COMPARATIVE STUDY OF TESTICULAR DIMENSIONS, GONADAL AND EPIDIDYMAL SPERM RESERVES OF BUNAJI, BOKOLOJI AND RAHAJI BREEDS OF ZEBU CATTLE

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN THERIOGENOLOGY

DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION
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ZARIA, NIGERIA

APRIL, 2021

DECLARATION

I declare that the work reported in this Dissertation, entitled: **"Comparative Study of Testicular Dimensions, Gonadal and Epididymal Sperm Reserves of Bunaji, Bokoloji and Rahaji Breeds of Zebu Cattle''** was done by me in the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria – Nigeria, under the supervision of Professor D. Ogwu and Professor P. I. Rekwot. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Taiwo Adebola IGE		
Name of student	Signature	Date

CERTIFICATION

This Dissertation, entitled **'COMPARATIVE STUDY OF TESTICULAR DIMENSIONS, GONADAL AND EPIDIDYMAL SPERM RESERVES OF BUNAJI, BOKOLOJI AND RAHAJI BREEDS OF ZEBU CATTLE',** by Taiwo Adebola IGE meets the regulations governing the award of the degree of Master of Science in Theriogenology and Production of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to God, my parents (Late Mr Emmanuel Oladele Ige and Mrs Margaret Omolola Ige) and Dr (Mrs) Kemi Iyun for being flawless sources of support and inspiration for my advancement in life and career.

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ABSTRACT

This study was undertaken to comparatively evaluate testicular dimensions (TD), gonadal sperm reserves (GSR) and epididymal sperm reserves (ESR) in the three major Nigeria indigenous breeds of Zebu cattle. Sixty (60) bulls comprising of 20 Bunaji, 20 Bokoloji and 20 Rahaji breeds of 3-6 years of age were used for this study. Age was estimated using their dentition. Their body weights were estimated using a girth tape. Body condition score was measured using the five-score scale. Scrotal circumference (SC) measurements were done using a flexible measuring tape. The testicles were collected post-slaughter at the abattoirs and conveyed to the laboratory using an ice insulated container and the testicles were analysed for their TD, GSR and ESR. The overall mean values for age, body weight, body condition score and SC for the Bunaji bulls were 3.50 ± 0.12 years, 329.50 ± 11.28 kg, 3.05 ± 0.20 score and 28.33 ± 0.69 cm respectively; for Bokoloji bulls were 4.10 ± 0.23 years, 392.90 ± 18.59 kg, 3.00 ± 0.11 score and 33.70 ± 1.47 cm respectively; and for the Rahaji bulls were 5.4 ± 0.17 years, 396.8 ± 13.15 kg, 3.30 ± 0.06 score and 30.90 ± 0.78 cm, respectively. Bokoloji bulls had the highest mean value of SC than Rahaji and Bunaji breeds. The overall mean values for the paired testicular volume (PTV), paired testicular weight (PTW), average testicular length (ATL) and GSR were 354.50 ± 30.52 cm^3, 347.43 ± 25.37 g, 10.72 ± 0.31 cm and 55.75 ±6.19 x 10^9/g respectively for the Bunaji bulls; 288.50 ± 18.59 cm^3, 292.55 ± 15.89 g, 10.11 ± 0.20 cm, and 45.90 ±13.31 x 10^9/g respectively for the Bokoloji bulls; and, 348.50 ± 14.31 cm^3, 331.38 ± 14.77 g, 10.89 ± 0.22 cm and 53.97 ±6.33 x 10^9/g respectively for the Rahaji bulls. Bokoloji bulls had lower values for PTV, PTW and ATL than Rahaji and Bunaji bulls and they all had similar GSR. The mean value of epididymal weights were all similar across the breed. Bunaji bulls recorded lower mean values of epididymal length when compared with the others. The overall mean count for the ESR were 178.50 ±39.22 x 10^9/gm, 24.23 ± 2.77 x 10^9/gm and 13.44 ± 2.24 x 10^9/gm for

Bunaji, Bokoloji and Rahaji bulls respectively. Bunaji bulls had significant higher mean count when compared with the others. The inter relationship between independent and dependent variables showed significant positive correlation of age with TD, GSR and ESR mostly in Bunaji bulls, few in Bokoloji bulls and mostly negative insignificant correlations in Rahaji bull. Positive and significant correlations were seen between SC with TD, GSR and ESR except between SC and ATL in Bunaji, SC and GSR in Rahaji and several in the Bokoloji bulls. Positive and significant correlations were seen between GSR with PTV and PTW in Bunaji and Bokoloji bulls, GSR with ATL in Bokoloji bulls, ESR with PEW and AEL in Bunaji and Rahaji bulls. The result of this study demonstrated that Bunaji bulls had higher mean counts of sperm reserves and a better testicular dimension in relation with the sperm reserves.

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ABBREVIATION

AEL	Average Epididymal Length
AI	Artificial Insemination
ATL	Average Testicular Length
ATP	Adenosine Triphosphate
BBSE	Bovine Breeding Soundness Examination
BCS	Body Condition Score
BSE	Breeding Soundness Evaluation
BTB	Blood-Testis Barrier
BW	Body Weight
cm	Centimetre
DBCP	Dibromochloropropane
DDT	Dichlorodiphenyl- trichloroethane
DNA	Deoxynucleic acid
ESR	Epididymal/Epididymal Sperm Reserve
FAO	Food and Agriculture Organization
FSH	Follicle-Stimulating Hormone
FSH-R	Follicle-Stimulating Hormone Receptors
g/gm	Gramme
GnRH	Gonadotropin-Releasing Hormone
GnRH-R	Gonadotropin-releasing Hormone Receptors
GSR	Gonadal Sperm Reserve
HPG	Hypothalamo-Pituitary-Gonadal
kg	Kilogramme
LH	Luteinizing Hormone
LH-R	Luteinizing Hormone Receptor
mL	Millilitre
mRNA	messenger Ribonucleic acid
NNLRS	Nigerian National Livestock Resources
PEW	Paired Epididymal Weight

PTV	Paired Testicular Volume
PTW	Paired Testicular Weight
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SC	Scrotal Circumference
SEM	Standard Error of Mean
SNP	Single Nucleotide Polymorphism
SSC	Spermatogonial Stem Cell
STF	Society for Theriogenology
TD	Testicular Dimension
TGF-β3	Transforming Growth Factor-β3
TNFα	Tumour Necrosis Factor α

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Nigeria's cattle population was estimated as 19.5 million (Rekwot, 2016) and most of the cattle herds are under the small-holder pastoral and agro-pastoral production systems. A few herds could be classified under the intensive cattle production systems (Rekwot, 2000). Nearly 90% of the cattle are located in the North (Lawal-Adebowale, 2012; Musa *et al*., 2014).

There are several indigenous breeds of cattle in Nigeria and they include Bunaji, Bokoloji, Rahaji, Adamawa Gudali, Azawak and Wadara belonging to the *Bos taurus indicus* group. Others are: Muturu, N'dama, Keteku and Kuri cattle all of *Bos taurus taurus* group (Babayemi *et al*., 2014; Gwaza and Momoh, 2016). *Bos taurus indicus* group are humped, adapted to tropical climate and are able to withstand heat and poor diet (Maina *et al*., 2010). Zebu cattle belonging to the *Bos taurus indicus* group are the majority of cattle types in Africa. They have a fatty thoracic hump on their shoulders and a large dewlap. The taurines (*Bos taurus taurus*) are humpless, long- or short-horned and shot-legged; and are usually trypanotolerant (Lawal-Adebowale, 2012).

