00 | -0.07 | -0.04 | -0.02 | -0.03 | 0.46 | 0.90 | 0.81 | | | | |
| 20,RT | 0.07 | 0.13 | 0.13 | 0.19 | 0.13 | -0.05 | 0.41 | 0.30 | 0.02 | 0.04 | 0.53 | 0.05 | 0.18 | 0.03 | 0.27 | 0.11 | 0.01 | 0.05 | 0.02 | | | |
| 21,CT | 0.20 | 0.26 | 0.27 | 0.29 | -0.03 | 0.10 | 0.39 | 0.25 | -0.02 | 0.28 | 0.38 | -0.02 | 0.21 | 0.07 | 0.35 | 0.37 | 0.19 | 0.22 | 0.24 | 0.54 | | |
| 22,TCT | 0.29 | 0.32 | 0.32 | 0.34 | 0.19 | 0.15 | 0.49 | 0.18 | -0.03 | 0.13 | 0.48 | 0.18 | 0.04 | 0.24 | 0.36 | -0.01 | -0.06 | 0.16 | 0.35 | 0.29 | | |

R-values greater than 0.24 are significant at 5%

**Table 4.10 : Prediction equations for all the parameters**

| | | |
|---|---|---|
| Testosterone (nmol/l) | = | 1.63+ 0.82 Semen volume(ml) |
| Testosterone (nmol/l) | = | 2.33+ 0.34 abnormal acrosome(%) |
| Body weight (kg) | = | 66.6+ 2.03 Age (weeks) |
| Heartgirth (cm) | = | 98.9+ 0.50 Age (weeks) |
| Scrotal circumference (cm) | = | 13.1+ 0.16 Age (weeks) |
| Heartgirth (cm) | = | 78.43 + 0.26 Body weight (kg) |
| Scrotal circumference (cm) | = | 2.43 + 4.63 Body condition score |
| Scrotal circumference (cm) | = | 8.20 + 0.08 Body weight (kg) |
| Scrotal circumference (cm) | = | -17.7 + 0.314 Heartgirth (cm) |
| Semen volume (ml) | = | 0.01 Bodyweight(kg) |
| Semen volume(ml) | = | 0.04 Heartgirth(cm) |
| Semen volume(ml) | = | 0.15 Scrotal circumference(cm) |
| Semen colour | = | 0.01Bodyweight(kg) |
| Semen colour | = | 0.03 Heartgirth (cm) |
| Semen colour | = | 0.09 Scrotal circumference(cm) |
| Live sperm Cell (%) | = | 38.0+ 0.18 Semen concentration/mlx$10^6$ |
| Live sperm Cell (%) | = | 4.03 Scrotal circumference (cm) |
| Live sperm cell(%) | = | 1.2 Heartgirth(cm) |
| Live sperm cell(%) | = | 0.31 Bodyweight(kg) |
| Live sperm Cell (%) | = | 11.9+ 0.79 Normal sperm (%) |
| Live sperm cell(%) | = | 27.7+1.04 motility(%) |
| Live sperm cell(%) | = | 21.7+15.1 Semen volume(ml) |
| Normal sperm (%) | = | 0.27 Bodyweight(kg) |
| Normal sperm (%) | = | 1.04 Heartgirth(cm) |
| Normal sperm (%) | = | 3.43 Scrotal circumference(cm) |
| Normal sperm (%) | = | 18.3+14.8 semen volume |
| Normal sperm (%) | = | 34.3+0.18 semen concentration/mlx$10^6$ |
| Sperm motility (%) | = | 11.2+ 0.13 Semen concentration/mlx$10^6$ |
| Sperm motility(%) | = | 0.09 Bodyweight(kg) |
| Sperm motility(%) | = | 0.37Heartgirth(cm) |
| Sperm motility(%) | = | 1.30 Scrotal circumference(cm) |
| Sperm motility(%) | = | 6.16+5.94 semen volume(ml) |
| Sperm concentration/mlx$10^6$ | = | 3.04 Scrotal circumference(cm) |
| Sperm concentration/mlx$10^6$ | = | 3.30+16.2 semen volume(ml) |

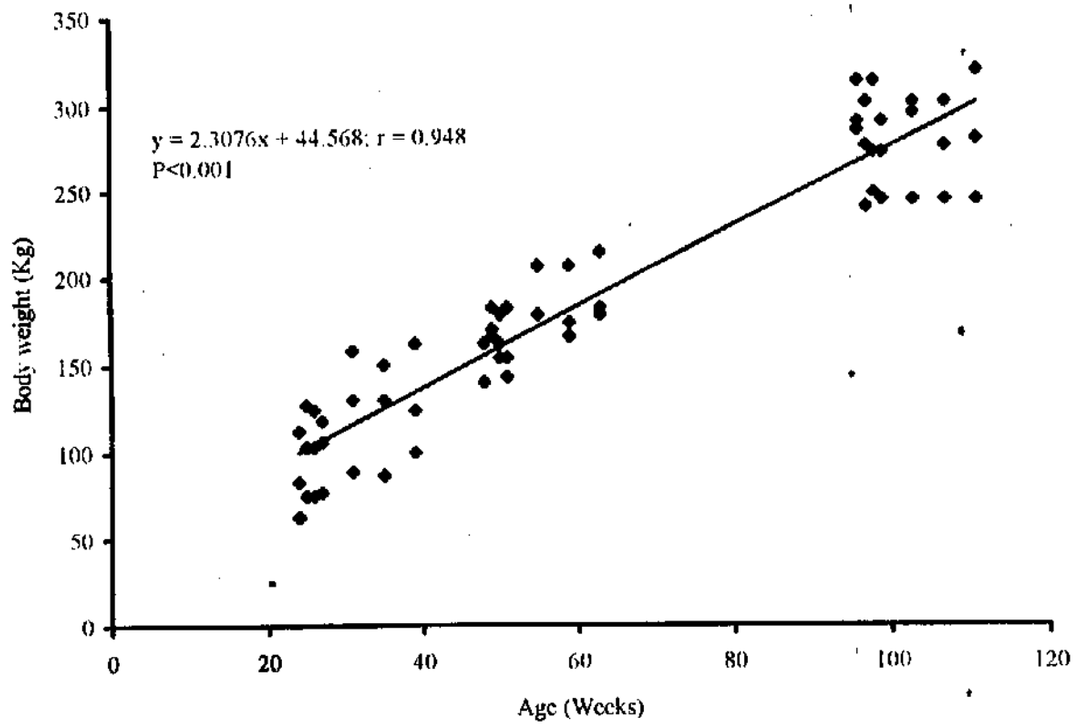



Figure 4.1: Relationship between age and body weight in Bunaji bulls

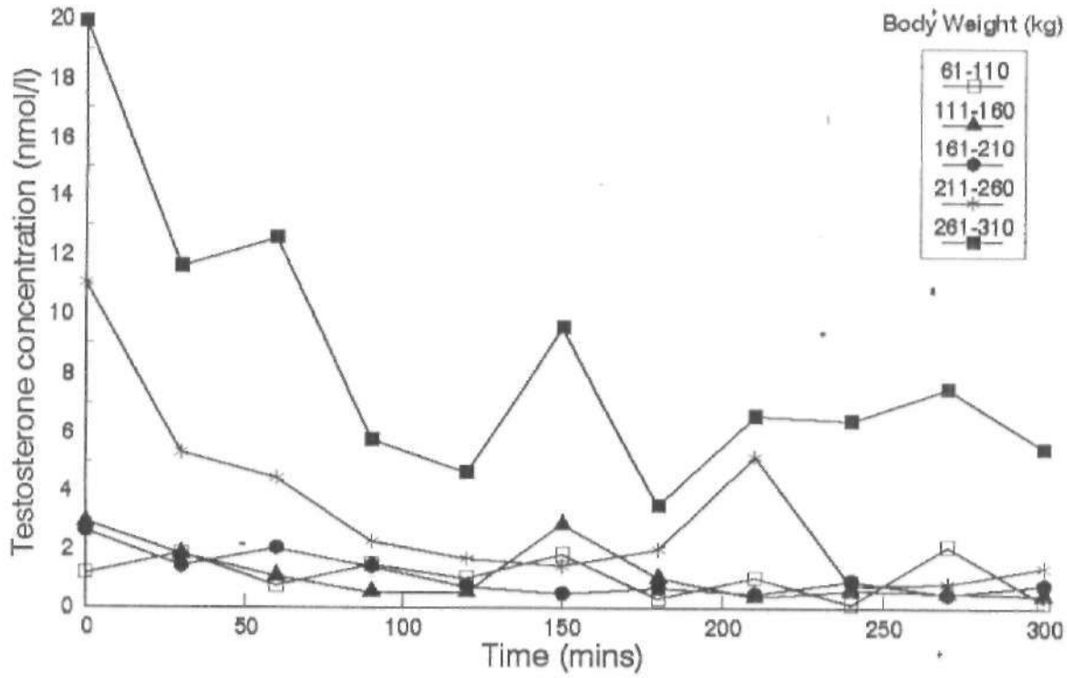



Figure 4.2: Testosterone secretion pattern in relation to body weight.

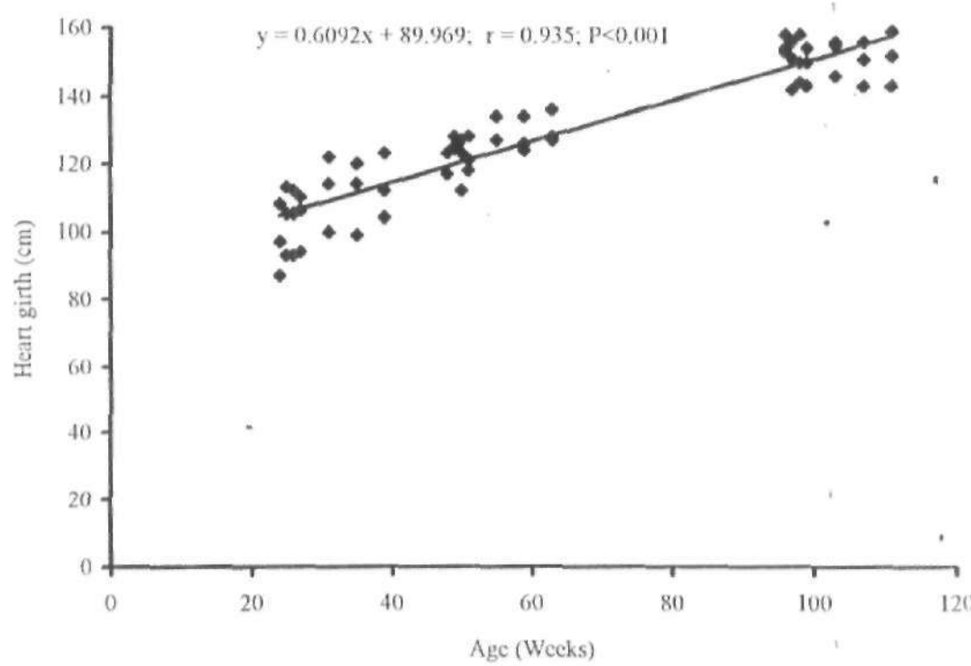



Figure 4.3 Relationship between age and heart girth in Bunaji bulls

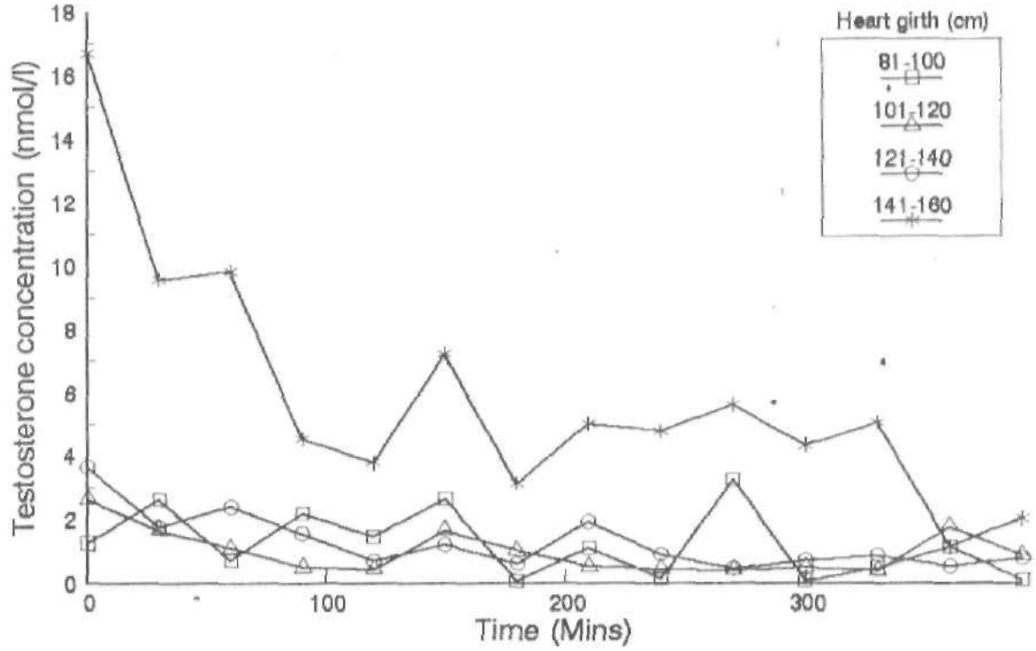



Figure 4.4: Testosterone secretion pattern in relation to heartgirth.