Cattle are kept in Nigeria for milk and beef production and the hides used for leather works while the dung from cattle are used for fuel and manure, and the bulls are used for drought purposes. In many cattle owning tribes, cattle are regarded as a means of wealth and prestige and they serve as means of fulfilment and a mark of respectability and status in the society (Maina *et al*., 2010).

Reproduction is one of the most important factors affecting livestock production and its success greatly depends on a mixture of factors including genetics (Peddinti *et al*., 2008; Amann and DeJarnette, 2012), physical environment (Fuerst-Waltl *et al*., 2006), nutrition (Rekwot *et al*., 1994) and management (Rekwot *et al*., 2004; Brito *et al*., 2007; Barth *et al*., 2008; Walker *et al*., 2009). The growth and development of testes and epididymis in farm animals have been well documented by many workers (Hahn *et al*., 1969; Coulter and Foote, 1979; Osinowo *et al*., 1981; Lunstra *et al*., 1988; Bailey *et al*., 1996; Vásquez *et al*., 2003; Perumal, 2014). Measurable reproductive criteria of breeds of bulls in the tropics have been reported to include growth, scrotal circumference, testicular development, sperm morphology and fertility (Osinowo *et al*., 1981; Daudu and Shoyinka, 1983). Testicular weight is an important trait that provides an accurate estimate of the amount of parenchyma in the testis (Almquist and Amann, 1961; Amann, 1970; Smith *et al*., 1981). The testes are the biological industry in male species involved in spermatogenesis and hormonal secretion (Etim, 2015). The amount of good quality live spermatozoa produced by the testis and the ability to store them effectively, are the basic index of selection of male animal for breeding purposes (Osinowo *et al*., 1981). The size of the testis has been reported to be a good indicator of present and future sperm production as well as breeding quality of the male (Ezekwe, 1998; Perry and Petterson, 2001; Togun and Egbunike, 2006). In the adult male, Sertoli cells determine testicular size and daily sperm production (Sharpe *et al*., 2003; Johnson *et al*., 2008) but they may also account for the intra-male variation in sperm size (Mossman *et al*., 2013). Rajak *et al*. (2014) found that, in adult bulls, the Sertoli Cell Index (SCI) was positively associated with ejaculate sperm concentration, mass activity, individual motility, viability, and membrane integrity.

There is general agreement that semen characteristics are not the only criteria on which to base an evaluation of the reproductive capacity of the male (Chenoweth *et al*., 1992;

Chenoweth *et al*., 1993; Raji and Njidda, 2014). Knowledge about gonadal and epididymal sperm reserves seems to be essential for a careful assessment of male fertility (Osman and El Azab, 1974; Jindal and Panda, 1980). Togun (2009) asserted that the importance of the breeding male for fertilizing eggs is rivalled only by his genetic influence on the progeny performance.

Epididymal Sperm Reserves (ESR) represents sperm stored in the caput, corpus and cauda epididymis, and the number of spermatozoa stored in the epididymis has been said to be related to sperm production by the testes (Jindal and Panda, 1980; Ezekwe, 1998).

The Scrotal Circumference (SC) has been the most widely used and studied criterion in screening programmes as a measure indicative of morphological and physiological characteristics of gonads and quantitative and therefore, the qualitative characteristics of semen in cattle (Perumal, 2014). Measuring the scrotal circumference of young bulls is an adequate, reputable method to assess current and future sperm-producing ability. The measurement gives an estimate of the weight of the testes, which is directly related to the level of sperm production (Hamilton, 2009). Scrotal circumference is also positively correlated with semen volume and quality, and it is intimately correlated to capacity of sperm production, number of sperms ejaculated and sperm reserves (Wildeus and Entwistle 1982; Palasz *et al.*, 1994; Latif *et al*., 2009). Scrotal circumference measurements are highly correlated with factors such as age at onset of puberty in male and it is also correlated to their female progeny (Lunstra *et al*., 1978; Moser *et al*, 1996; Van Melis *et al*., 2010), yearling weight in male progeny (Bourdon and Brinks, 1986), sperm output (Almquist *et al*., 1976), and pregnancy rates (Mateos *et al*., 1978). Bulls with adequate scrotal development for their age have a higher probability of becoming satisfactory breeders than bulls with smaller scrotal circumferences (Hamilton, 2009). A positive correlation between testicular

development and semen quality has been documented through numerous studies (Fields *et al.,* 1979; Lunstra and Echternkamp 1982; Neely *et al.,* 1982; Spitzer *et al.,* 1988; Bailey *et al.,* 1996; Coe 1999; Arteaga *et al.,* 2001). Data reported by Lunstra *et al.* (1978) in *Bos taurus* bulls, indicated that 52, 74 and 92% reached puberty with 28, 29 and 30 cm of scrotal circumference, respectively.

Bailey *et al*. (1996) suggested that scrotal circumference might not be the most appropriate measure to represent the sperm production of young bulls and, consequently, the reproductive potential of these animals. According to these authors, longer testis present greater contact surface with the environment, which facilitates thermoregulation, in addition to the distribution of blood vessels and the spermatic tissue is uniform, thus the more elongated testicular forms would be more advantageous for reproduction.

Scrotal circumference has shown moderate to high heritability and high genetic correlation with other testicular measurements and semen quality traits in *Bos índicus* (Quirino, 1999). The scrotal circumference of a bull is also positively related to the fertility of his daughters (Hamilton, 2009). Heifers from sires with larger than average scrotal circumference tend to reach puberty earlier than those from bulls with smaller scrotal circumferences (Hamilton, 2009). Increased scrotal circumference in sires is also favourably correlated to their daughter's age at first breeding, pregnancy rate and days to rebreeding after calving (Hamilton, 2009). Due to low heritability, direct selection for female fertility traits has not been successful. The strong genetic relationship between scrotal circumference and female reproductive traits provides an alternative selection method (Hamilton, 2009).

1.2 Statement of Research Problem

Bunaji, Bokoloji and Rahaji breeds of cattle are fairly large breeds of cattle that are very common in Nigeria, but most of these animals are reared under the extensive or pastoral system of management, in which detailed records on birth, age, growth and reproductive characteristics are usually not done. However, the relationship between growth, body condition score, testicular development, testicular dimensions, scrotal circumference and spermatogenesis and/or semen quality are of importance in Artificial Insemination Centres and progeny test stations. Bulls play a key role in cow-calf production and represent an important source of bio-economic capital. Knowledge is required on the relationship of testicular dimension and gonadal and epididymal sperm reserves. This would enhance the possibility of early commencement of semen collection from bulls at a young age with adequate scrotal circumference and testicular dimensions and; also compare the breeds for the best reproductive parameters.

1.3 Justification of the Study

Nigerian cattle breeds are a neglected heritage that needs to be exploited more, with respect to conservation of livestock genetic resources and selection with the breeds of livestock species (Rekwot, 2016). The importance of bunaji, bokoloji and rahaji breeds to the national breeding programme made it mandatory to compare the Body Condition Score and Body Weight related changes in the scrotal circumference, testicular dimensions as well as the gonadal and epididymal sperm reserves. Scrotal Circumference (SC) measurement is an important feature of breeding soundness examination of bulls which has long been used as an indicator for the estimation of the sperm producing ability and fertility of bulls. The semen volume, sperm concentration, motility and morphology are attributes having positive association with the scrotal circumference of bulls (Chacon *et al.*, 1999). Although, the

standards for breeding soundness examination of bulls “set by Society for Theriogenology in 1993” have been used in various dairy and beef breeds for many years, to categorize the bulls based on their breeding potential (Sylla *et al.,* 2007), these standards have great variation in their use at field level for Artificial Insemination sires selection due to the fact that every breed has its minimum acceptable scrotal circumference size which is a potential indicator of semen production ability. Therefore, it was imperative to study the relationship of scrotal circumference, body condition score and testicular dimensions with gonadal and epididymal sperm reserves and variability that exist within and between these breeds in order to classify the bulls according to their breeding potential, which this study was sets out to do.

This study was also designed to provide the basis for investigation of parameters useful in approximate prediction of future reproductive performance and in establishing the extent to which differences in these parameters are varied.

These needs served as the basis for this study and to allow for an effective prediction of the sperm production capabilities of these breeds in their natural environment, based on their testicular dimensions, scrotal circumferences and their gonadal and epididymal sperm reserves.

1.4 Aim and Objectives

1.4.1 Aim of the study

The aim of this study was to compare the body weight, body condition score, scrotal circumference, testicular dimension against gonadal and epididymal sperm reserves of bunaji, bokoloji and rahaji breeds of zebu cattle from Zango-Zaria and Kano Central Abattoirs.

1.4.2 Objectives of the study

The objectives of this study were to comparatively evaluate:

i. The body weights and body condition scores in relation to the gonadal and epididymal sperm reserves of Bunaji, Bokoloji and Rahaji breeds of cattle from Zango-Zaria and Kano Central abattoirs.

ii. The scrotal circumference in relation to the gonadal and epididymal sperm reserves of Bunaji, Bokoloji and Rahaji breeds of zebu cattle from Zango-Zaria and Kano Central Abattoirs.

iii. The testicular dimensions in relation to the gonadal and epididymal sperm reserves of Bunaji, Bokoloji and Rahaji breeds of zebu cattle from Zango-Zaria and Kano Central Abattoirs.

iv. The gonadal sperm/spermatid reserves of Bunaji, Bokoloji and Rahaji breeds of zebu cattle from Zango-Zaria and Kano Central Abattoirs.

v. The epididymal sperm/spermatid reserves of bunaji, bokoloji and rahaji breeds of zebu cattle from Zango-Zaria and Kano Central Abattoirs.

1.5 Statement of Research Hypothesis

The Null hypothesis (H_0) states that there is no difference in the body weights, body condition score, testicular dimensions, gonadal and epididymal sperm reserves of Bunaji, Bokoloji and Rahaji breeds of cattle from Zango-Zaria and Kano Central abattoirs.

CHAPTER TWO

2.0. LITERATURE REVIEW

FAO (2013) estimated global cattle population to be about 1.47 billion and Africa having over 307 million. There are over 800 breeds of cattle recognised worldwide (Loftus and Scherf, 1993; *List of Breeds of Cattle*, 2010) some of which were bred by human for specialized purpose. Scott (2008) reported that there are more than 250 breeds of beef cattle worldwide.

2.1 Taxonomical Classification of Cattle

The scientific classification of zebu cattle is:

Kingdom: *Animalia*

Phylum: *Chodata*

Class: *Mammalia*

Order: *Artiodactyla*

Family: *Bovidae*

Subfamily: *Bovinae*

Genus: *Bos*

Species: *Bos taurus indicus* (Lühken, 2009)

2.2 Population, Breeds and Distribution of Cattle in Nigeria

Nigeria's cattle population was estimated as 19.5 million (Rekwot, 2016) and most of the cattle herds are under the smallholder pastoral and agro-pastoral production system. The Nigeria cattle breed is broadly divided into two, the zebu breeds (*Bos taurus indicus*) which include bunaji (White Fulani), rahaji (Red Bororo), Sokoto Gudali (bokoloji), Adamawa Gudali, Azawak, Jali and Ambala while the taurine breeds include humpless longhorn (N'dama), West African Dwarf Shorthorn (muturu), Borgu Keteku (Shorthorn x Zebu), Lagos Keteku (N'dama x Muturu) and Kuri (Blench, 1999) and they are generally known to be trypanotolerant breeds of cattle.

About 90% of the country's cattle population are concentrated in the Northern part of Nigeria (Girei *et al*., 2013), the concentration of Nigeria's livestock-base in the Northern region is most likely to have been influenced by the ecological condition of the region which is characterized by low rainfall, lighter sandy soils and longer dry season (Lawal-Adebowale, 2012). Above 80 percent of the cattle population in Nigeria are kept in the hands of traditional pastoralists with about 60-75 percent of the herds kept in these traditional pastoral systems as females (Kubkomawa, 2017).

2.2.1 Bunaji (White Fulani) breed

White Fulani also called Bunaji or Yakanaji population in Nigeria, Cameroun and the Central Africa republic is estimated at about 9.65 million. Bunaji are the most numerous and widely distributed breed of cattle in Nigeria (Ahamefule *et al*., 2007), representing about 37 % of the national cattle population (Ikhatua, 2010). The breed is owned mainly by the nomadic Fulani people. The coat colour of White Fulani is commonly white on a black skin with black ears, eyes, muzzle, hooves, horn tips and tip of tail. Their thoracic or sometimes intermediate hump and dewlap are well developed. The head is long, wide across the forehead and with a

straight or concave appearance; average adult wither height is 130 cm; the neck is strong providing an upward carriage for the head; horns are slender, medium to long (81 to 107 cm), lyre shaped curved outwards and upwards, with an outward turn at the tip (Faulkner and Epstein 1957). The White Fulani are generally taller and narrower bodied cattle; the rump is of good length but has a marked slope from hook to pin bones. The general shallowness of the body and lack of width give the breed a leggy appearance which has been described as an adaptation to long distance trekking under the pastoral management (Tawah and Rege 1996).

The breed is of interest in that it is more tolerant to heat as compared to N'Dama and Gudali, more resistant to dermatophilosis than the Muturu and N'Dama breeds, resistant to intestinal helminth parasites, and has low mortality rate. Although it is less resistant to trypanosomosis than the N'Dama, it is more tolerant than the Gudali and other Zebu types (Tawah and Rege, 1996).

The White Fulani are used for milk, meat and draught although the traditional owners keep them mainly for milk. Their dairy potential is better than most Zebus, and is comparable to Kenana of the Sudan. Their conformation and body size make them suitable for draught. They are good beef animals, which fatten quite well in feedlots and on natural pastures. The average birth weights computed in the different regimes, range from 18.2 to 24.2 kg; mature weight of bulls and cows in the improved system of management is 350-665 and 250-380 kg, respectively (Tawah and Rege, 1996). Feedlot studies indicate that these cattle can achieve growth performance of 1 kg per day. Slaughter and carcass weights of 325 and 166 kg were reported in well-finished steers. The dressing percentage is reported to be 50-60, average age at first calving 40-49 months and average calving interval 403 days (Tawah and Rege, 1996).

Plate I: Bunaji (White Fulani) cattle

2.2.2 Sokoto Gudali (Bokoloji) breed

Gudali is a Hausa word for "short-horned and short-legged animals". They are also referred to as Fulbe or Peuhl zebu in West and Central Africa. Population estimate based on breed level survey in 1992 for the Sokoto Gudali in Nigeria is 4.4 million (DAD-IS, 2005). The Nigerian National Livestock Resources (NNLRS) of 1999 estimated that Gudali represents 32% of the national herd (Kubkomawa *et al*., 2015). About 90% of the Sokoto Gudali are owned and managed by Fulani and Hausa pastoralists and transhumant herders (Ngere, 1985), who feed their cattle on communally owned grazing lands and browse especially in the dry season (Tawah and Rege 1996). They and are also known for their hardiness to the arid northern environments (Ikhatua, 2010).