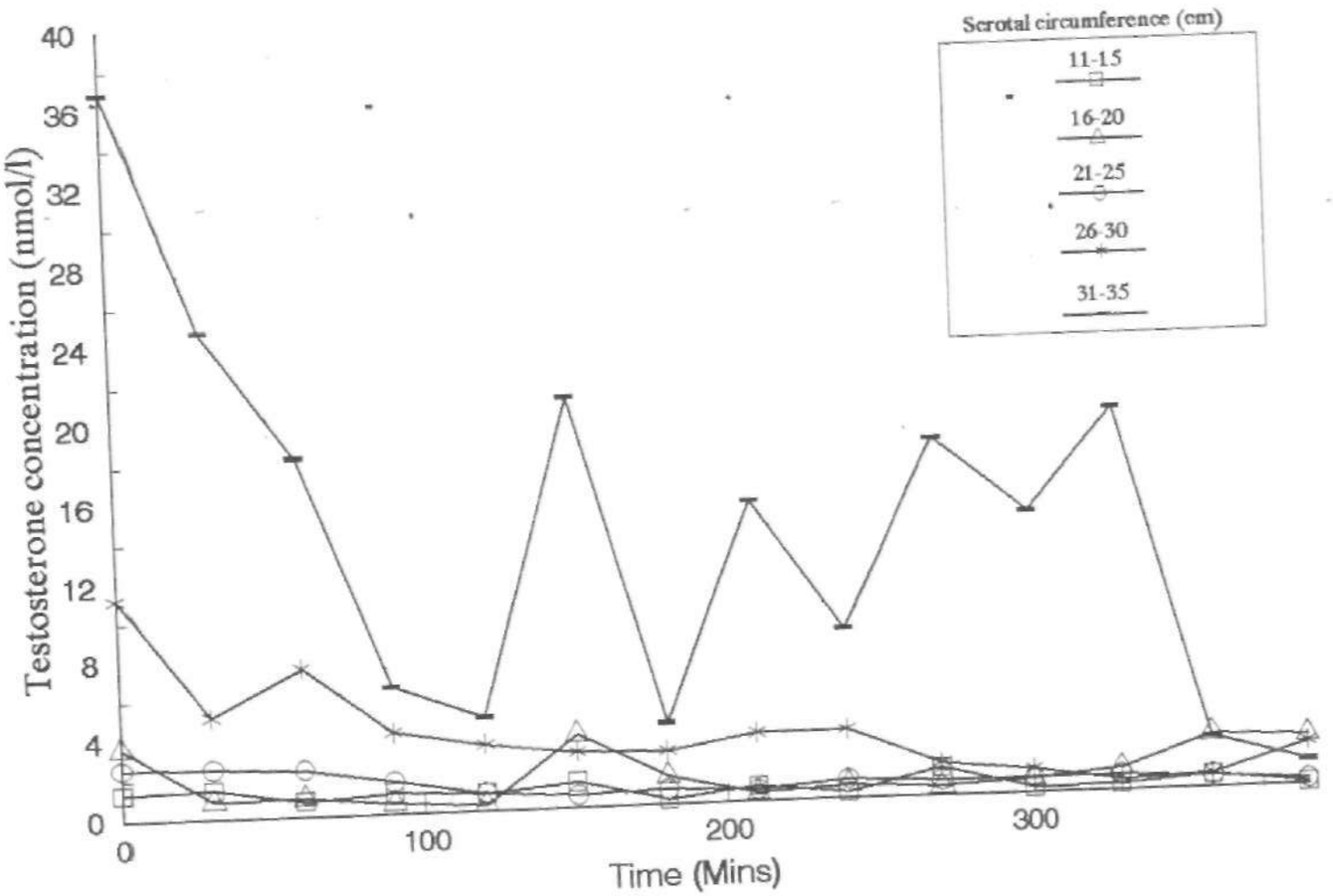



Figure 4.6: Testosterone concentration in relation to scrotal circumference.

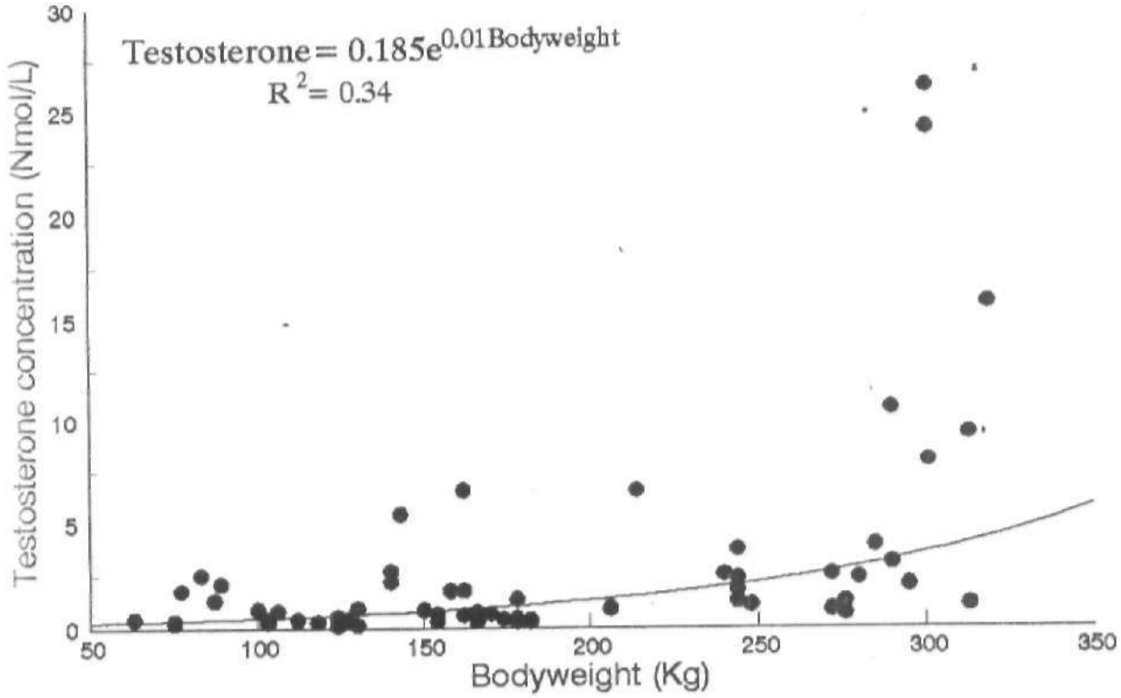



Figure 4.7: Relationship between testosterone concentration and bodyweight of Bunaji bulls.