The Sokoto Gudali has multiple coat colours although the most common one is black and white coating with light underside. The usually have highly developed dewlap and their horns are almost absent (Wosu, 2002). The head is long and wide between the eyes and across the forehead, with a straight or slightly convex facial profile. Ears are long, large and convex, sometimes pendulous, although not to the same degree as in some of the Indo-Pakistan zebus. The hump is thoracic in position and the average height at withers ranges from 130-138 cm for males and 116-132 cm for females (Tawah and Rege 1996).

The Sokoto Gudali cattle are known for their meat and milk. Mature weights range from 495-660 kg for males and 240-355 kg for females. They are also reputed for their beef quality. Their slow and sluggish nature allows them to be used for draft, including ploughing and carting.

Plate II: Bokoloji (Sokoto Gudali) Bull

2.2.3 Red Bororo (Rahaji) breed

The Red Bororo (Rahaji) is the third most numerous breed of cattle in Nigeria, representing 22% of the national herd (Kubkomawa *et al.,* 2015). The Rahaji is adapted to arid and semi-arid regions and are rarely found further south in the wet season, except for the isolated population on the Mambila Plateau in the south-east (Blench, 1993; Meghen *et al.*, 1999). The Rahaji is one of the largest zebu breeds and is distinguished by its deep burgundy-coloured coat, pendulous ears and long, thick horns (Katie and Alistair, 1986; Payne and Wilson, 1999; Wosu, 2002). Fulani pastoralists consider the Rahaji an extremely prestigious breed and many herds of 'white' cattle include a few Rahaji for crossbreeding. Nonetheless, it tolerates neither humidity-related diseases nor poor nutrition (Blench, 1993).

Plate III: Rahaji (Red Bororo) cattle

2.2.4 Adamawa Gudali breed

The Adamawa Gudali, as its name implies, is restricted to Adamawa state (Blench, 1993; Meghen *et al.*, 1999). The Nigerian National Livestock Resources (NNLRS) of 1999 estimated that Adamawa Gudali represent 2% of the national herd. At least two local types were originally recognized in Nigeria: the Banyo type, with Rahaji blood and rather large horns, often with a white face and red eye patches; and the Yola type, which has a mixture of Muturu (Kubkomawa *et al*., 2015). The Muturu element has been progressively diluted since the 1950s and the Yola breed is no longer recognized as a distinct variety by local herders. The Adamawa Gudali resembles the Bunaji in conformation. It is medium to large sized, with medium length horns, and usually pied, or with a white, black, red or brown coat. It has thick, crescent-shaped horns, a pendulous hump, and a short head and muzzle (Katie and Alistair, 1986; Payne and Wilson, 1999; Wosu, 2002), however, the pendulous hump is the feature that most reliably distinguishes it from the Bunaji. Both Kanuri and Fulani pastoralists own Adamawa Gudali. It is rare for them to have complete herds of Adamawa Gudali, and often they are mixed with Wadara, Bunaji or Rahaji. Adamawa Gudali is regarded by many farmers as the indigenous race of the region and they are common in villages, where they are favoured for ploughing, but when they become too large to pull a plough effectively they are further fattened in the compound and sent to market (Katie and Alistair, 1986; Payne and Wilson, 1999 and Babayemi *et al*., 2014).

2.2.5 Wadara breed

Wadara cattle, another Nigerian breed, is a medium sized, lightly built cattle, and is usually dark red, black, pied or brown (Kubkomawa *et al.*, 2015). They are shorthorned and have a small erect hump, representing some 6.6% of the national herd. Wadara cattle are the ‘indigenous’ cattle of Borno and are referred to by the Koyam and related pastoralists as ‘our’

cattle (Kubkomawa *et al.,* 2015). They are frequently called 'Shuwa' in the literature, after the Shuwa Arabs who also herd the cattle. A related breed with a white coat, the Ambala, is often traded into Nigeria from Chad (Blench, 1993; Meghen *et al.*, 1999).

2.2.6 Azawak breed

The Azawak is another breed found in Nigeria and is said to be native to the Azawak Valley north-east of Nigeria and is distributed along its north-western border. It is lightly built with medium-length horns (Kubkomawa *et al.,* 2015). Although Azawak in Niger republic is commonly described as red, the Azawak that enter Nigeria are usually a light fawn colour, though they can also be white, brown, pied and black (Kubkomawa *et al.,* 2015). The Nigerian National Livestock Resources (NNLRS) of 1999 estimated that they represent just 0.7% of the national herd (Kubkomawa *et al.*, 2015). A small population of Azawak cattle exists in Nigeria throughout the year, but the majority are seasonal transhumants. Azawak are generally only found on the border north and west of Sokoto but there are also some in the north-west of Borgu and dotted along the frontier from Sokoto to Katsina (Blench, 1993; Meghen *et al.*, 1999).

2.2.7 N'dama breed

The N'dama cattle are native to Senegal, Gambia and also West Africa (Starkey, 1984; Blench *et al*., 1998; Babayemi *et al*., 2014). They were first brought into Nigeria from Guinea in 1939 on an experimental basis, because they were trypanotolerant and yet were larger than muturu (Starkey, 1984; Blench *et al*., 1998).

The N'dama has a medium-sized compact body with lyre shaped black-tipped horns and no hump. There is a small dewlap in the male, but a fairly large head. Although those imported into Nigeria are generally light brown, there are black and pied animals in Guinea (Kubkomawa *et al*., 2015). N'dama cattle have been sold to farmers and pastoralists with a

view to improving the resistance of local herds to trypanosomiasis. In most cases, herders cross them with Zebu and there are now few pure N'dama outside institutions, although some were recorded in northern Yoruba land (Blench, 1993; Meghen *et al.*, 1999).

The breed is known for its tolerance to trypanosomosis (Ngamuna *et al*., 1988; Claxton and Leperre 1991; Dwinger *et al*., 1992). It is also markedly resistant to tick-borne infections (Mattioli *et al*., 1995), but not to rinderpest. In addition, the breed is well adapted to stressful humid and dry tropical climates. Studies have shown that their trypanotolerance is due to their ability to control parasitaemia and limit anaemia which are inert abilities to trypanosomosis (Paling *et al*., 1991).

The N'Dama cattle are known for their beef conformation and the average adult weights range from 320 to 360 kg and 250 to 270 kg for males and females, respectively (Payne, 1970; Starkey, 1982; 1984; Maule, 1990; Mason, 1996). The dressing percentage is around 50% and the meat has a very good flavour without much fat (Maule 1990; Rege and Tawah 1999).

2.2.8 Muturu breed

`Muturu', meaning humpless, is the Hausa name for the West African Shorthorn in Anglophone West Africa. The breed is a variety of West African Shorthorn and the Nigerian Forest Muturu is also called Kirdi. The number of Muturu cattle in 1990 in Nigeria was between 75 000 to 120 000 (DAD-IS 2005).

The Muturu is found in areas heavily infested with tsetse, as a result of which this breed has adapted and naturally selected to be tolerant to trypanosomosis, ticks and tick-borne diseases (Adeniji, 1983) although it is susceptible to rinderpest.

The typical coat colour of forest Muturu is black and that of the Savannah Muturu is black and white. Height at withers is 95 cm for males and 88 cm for females (Maule, 1990). It is the

smallest cattle breed known. They are used for socio-cultural purposes. In Igboland these cattle are traditionally sacred and considered as properties of the local deities or they are dedicated to a shrine. Muturu cows are seldom milked. As beef cattle they perform well, dressing out at 64%.The animals and their hides are used mainly for ritual sacrifices and ceremonies particularly funerals. They are also commonly kept as pets or, frequently, they are used for prestige or dowry purposes (Rege and Tawah, 1999).

2.2.9 Kuri breed

This is a hump-less longhorn breed (Malbrout *et al*., 1947). They were introduced into Nigeria in 1944 by the importation of a nucleus breeding herd consisting of 10 cows and a bull (Adeniyi, 1985). The Kuri breed is either generally white coloured or white speckled with black or grayish black in particular, around the neck ears, the head and front part of the chest (Adeniyi, 1985). It's a heavily built animal of 151 cm in height, and massive vertical high bulbous horns (Gwaza and Momoh, 2016).

2.2.10 Biu cattle breed

This breed is restricted to a hilly volcanic area of Borno State, Nigeria. Number not more than 1000-2000 heads (Gwaza and Momoh, 2016). Not much information is available for this breed.

Table 2.1: Nigerian breeds of cattle

Breed	Local name	Homeland	Features
Bunaji	Akuji, Zebu, Daneji, Yakaneji, Lyrehorn-Zebu, White Fulani	Northern Nigeria Middle belt	Usually white with long horn, have hump. Have black colour at their eyes, mouth and ears. Most populous
Bokoloji	Sokoto Gudali, Zebu, Short horn	Northern-western Nigeria	Humped with no horns and have dewlaps
Adamawa Gudali	Adamawa Fulani, Cameroon Fulani, Bamenda	Adamawa Ngundere Cameroon high land, Banyo	
Keteku	Borgu, Kaiama, Borgawa (Ketaria)	Borgu, Niger state	
Muturu	West African Dwarf, Short horn	Middle belt, Southern Africa	Short horn Humpless
Kuri	Bare, Buduma, Jotko	Lake Chad basin Borno state	Very large hollow horns
Azaouak	Adar, Buzaye, Azawal, Tagama	Azaouak Valley Sokoto-Niger border	
Ndama	Fouta long horn, Hamitic long horn	Fouta Djallon, Plateau of Rep. Guinea	Humpless
Wadara	Shuwa, Turchoa	Borno State North East	
Rahaji	Red Bororo, Abore, Daneji, Red Fulani	Northern bordering Nigeria-Chad and Niger Republic	

(Rekwot, 2016)

Fig 2.1: Primary area of cattle breeds in Nigeria and major vegetation zones (Adapted from Blench, 1999)

2.3 Cattle Production in Nigeria

Specifically, about 90% of the country's cattle population are concentrated in the Northern part of the country (Girei *et al*., 2013). The concentration of Nigeria's livestock-base in the Northern region is most likely to have been influenced by the ecological condition of the region which is characterized by low rainfall duration, lighter sandy soils and longer dry season (Lawal-Adebowale, 2012). All types of cattle interbreed and can therefore be regarded as a single species (Blench *et al.*, 1998). Breeds of locally available cattle in Nigeria are basically indigenous and are grouped as the Zebu and Taurine. The Zebus are locally recognized by the cattle rearers in Northern part of Nigeria include Bunaji, Rahaji, Sokoto Gudali, Adamawa Gudali, Azawak and Wadara. FAO (2004) reported that cattle contribute over 50% of the national meat supply. Despite the large population of livestock in Nigeria, the protein intake is still below the minimum requirement (FAO, 2001) and this may be attributed to the low number of cattle production in the country as a result of regionalized suitability to humid areas.

2.4 Anatomy and Physiology of the Reproductive Tract of the Bull

2.4.1 Testes

The testes are the primary organs of reproduction in the male because they produce male gametes (spermatozoa) and male sex hormones (androgen). The outer layer of the testes, the *tunica albuginea*, is a thin white membrane of elastic connective tissue (Cornwall, 2009).

The testes are housed in the scrotum which is suspended between the thighs in the inguinal region. The scrotum consists of external and internal layers. The external layer is made up of the skin, *tunica dartos*, superficial perineal fascia, external spermatic fascia, cremasteric fascia, internal spermatic fascia, and parietal vaginal tunic. The skin of the scrotum and *tunica dartos* muscle are closely adhered whereas the fascial layers are easily separated from

the skin and the parietal vaginal tunic (Johnson *et al*., 1970). The coverings of the testicle itself consist of the visceral vaginal tunic and the *tunica albuginea* (Nabors and Linford, 2015). The visceral vaginal tunic is the innermost layer of the vaginal tunic, an out-pouching of abdominal peritoneum that passes through the inguinal canal into the scrotal sac. The potential space between the parietal and visceral vaginal tunic is the vaginal cavity. The purpose of the vaginal cavity is for temperature regulation of the testicle by raising it closer to the body through contraction of the *tunica dartos* and cremaster muscles. The *tunica albuginea* is a thick fibrous capsule that covers the testicle and maintains the testicular contents under pressure (McGavin and Zachary, 2007). Internally, the *tunica albuginea* forms the axially positioned mediastinum testis from which connective tissue septa divide the testis into indistinct lobules. This connective tissue framework supports the vasculature, nerves, parenchyma, and tubular system of the testicle. The scrotum of the bull is pendulous due to the dorsoventral orientation of the testes contained within (Nickel *et al*., 1973).

The testicular parenchyma contains the cellular machinery for spermatogenesis and hormone production. The parenchyma is arranged in indistinct lobules of convoluted tubules called seminiferous tubules. The seminiferous tubules contain the spermatogonia from which the mature sperm cells develop (Nabors and Linford, 2015). Sertoli cells are also located within the lumen of the seminiferous tubules. The Leydig cells that are responsible for the production of the male hormone testosterone are located between the seminiferous tubules in the interstitial space (Nickel *et al*., 1973).