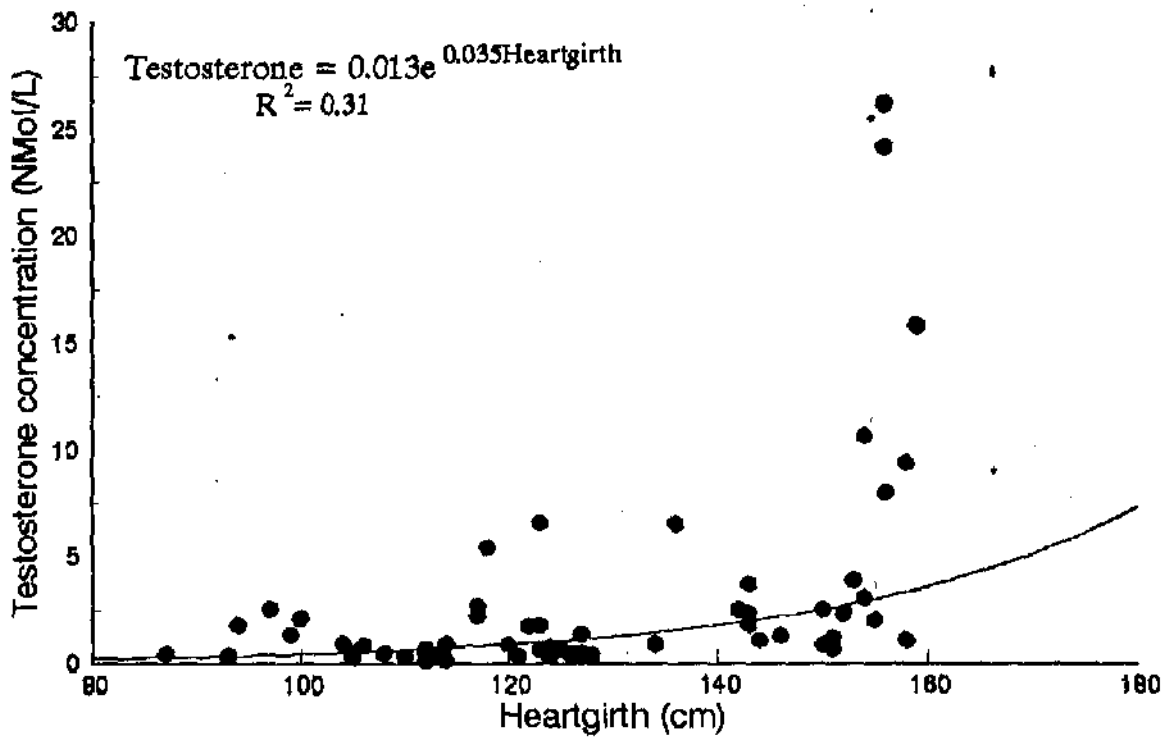



Figure 4.8: Relationship between testosterone concentration and heartgirth of Bunaji bulls.

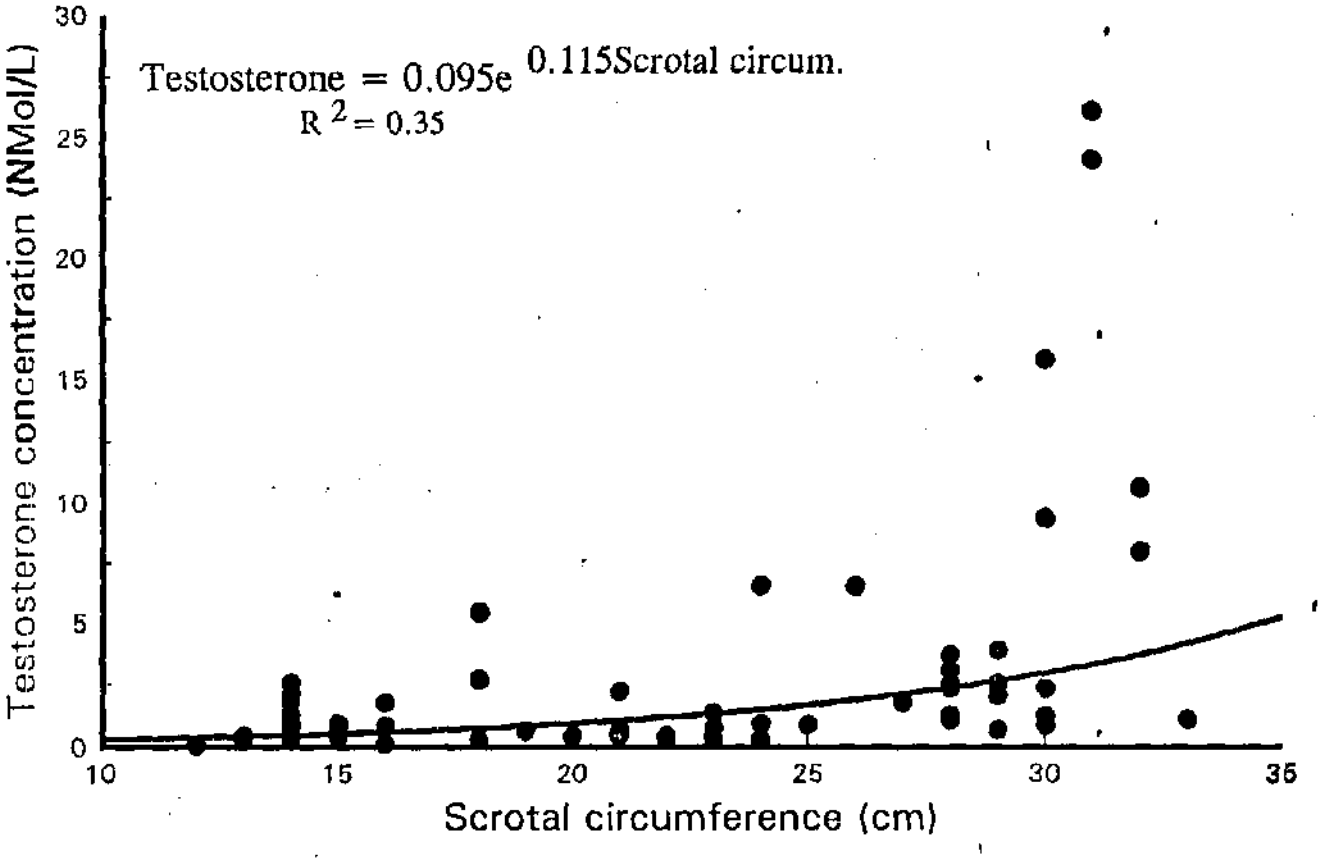



Figure 4.9: Relationship between testosterone concentration and scrotal circumference of Bunaji bulls.