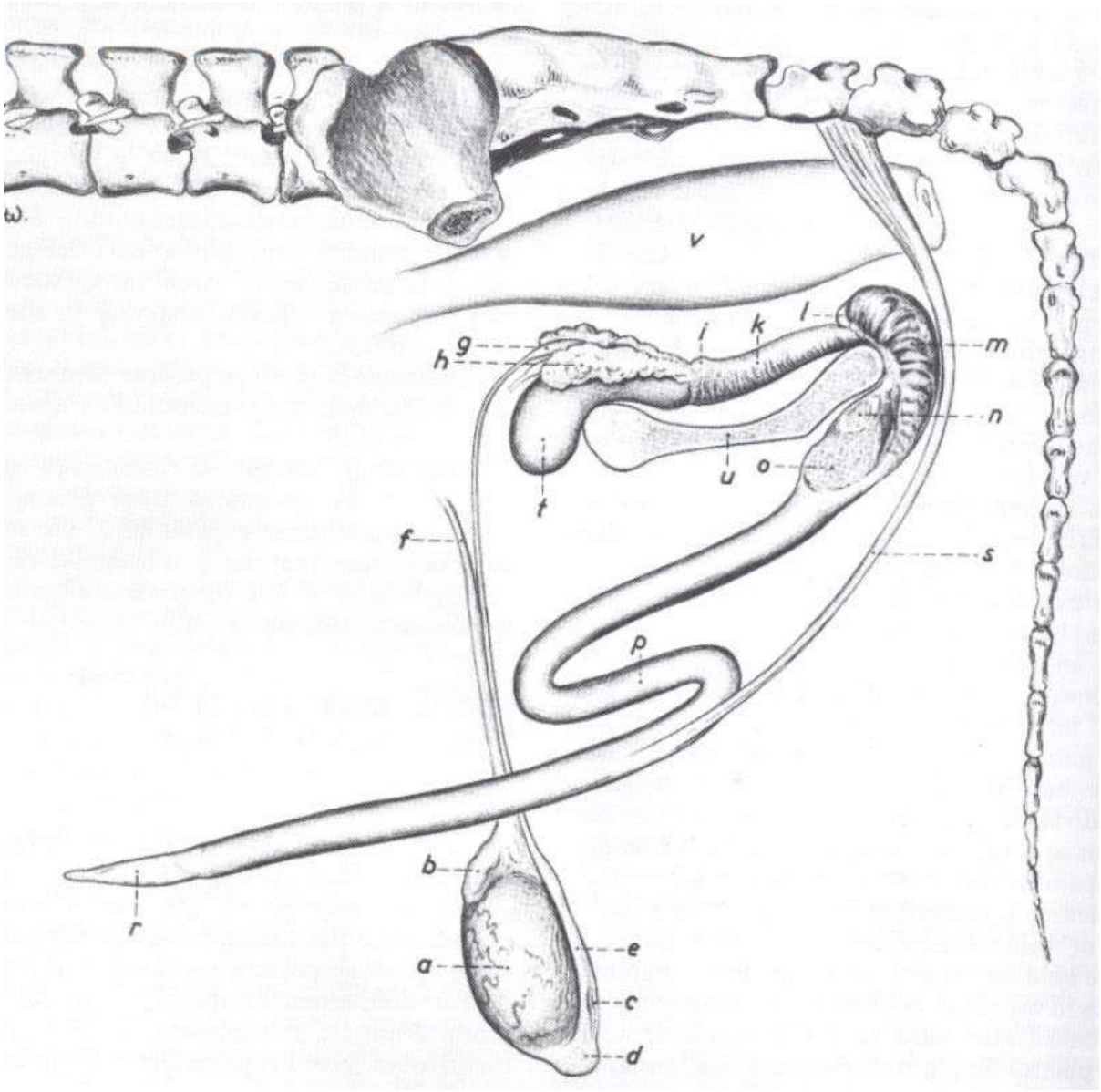

Figure 2.2: Reproductive anatomy of the bulls (a=testicle, b-head of epididymis, c-body of epididymis, d-tail of epididymis, e-*ductus deferens*, f-mesorchium, g-ampulla, h-vesicular gland, i-prostate, k-urethra, l-bulbourethral gland, m-bulbospongiosus, n-crus penis, o-ishiocavenosus, p-penis, r-glans penis, s-retractor penis, t-urinary bladder, u-pelvic symphysis, v-rectum) (Amann, 1983).

Testes size has been regarded as the trait of choice in males to genetically improve female reproductive performance (Matos and Thomas, 1992). Testicular size is the main factor determining the number of sperm and volume of ejaculate (Ashwood, 2009). Akpa *et al*. (2012) reported that testicular size may be useful as a selection criterion for improvement of the reproductive ability. Testicular size has been found to be significantly correlated with luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone concentrations of the blood. From the genetic perspective, testicular size when measured at a young age, could be a useful selection trait in order to improve female reproduction. According to Akpa *et al.* (2012), a positive and significant correlation was observed between testicular dimensions and body measurements. Bratte *et al.* (1999) stated that testicular length and circumference have measures of testicular size which was found to be significantly correlated with body weight. Also, Akpa *et al*. (2012) stated that a positive relationship existed between semen quality and testicular dimension, giving an indication that improvement in one would lead to improvement in the other. Oyeyemi *et al.* (2012) observed a positive correlation between testicular weight, diameter, length and epididymis length and scrotal circumference. Salheb *et al.* (2001) observed that parental size, age and body weight affected testicular growth with no significant differences between the left and right testes. Testicular weight and sperm concentration were positively correlated (Vidament *et al.,* 2007; Akpa *et al*., 2012). Teodoro *et al*. (2013) stated that testicular length can be considered as a viable indicator of the effect of thermal stress on the gonads. The testicular volume is a parameter that minimizes the error of SC when the testicles have different shape (Teodoro *et al*., 2013). Scrotal volume was estimated by the volume of liquid displaced, by immersing the whole scrotal sac of a standing ram in a 1 litre container filled with warm water according to Archimedes Law of Bouyancy (Piperelis *et al*., 2008; Azawi *et al*., 2012) while testicular volume was estimated by the volume of liquid (water) displaced, by immersing the testes of

an animal in a 500 ml measuring cylinder (Etim, 2015). In adult males, testicular volume is measured in relation to spermatogenic activity, whereas in younger males, testicular volume measurement is mainly important in assessing the onset of puberty or puberty development (Bree and Hoang, 1996). Testicular volume can also be used to evaluate testicular abnormalities such as cryptorchidism among others (Bree and Hoang, 1996).

Mortons (2006) and Ogbuewu (2008) reported that a decreased weight of the testes indicates wide spread or diffuse loss of seminiferous epithelial cells. Perry and Petterson (2001) and Ogbuewu (2008) documented that testis size is a good indication of present and future sperm production as well as the breeding potential of the male. Ibrahim *et al*. (2012a) in a study observed that the paired testes weights and gonadal sperm reserves showed a highly significant positive correlation.

Ibrahim *et al*. (2012b) also reported that testicular weight is a reliable index of semen producing ability and that it has been shown to vary according to breeds. Males with larger testes tend to sire daughters that reach puberty at an earlier age and ovulate more ova during oestrus period. Testicular weight and size vary with breed, age and time of the year. Variations in the testes weight are markedly greater between younger bulls and decreases with advancement in age. There was evidence that the larger, faster growing bulls would have larger testes than smaller bulls of comparable ages (Ibrahim *et al.*, 2012b). Also, Britto *et al.* (2004) stated that heavier testes produce more spermatozoa than the smaller testes. Berndson *et al*. (1987) and Ibrahim *et al.* (2012b) documented that testes which possess greater number of sustenocytes were heavier and produced more spermatozoa than testes with fewer sustenocytes. Higher testes weight would mean that the testes could contain more seminiferous tubules, interstitial endocrine cells, sustenocytes and possibly more spermatozoa.

2.4.2 Spermatogenesis

Spermatogenesis is the term used to describe the formation of male gametes and is the process whereby diploid undifferentiated spermatogonia give rise to haploid spermatozoa. This is a highly regulated and productive process that results in approximately 100 million sperm each day in men and even greater numbers in most livestock species (Sharpe, 1994). Spermatogenesis is established in the bull by 32 weeks postpartum but varies slightly by breed (Curtis and Amann, 1981). The ultimate goal of spermatogenesis is to transmit genetic information to future generations. In order for this to be successful, germ cells must undergo differentiation and specialization in a three-stage process including the spermatocytogenesis, the meiotic phase, and the spermiogenesis (Oatley and Brinster, 2006). This process is maintained from puberty through the majority of adult life, under the regulated balance of spermatogonial stem cells (SSCs) self-renewal and differentiation.

2.4.2.1 Spermatocytogenesis

Spermatocytogenesis is a proliferative phase marked by multiple mitotic divisions which account largely for the immense number of spermatozoa that are produced each day. Cells recruited for spermatogenesis come from the stem cell pool, which resides on the basement membrane of the seminiferous tubule. SSCs, by signalling which is not fully understood, begin the process of differentiation and go through mitotic phases, forming a syncytium of 4, 8 and then 16 A_{al} cells, connected by intercellular bridges after each cell division. Without dividing, the 16 A_{al} cells differentiate into A_1 spermatogonia and regain c-kit expression (Yoshinaga *et al*., 1991). It is the A_1 spermatogonia which are first referred to as differentiated cells. These cells proliferate extensively through multiple mitotic divisions, with 16-cell A_1 chains dividing to make 32 A_2 cells which divide again, forming 64 A_3 cells and yet again to create chains of 128 A_4 cells. At this point, the A_4 cells divide yet again to form an intermediate cell type which divide again to form type B spermatogonia. At this

point, there are 512 type B spermatogonia for every A_1 cell that entered mitosis. These cells will undergo one final cycle of mitosis resulting in 1024 round cells called primary spermatocytes. Consequently, for every chain of A_{al} (16) spermatogonia, a possible 1024 primary spermatocytes could be produced. Many of these cells will undergo apoptosis throughout this process, hence, significantly reducing this number (Allan *et al*., 1987). During spermatocytogenesis, stem germ cells engage in the spermatogenetic process by performing a first mitotic division that generates spermatogonia. The latter proliferate in turn by performing several successive mitotic divisions which result in the production of preleptotene spermatocytes. The preleptotene spermatocytes cross the blood-testis barrier and engage in meiotic prophase (Staub and Johnson, 2018). Spermatocytogenesis in the bulls takes about 21 days.

2.4.2.2 Meiotic phase

Following the highly proliferative, initial phase of spermatogenesis, primary spermatocytes then enter the meiotic phase. This is the second and longest phase of spermatogenesis and it is during this time that the cells undergo two meiotic divisions. During the prophase of the first division of meiosis, the germinal cells differentiate successively into different stages (leptotene, zygotene, pachytene, diplotene) before undergoing the two meiotic divisions. The first meiotic division is the reductional division (reduction of chromosome number, separation of homologous chromosomes), while the second meiotic division is the equational division (separation of the daughter chromatids). Meiosis therefore allows the production of round haploid spermatids (Staub and Johnson, 2018). Testosterone, in conjunction with cytokines such as tumour necrosis factor α (TNFα) and transforming growth factor-β3 (TGF-β3), remodel the sertoli cell tight junctions of the blood-testis barrier (BTB), allowing primary spermatocytes to move from the basement membrane to the immune-privileged, adluminal area of the seminiferous tubule without disrupting the immunological barrier (Li *et*

al., 2009). Once inside the BTB, the primary spermatocytes immediately enter prophase I, which has five sub-phases designated preleptotene, leptotene, zygotene, pachytene, and diplotene (Bellve *et al*., 1977). During these phases, DNA is replicated, homologous chromosomes are paired, genetic recombination occurs via crossing over, and the chromosomes are separated to opposite poles of the cell for cell division, resulting in two secondary spermatocytes which rapidly enter a second meiotic division.

No DNA replication occurs during prophase II, therefore, meiosis II produces four small, haploid round spermatids that enter the next phase of spermatogenesis called the transformative phase. This phase take 23 days in the bulls to be completed.

2.4.2.3 Transformative phase

The transformative phase or spermiogenesis is the third and final stage of spermatogenesis. It consists of the differentiation of round spermatids into spermatids at various degrees of elongation and finally into spermatozoa (Staub and Johnson, 2018). During this phase, the round spermatids will undergo a remarkable morphologic differentiation with functional sperm as the end product. This process of differentiation consists of the following four sub-phases: the Golgi phase, the cap phase, the acrosomal phase, and the maturation phase (Hess, 1999). The Golgi phase is characterized by the initial formation of the acrosomal vesicle through proliferation of membrane-bound vesicles by the Golgi apparatus (Nagano, 1962). Also during this phase, the mother-daughter centriole pair migrates from the base of the nucleus. The mother centriole forms an initiation site for the future flagellum and the daughter centriole forms the axoneme, which is the central segment of the flagellum (Nagano, 1962). During the cap phase, the acrosome spreads out over the anterior surface of the nucleus and continues to spread down the sides of the nucleus during the acrosomal phase, which is characterized by nuclear and cytoplasmic elongation. The final phase, maturation, involves the assembly of the 3-piece tail, which extends from the Sertoli cells out

into the seminiferous tubule lumen (McIntosh and Porter, 1967). Finally, the elongated spermatid is ready to be released from the Sertoli cell into the tubule lumen, called spermiation. This process involves final modifications to the spermatid's nucleus and cytoplasm and removal of Sertoli-germ cell connections, called ectoplasmic specialization junctions (O'Donnell *et al*., 2011). Spermiation concludes with release of the spermatid, which is now called a spermatozoan, into the lumen. Spermatozoa then travel to the epididymis for final maturation and storage (Russell, 1993).

For sperm production within the testis to be continuous, different areas along the length of the seminiferous tubules have to be in different stages of production at any given time. Each entire round of spermatogenesis in the bull can be divided into 12 stages and is referred to as the cycle of the seminiferous epithelium (Berndston and Desjardins, 1974). Each stage is characterized by the different types of spermatogonia present within a given area of the seminiferous tubules. This continuous, cyclic nature of spermatogenesis is supported by the self-renewal and differentiation of SSCs. Spermiogenesis takes 17 days to be completed in the bulls.

2.4.2.4 Seminiferous epithelial cycle

The seminiferous epithelium is composed of several generations of germ cells because of the engagement of new generations of sperm cells, from spermatogonia to spermatozoa, which continues throughout the inner surface of the membrane of the seminiferous tubules without waiting for the preceding generations to complete their evolution and to disappear as spermatozoa into the lumen of the tubules (Staub and Johnson, 2018). The fate of a generation of germ cells is closely related to the development of the other generations of neighbouring cells in a section of seminiferous tubule. The result is the presence of cellular associations which follow one another in time, at a given point of the seminiferous tubule, in

a perfectly regular order, characterizing the so-called cycle of the seminiferous epithelium. The consistency of these cellular associations results from two phenomena; the first which is at one point of the tubule, new spermatogonia begin their divisions and engage in spermatogenesis at time intervals of constant duration. And the second occurs once the germinal cells are involved in spermatogenesis, their rate of differentiation is always the same and each step has a fixed and constant duration (Staub and Johnson, 2018).

The cell associations identified within a cycle of the seminiferous epithelium are used to divide the cycle into phases or stages allowing a chronological division of the latter. In the bull, the classification is into eight stages, using spermiation as a reference point (Hochereau-de Reviers, 1970). However, there is a second classification (Berndston and Desjardins, 1974), using the meiotic divisions as a reference point, and this is based on the development of the acrosome during spermiogenesis.