## CHAPTER FIVE

## 5.0 GENERAL DICUSSION

This study has provided the missing link in research on the Bunaji male animals. Although there remains a paucity of data on the mature Bunaji bulls (as all the experimental animals were growing), these data on the developing bulls may serve as a baseline information with respect to the endocrine activity of the breed.

The basal, peak and total T concentration were mainly associated with age, BWT, HGT and SC of the animals. Selecting a male on the basis of its BWT and SC will put a high selection pressure as the study showed these influenced T concentration and consequently semen parameters. The quality of semen improved with age, SC and T concentration. The larger and heavier the testes, the higher the sperm producing parenchyma, the higher the T concentration, the lower the percent abnormality. However, some studies have shown that the percentage of live sperm was less with increased BWT for buffalo bulls and goats (Pant and Mukherjee, 1973; Misra, 1976).

The animals in this study could be said to have passed the breeding soundness examination of the Society for Theriogenology (1980) as over 60% of the sperm cells were normal and alive.

Correlation between reproductive characteristics have often been obtained with bulls at commercial artificial insemination centres. Although such information may be of immense value, such bulls typically have survived rigorous culling for testicular size, semen

quality and fertility. In contrast, selection intensity was minimal for the bulls examined in this study. Selection was not even limited to the passing of the breeding soundness examination of the Society for Theriogenology (1980) which is based on 3 criteria; viz scrotal circumference (40 points), spermatozoal morphologic features (40 points) and gross motility of unfrozen semen (20 points). A score of $\geq 60$ was regarded as satisfactory.

The variations observed in the standard errors for plasma testosterone could be due to assay technique, and variation among bulls (Physiological maturity).

The difference observed in plasma testosterone levels between this work and other reports (Swanson *et al.*, 1971 Rawlings *et al.*, 1972; Mongkonpunya *et al.*, 1975; Amann and Walker 1983; Cooper *et al.*, 1987, Rekwot *et al.*, 1997) can be attributed to breed differences.

## CONCLUSION

T concentration has exponential relationship with BWT, HGT and SC. The larger and heavier the animals the higher the basal, peak and total T concentrations.

Semen quality improved with increased T concentration as the study showed that T is positively correlated to semen characteristics but negatively correlated to cytoplasmic droplets and tail abnormalities. The incidence of abnormal sperm reduced with age accompanied by increased T concentration.

From the results obtained in this study,

1. Yearling Bunaji bull can be selected for natural mating or artificial insemination. This will help to reduce generation interval and increase the number of offspring from desired

sires. Moreover, the bull can be used for a longer period.

2. The weight gain of the prepubertal bulls makes them good for fattening programme.

3. The Bunaji bull can be selected for use in AI centres as their T concentration, body growth and semen pictures were not inferior to the other tropical breeds and competes favourably with that of the *Bos taurus* breeds.

**Summary**

The pattern of testosterone secretions in 6(A), 12(B), and 24(C) months old Bunaji bulls; and its relationship with body growth and semen parameters were determined for B and C group of animals.

The basal and peak T concentrations for the Bunaji breed of cattle are 2.77±8.18 and 5.69±4.51nmol/l. The basal, peak and total T concentrations for groups A, B and C animals were 1.47±5.62, 1.49±1.54 and 0.87±0.41nmol/l; 0.55±1.46, 2.89±3.11 and 1.54±1.18nmol/l; and 1.62±14.16, 11.37±10.11 and 5.93±4.32nmol/l. The groups B and C animals displayed markedly higher number of peaks, basal and peak T concentrations than the younger group. The groups exhibited significant diurnal fluctuation in plasma T concentration.

T concentration had exponential relationship with age, BWT, HGT, and SC ($P^3 < 0.001$). A negative correlation exists between T and the incidence of cytoplasmic droplets and tail abnormalities. T concentrations were higher in the dry season than in the wet season associated with higher BWT, HGT and SC but poor BCS with the latter being higher in the wet season for all the groups.

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# APPENDIX

**Appendix 1:** Composition of the stains used for semen analysis

1 Nigrosin - Eosin stain

a) Nigrosin solution: 20g Nigrosin added to 100ml distilled $H_20$and dissolved by boiling.

b) Stock Buffer Solution

(1) $N_{a2}$ $HP0_4$ .2H2O 21.628g to 500ml Mix 200ml(1)+80ml (2)

(2) $KH_2P0_4$ 22.254g to 500ml

c) Stock glucose solution

Glucose 43.3g to 500ml with Distilled $H_20$.

Nigrosin - Eosin stain

Nigrosin solution 150 ml

Eosin yellow 59ml

Stock buffer solution 30ml

Stock glucose solution 20ml

Dist. $H_20$ to 300ml

2 Buffered formal saline

| | |
|---|---|
| Stock buffer solution (as for Nigrosin - eosin) | 100ml |
| Stock Nacl (9.01g Nacl/500ml) | 150ml |
| Commercial formalin | 62.5ml |
| Dist. H20 to | 500ml |

3 Live:Dead

1) 1 drop of semen was added to 4 - 5 drops of stain.
2) It was mixed with a pasteur pipette.
3) It was allowed to stand for 3 minutes.
4) Two smears were prepared by placing a small drop of semen on the end of each slide.
5) A second slide held at an angle of 45° was used to draw out the drops along the slide.
6) It was air dried and stored for later examination.
7) Smears were examined under microscope counting 100 spermatozoa in random fields on the slide. Spermatozoa in which the head took up the stain were classed as dead.

8) The count was expressed as percentage live.

4 Morphology

1) At collection 0.1ml semen was added to 4.9ml buffered formol saline.
2) A drop of the mixture was added to a slide, with a coverslip and examined under phase contrast at high power (x 450).
3) 400 spermatozoa were counted and the percentage of abnormal sperm based on the morphology of the midpiece and tail, proximal or distal droplets decapitated sperm, and bent and coiled tails were recorded.

5 Concentration

1) Both chambers of the haemocytometer carefully was filled with the diluted semen.
2) It was allowed to stand for 4 - 5 minutes to allow the spermatozoa to settle.
3) The counting chamberlines was located using a magnification of x 320.
4) 5 large squares each containing 16 small squares were counted.
5) Only sperm heads touching top or right hand lines,ejecting those which touch the left hand or bottom lines of the larger squares were counted.
6) Both chambers were counted and the mean determined.
7) Spermatozoa/ml was calculated.

**Appendix 2: The general hormone profile, and the groups hormone profile values with time**

| Time(mins) | General | 6mnths(A) | 12mnths(B) | 24mnths(C) |
|---|---|---|---|---|
| 0 | 7.49 | 1.48 | 4.28 | 16.70 |
| 30 | 4.45 | 1.22 | 2.54 | 9.57 |
| 60 | 4.37 | 0.90 | 2.38 | 9.84 |
| 90 | 2.36 | 0.99 | 1.55 | 4.54 |
| 120 | 1.77 | 0.75 | 0.73 | 3.82 |
| 150 | 3.50 | 1.75 | 1.51 | 7.24 |
| 180 | 1.51 | 0.22 | 1.17 | 3.15 |
| 210 | 2.54 | 0.59 | 1.96 | 5.05 |
| 240 | 2.01 | 0.43 | 0.79 | 4.80 |
| 270 | 2.49 | 1.24 | 0.59 | 5.64 |
| 300 | 1.80 | 0.30 | 0.75 | 4.35 |
| 330 | 2.12 | 0.57 | 0.71 | 5.08 |
| 360 | 1.13 | 0.57 | 1.67 | 1.15 |
| 390 | 1.17 | 0.62 | 0.83 | 2.05 |

Appendix 3: Hormone profiles for the different animals in the different groups

| | | Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | | | B | | | | C |
| Time(mins) | 1082 | 1280 | 1274 | 1601 | 1658 | 1278 | 0114 | 0115 | 0116 |
| 0 | 1.05 | 1.24 | 2.14 | 7.05 | 1.47 | 4.33 | 34.60 | 6.97 | 8.52 |
| 30 | 2.43 | 0.81 | 0.42 | 4.10 | 2.30 | 1.23 | 19.93 | 4.28 | 4.50 |
| 60 | 0.31 | 1.14 | 1.24 | 5.52 | 1.08 | 0.54 | 22.18 | 4.23 | 3.10 |
| 90 | 0.01 | 2.51 | 0.46 | 3.52 | 0.76 | 0.38 | 11.00 | 1.03 | 1.58 |
| 120 | 0.48 | 1.51 | 0.27 | 1.03 | 0.87 | 0.29 | 7.37 | 2.10 | 2.00 |
| 150 | 2.31 | 0.78 | 2.17 | 0.87 | 0.47 | 3.20 | 19.27 | 0.92 | 1.52 |
| 180 | 0.02 | 0.50 | 0.14 | 0.51 | 1.00 | 2.03 | 5.48 | 1.50 | 2.46 |
| 210 | 1.07 | 0.56 | 0.14 | 4.49 | 0.85 | 0.55 | 11.94 | 0.98 | 2.23 |
| 240 | 0.01 | 0.31 | 0.98 | 0.58 | 1.46 | 0.33 | 11.95 | 1.34 | 1.11 |
| 270 | 3.23 | 0.14 | 0.34 | 0.42 | 1.10 | 0.26 | 14.75 | 0.88 | 1.29 |
| 300 | 0.02 | 0.39 | 0.49 | 1.30 | 0.26 | 0.70 | 10.50 | 1.24 | 1.29 |
| 330 | 0.30 | 0.28 | 1.14 | 1.14 | 0.45 | 0.53 | 14.11 | 0.71 | 0.42 |
| 360 | 1.21 | 0.07 | 0.43 | 0.82 | 0.28 | 3.90 | 1.96 | 0.55 | 0.94 |
| 390 | 0.13 | 0.04 | 1.70 | 0.17 | 0.29 | 2.03 | 5.30 | 0.33 | 0.51 |

**Appendix 4: Profiles for the different bodyweight(kg) and heartgirth(cm) classes**

| | Bodyweight classes | | | | | Heartgirth classes | | | |
|---|---|---|---|---|---|---|---|---|---|
| Time | 61-110 | 111-160 | 161-210 | 211-260 | 261-310 | 81-100 | 101-120 | 121-140 | 141-160 |
| 0 | 1.17 | 2.92 | 2.62 | 11.03 | 19.91 | 1.28 | 2.64 | 3.67 | 16.70 |
| 30 | 1.85 | 1.83 | 1.43 | 5.32 | 11.61 | 2.63 | 1.68 | 1.78 | 9.57 |
| 60 | 0.75 | 1.07 | 2.03 | 4.43 | 12.57 | 0.73 | 1.11 | 2.41 | 9.84 |
| 90 | 1.48 | 0.54 | 1.41 | 2.27 | 5.77 | 2.20 | 0.52 | 1.57 | 4.54 |
| 120 | 1.01 | 0.53 | 0.73 | 1.67 | 4.63 | 1.48 | 0.45 | 0.71 | 3.82 |
| 150 | 1.83 | 2.85 | 0.49 | 1.42 | 9.53 | 2.67 | 1.68 | 1.22 | 7.24 |
| 180 | 0.31 | 1.02 | 0.69 | 1.99 | 3.50 | 0.09 | 1.04 | 0.63 | 3.15 |
| 210 | 1.03 | 0.38 | 0.48 | 5.19 | 6.61 | 1.12 | 0.55 | 1.95 | 5.05 |
| 240 | 0.14 | 0.59 | 0.92 | 0.78 | 6.44 | 0.15 | 0.45 | 0.92 | 4.80 |
| 270 | 2.11 | 0.51 | 0.47 | 0.84 | 7.54 | 3.26 | 0.42 | 0.47 | 5.64 |
| 300 | 0.22 | 0.45 | 0.80 | 1.38 | 5.48 | 0.08 | 0.50 | 0.72 | 4.35 |
| 330 | 0.36 | 0.87 | 0.64 | 0.48 | 6.91 | 0.49 | 0.41 | 0.89 | 5.08 |
| 360 | 0.80 | 2.04 | 0.56 | 1.03 | 1.18 | 1.16 | 1.78 | 0.54 | 1.15 |
| 390 | 0.09 | 1.04 | 0.92 | 0.55 | 2.63 | 0.10 | 0.90 | 0.81 | 2.05 |

**Appendix 5: Profile for the different scrotal circumference classes (cm)**

| | Scrotal circumference classes | | | | |
|---|---|---|---|---|---|
| **Time** | **11-15** | **16-20** | **21-25** | **26-30** | **31-35** |
| 0 | 1.34 | 3.66 | 2.60 | 11.26 | 36.88 |
| 30 | 1.46 | 0.85 | 2.52 | 5.16 | 24.73 |
| 60 | 0.77 | 0.94 | 2.37 | 7.55 | 18.27 |
| 90 | 1.06 | 0.55 | 1.63 | 4.12 | 6.47 |
| 120 | 0.88 | 0.28 | 0.89 | 3.37 | 4.77 |
| 150 | 1.30 | 3.71 | 0.61 | 2.84 | 20.89 |
| 180 | 0.24 | 1.43 | 0.74 | 2.70 | 4.12 |
| 210 | 0.73 | 0.38 | 0.58 | 3.46 | 15.32 |
| 240 | 0.28 | 0.66 | 0.94 | 3.47 | 8.63 |
| 270 | 1.38 | 0.42 | 0.73 | 1.65 | 18.19 |
| 300 | 0.24 | 0.68 | 0.76 | 1.18 | 14.36 |
| 330 | 0.28 | 1.10 | 0.77 | 0.55 | 19.54 |
| 360 | 0.63 | 2.73 | 0.60 | 0.70 | 2.58 |
| 390 | 0.09 | 2.51 | 0.25 | 2.18 | 1.24 |

Appendix 6: Effect of body weight on semen characteristics.

| Body weight (KG) | LSC,% | SV,ml | SCo | SC x 106/ml | SM,% | NS, % |
|---|---|---|---|---|---|---|
| 111 - 160 | 68.00±6.62 | 2.03±0.87 | watery | 10.33±47.96 | 0.67±11.70 | 51.33±16.11 |
| 161 - 210 | 73.86±3.06 | 1.89±0.40 | Milky | 25.36±22.20 | 29.07±5.42 | 64.43±7.46 |
| 211 - 260 | 84.00±4.33 | 3.40±0.57 | Milky | 162.86±31.40 | 48.57±7.66 | 76.71±10.55 |
| 261 - 310 | 72.75±3.31 | 2.51±0.44 | Milky | 9.83±23.98 | 13.75±5.85 | 65.17±8.06 |

Appendix 7: Percent sperm abnormalities for different Body weights (kg).

| BWT | FH | PH | SH | DH | PD | DD | ABD | RT | CT | TCT | AA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 111-160 | 1.00±3.47 | 4.00±5.70 | 2.00±3.00 | 2.33±1.18 | 0.00±3.98 | 0.33±0.84 | 0.00±1.98 | 3.67±1.23 | 1.33±1.11 | 0.33±1.19 | 0.33±2.82 |
| 161-210 | 3.36±1.61 | 7.93±2.64 | 0.29±1.39 | 1.14±0.55 | 3.64±1.84 | 0.71±0.39 | 2.36±0.92 | 2.43±0.57 | 1.57±0.51 | 2.14±0.55 | 3.00±1.30 |
| 211-260 | 1.43±2.27 | 4.86±3.73 | 0.00±1.96 | 1.00±0.77 | 0.00±2.61 | 0.00±0.55 | 0.00±1.29 | 0.43±0.80 | 0.29±0.73 | 0.43±0.78 | 0.43±1.84 |
| 261-310 | 6.25±1.74 | 5.25±2.85 | 3.33±1.50 | 2.58±0.59 | 0.58±1.99 | 0.42±0.42 | 0.083±0.99 | 1.42±0.61 | 1.67±0.55 | 2.25±0.60 | 3.17±1.41 |

Appendix 8: Effect of heartgirth on semen characteristics.

| Heartgirth (cm) | LSC,% | SV,ml | SCo | SC x 106/ml | SM,% | NS, % |
|---|---|---|---|---|---|---|
| 101 - 120 | 62.50±8.39 | 1.40±1.05 | watery | 10.50±70.42 | 1.00±16.92 | 41.00±19.49 |
| 121 - 140 | 74.88±2.97 | 1.91±0.37 | Milky | 27.81±24.90 | 30.44±5.98 | 66.56±6.89 |
| 141 - 160 | 76.44±2.80 | 2.95±0.35 | Milky | 65.44±23.47 | 23.61±5.64 | 68.22±6.50 |

**Appendix 9: Percent sperm abnormalities for different heartgirths (cm)**

| Heartgirth | FH | PH | SH | DH | PD | DD | ABD | RT | CT | TCT | AA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 101-120 | 0.00±4.35 | 3.50±6.91 | 1.50±3.72 | 0.50±1.49 | 0.00±4.84 | 0.00±1.02 | 0.00±2.42 | 3.50±1.54 | -0.00±1.36 | -0.00±1.52 | -0.00±3.48 |
| 121 - 140 | 3.19±1.54 | 7.38±2.44 | 0.44±1.32 | 1.50±0.53 | 3.19±1.71 | 0.69±0.36 | 2.06±0.86 | 2.38±0.54 | 1.69±0.48 | 1.94±0.54 | 2.88±1.23 |
| 141 - 160 | 4.67±1.45 | 5.28±2.30 | 2.22±1.24 | 2.00±0.50 | 0.39±1.61 | 0.28±0.34 | 0.06±0.81 | 1.11±0.51 | 1.17±0.45 | 1.67±0.51 | 2.11±1.16 |

Appendix 10: Effect of scrotal circumference on semen characteristics

| SC | LSC,% | SV,ml | SCo | SC x $10^6$/ml | SM,% | NS, % |
|---|---|---|---|---|---|---|
| 16-20 | 67.85±5.00 | 1.76±0.67 | watery | 8.15±44.73 | 18.04±10.25 | 61.94±12.20 |
| 21-25 | 75.42±3.22 | 1.94±0.43 | watery | 25.49±28.89 | 26.59±6.62 | 63.24±7.88 |
| 26-30 | 79.67±3.01 | 2.83±0.40 | Milky | 93.12±26.91 | 31.04±6.17 | 71.64±7.34 |
| 31-35 | 67.87±5.00 | 2.96±0.67 | Milky | 127±44.75 | 14.04±10.25 | 60.71±12.21 |

**Appendix 11: Percent sperm abnormalities for scrotal circumference of different sizes.**

| SC | FH | PH | SH | DH | PD | DD | ABD | RT | CT | TCT | AA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 - 20 | 3.59±2.90 | 6.17±3.10 | 0.31±2.17 | 0.12±0.86 | 0.78±3.13 | 0.09±0.61 | 0.41±1.38 | 1.52±0.82 | 0.63±0.90 | 1.23±1.00 | 2.11±1.82 |
| 21 - 25 | 2.90±1.86 | 7.22±2.00 | 0.69±1.40 | 2.01±0.55 | 3.90±2.02 | 0.84±0.39 | 2.55±0.89 | 3.02±0.52 | 1.90±0.58 | 2.02±0.65 | 2.85±1.18 |
| 26 - 30 | 4.96±1.73 | 5.35±1.87 | 0.66±1.31 | 2.19±0.52 | 0.54±1.89 | 0.42±0.37 | 0.13±0.83 | 1.17±0.49 | 1.22±0.54 | 1.30±0.60 | 2.18±1.10 |
| 31 - 35 | 2.56±2.90 | 5.51±3.10 | 6.18±2.17 | 1.14±0.86 | -0.06±3.14 | -0.09±0.60 | -0.08±1.38 | 0.96±0.82 | 0.99±0.90 | 2.49±1.00 | 1.74±1.82 |

Appendix 12: **Mean testosterone concentrations (NMol/L) for BWT,kg, HGT,cm andSC,cm of Bunaji bulls**

| Parameters | class | T concentration |
|---|---|---|
| Bodyweight | 61 - 110 | 0.94±2.55 |
| | 111 - 160 | 1.19±3.15 |
| | 161 - 210 | 1.01±2.62 |
| | 211 - 260 | 2.74±4.76 |
| | 261 - 310 | 7.45±13.91 |
| Heartgirth | 81 - 110 | 1.25±3.13 |
| | 101 - 120 | 1.01±2.81 |
| | 121 - 140 | 1.31±3.58 |
| | 141 - 160 | 5.93±12.09 |
| Scrotal | 11 - 15 | 0.76±2.11 |
| circumference | 16 - 20 | 1.42±3.60 |
| | 21 - 25 | 1.14±2.84 |
| | 26 - 30 | 3.59±6.60 |
| | 31 - 35 | 14.00±20.11 |

Appendix 13: **Mean body measurements and testosterone concentrations for all the experimental animals in the different groups**

| Group | Tag No. | BWT | HGT | SC | BCS | TT |
|---|---|---|---|---|---|---|
| A | 1082 | 80.86±12.06 | 95.71±5.65 | 13.57±0.53 | 3.79±0.39 | 1.01±0.74 |
| | 1274 | 135.86±20.33 | 115.43±6.11 | 15.29±0.76 | 3.93±0.35 | 0.86±0.64 |
| | 1280 | 111.29±17.45 | 107.57±6.19 | 14.14±1.21 | 4.14±0.38 | 0.74±0.86 |
| B | 1278 | 162.43±16.95 | 121.71±6.07 | 19.00±1.53 | 3.93±0.35 | 1.45±1.94 |
| | 1601 | 190.00±18.90 | 130.00±4.73 | 24.14±1.07 | 4.21±0.39 | 2.25±2.94 |
| | 1658 | 164.00±13.61 | 123.43±3.55 | 21.71±0.95 | 4.00±0.41 | 0.90±0.66 |
| C | 0114 | 305.43±9.96 | 156.71±1.70 | 31.29±1.11 | 4.36±0.38 | 13.60±9.02 |
| | 0115 | 279.43±8.24 | 151.71±1.80 | 29.43±0.53 | 4.29±0.39 | 1.93±1.13 |
| | 0116 | 250.57±17.54 | 145.00±4.16 | 27.86±0.38 | 4.29±0.39 | 2.25±0.95 |

Appendix 14: **Seasonal body measurement and testosterone changes for the animals in the different groups.**

| Season | Tag No. | BWT | HGT | SC | BCS | TT |
|---|---|---|---|---|---|---|
| Dry | 1082 | 92.00±7.00 | 101.00±2.65 | 14.00±0.00 | 3.5±0.50 | 1.44±0.61 |
| Wet | 1082 | 72.50±6.40 | 91.75±3.20 | 13.25±0.50 | 4.00±0.00 | 0.70±0.73 |
| Dry | 1274 | 156.67±6.11 | 121.67±1.53 | 16.00±0.00 | 3.83±0.29 | 1.46±0.53 |
| Wet | 1274 | 120.25±6.65 | 110.75±2.22 | 14.75±0.50 | 4.00±0.41 | 0.42±0.10 |
| Dry | 1280 | 128.00±3.46 | 113.33±1.15 | 14.33±2.08 | 3.83±0.29 | 0.37±0.48 |
| Wet | 1280 | 98.75±10.59 | 103.25±4.19 | 14.00±0.00 | 4.38±0.25 | 1.01±1.04 |
| Dry | 1278 | 178.00±4.00 | 127.00±1.00 | 20.00±2.00 | 4.00±0.50 | 0.37±0.10 |
| Wet | 1278 | 150.75±11.81 | 117.75±4.92 | 18.25±0.50 | 3.88±0.25 | 2.26±2.35 |
| Dry | 1601 | 208.67±4.62 | 134.67±1.15 | 25.00±1.00 | 4.00±0.50 | 2.77±3.25 |
| Wet | 1601 | 176.00±9.52 | 126.50±2.38 | 23.50±0.58 | 4.38±0.25 | 1.87±3.12 |
| Dry | 1658 | 174.00±6.93 | 126.00±1.73 | 22.33±1.15 | 4.00±0.50 | 0.85±0.46 |
| Wet | 1658 | 156.50±12.79 | 121.50±3.42 | 21.25±0.50 | 4.00±0.41 | 0.95±0.85 |
| Dry | 0114 | 307.00±10.39 | 157.00±1.73 | 31.00±1.00 | 4.00±0.00 | 15.98±8.07 |
| Wet | 0114 | 304.25±11.06 | 156.50±1.91 | 31.50±1.29 | 4.63±0.25 | 11.81±10.46 |
| Dry | 0115 | 283.67±10.02 | 152.67±2.08 | 29.67±0.58 | 4.00±0.00 | 1.87±0.57 |
| Wet | 0115 | 276.25±6.13 | 151.00±1.41 | 29.25±0.50 | 4.50±0.41 | 1.98±1.53 |
| Dry | 0116 | 244.00±0.00 | 144.00±1.73 | 27.67±0.58 | 4.00±0.00 | 1.79±0.54 |
| Wet | 0116 | 255.50±23.23 | 145.75±5.56 | 28.00±0.00 | 4.50±0.41 | 2.59±1.12 |