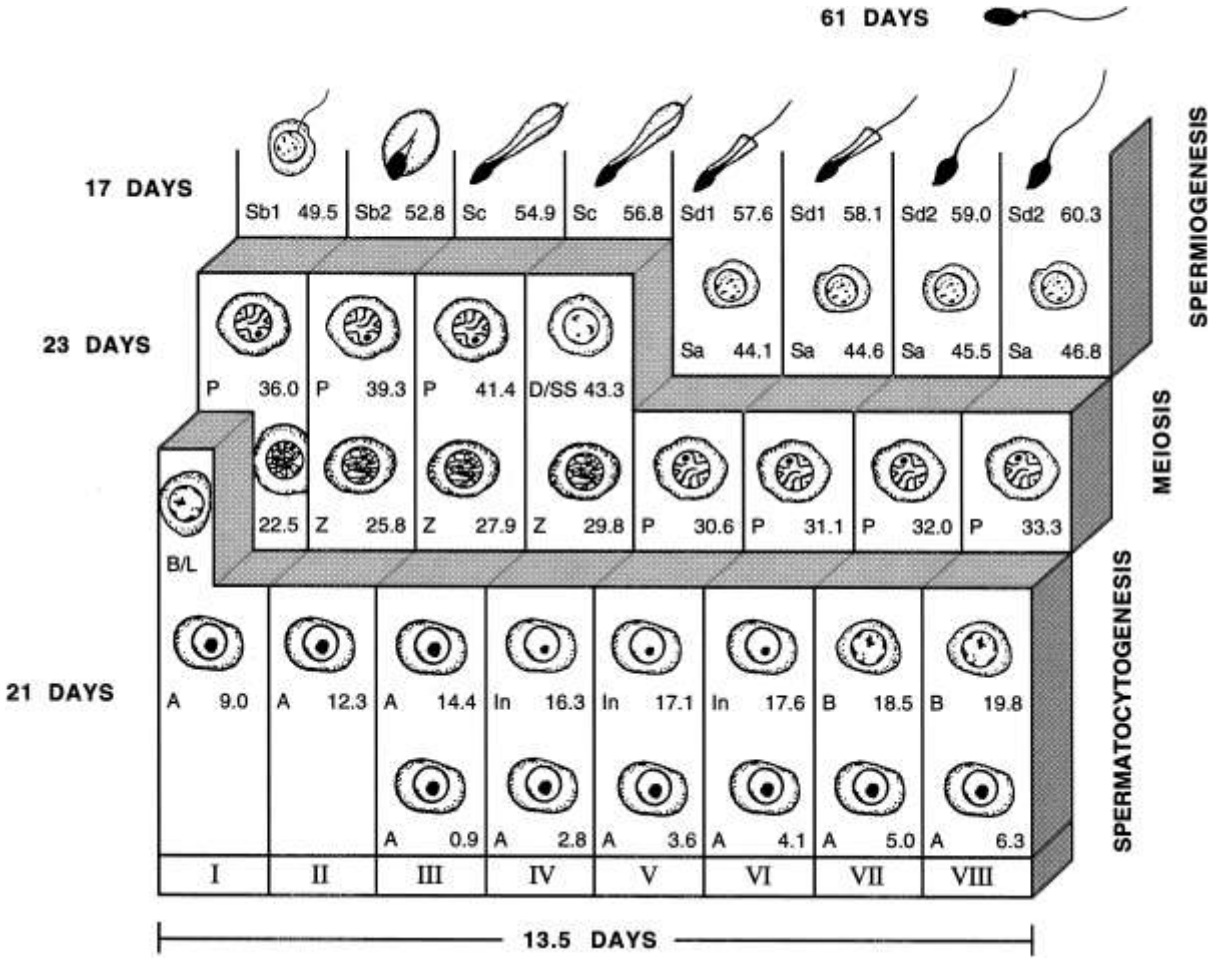

Fig 2.3: Drawings and classification of germ cells at different developmental steps in the three major divisions of spermatogenesis (spermatocytogenesis, meiosis, and spermiogenesis) combined to make the eight stages of the cycle of bull seminiferous epithelium. During the 21 days of spermatocytogenesis, A spermatogonia (A) enters cyclic (at 13.5-day interval) activity during stage III and undergo division to produce intermediate (In), B (B) spermatogonia, and leptotene primary spermatocytes (L). During the 23 days of meiosis, leptotene primary spermatocytes differentiate through zygotene (Z), pachytene (P), and diplotene (D) before the first meiotic division to produce secondary spermatocytes (SS), and the second meiotic division to produce Sa spermatids (Sa). During the 17 days of spermiogenesis, Sa spermatids differentiate through Sb1, Sb2, Sc, Sd1, and Sd2 steps of development before spermiation as spermatozoa. The letters indicate the developmental step, and the numbers associated with each germ cell step indicate the developmental age of each cell type in the middle of each spermatogenic stage. The cycle length is 13.5 days, and the duration of spermatogenesis is 61 days in the bull. (Johnson *et al*., 2000)

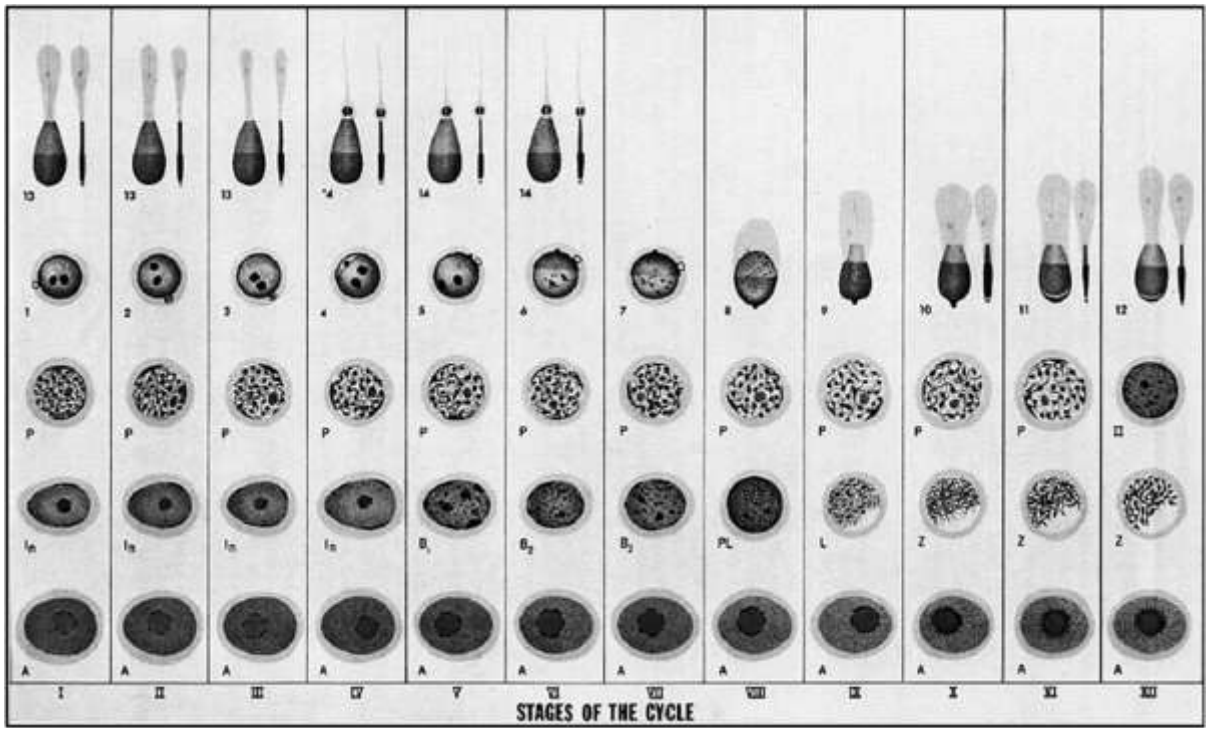

Fig 2.4: Cellular associations at each of the 12 stages of the bovine seminiferous epithelium cycle (Roman numerals I to XII) based on the development of the acrosome during spermiogenesis. The types of germ cells observed are A = type A spermatogonia; In = intermediate spermatogonia; B1 = type B1 spermatogonia; B2 = type B2 spermatogonia; PL = preleptotene primary spermatocytes; L = leptotene primary spermatocytes; Z = zygotene primary spermatocytes; P = pachytene primary spermatocytes; II = secondary spermatocytes; 1 to 14 = spermatids (Berndston and Desjardins, 1974).

Table 2.2: Number of seminiferous epithelial cycles, length of cycle and duration of spermatogenesis in some mammals.

Species	Number of seminiferous epithelial cycle	Cycle length (days)	Duration of Spermatogenesis (days)	References
Bulls	4.5	13.5	61	Staub and Johnson, 2018
Ram	4.5	10.4	47	Cardosa and Queiroz, 1988
Boar	4.5	8.6	39	Franca *et al.*, 2005
Buck	4.5	10.6	48	Franca *et al.*, 1999
Stallion	4.5	12.2	55	Amann and Schanbacher, 1983
Dog	4.5	13.6	61	Soares *et al*., 2009
Cat	4.5	10.4	47	França and Godinho, 2003
Man	4.5	16	72	Amann, 2008

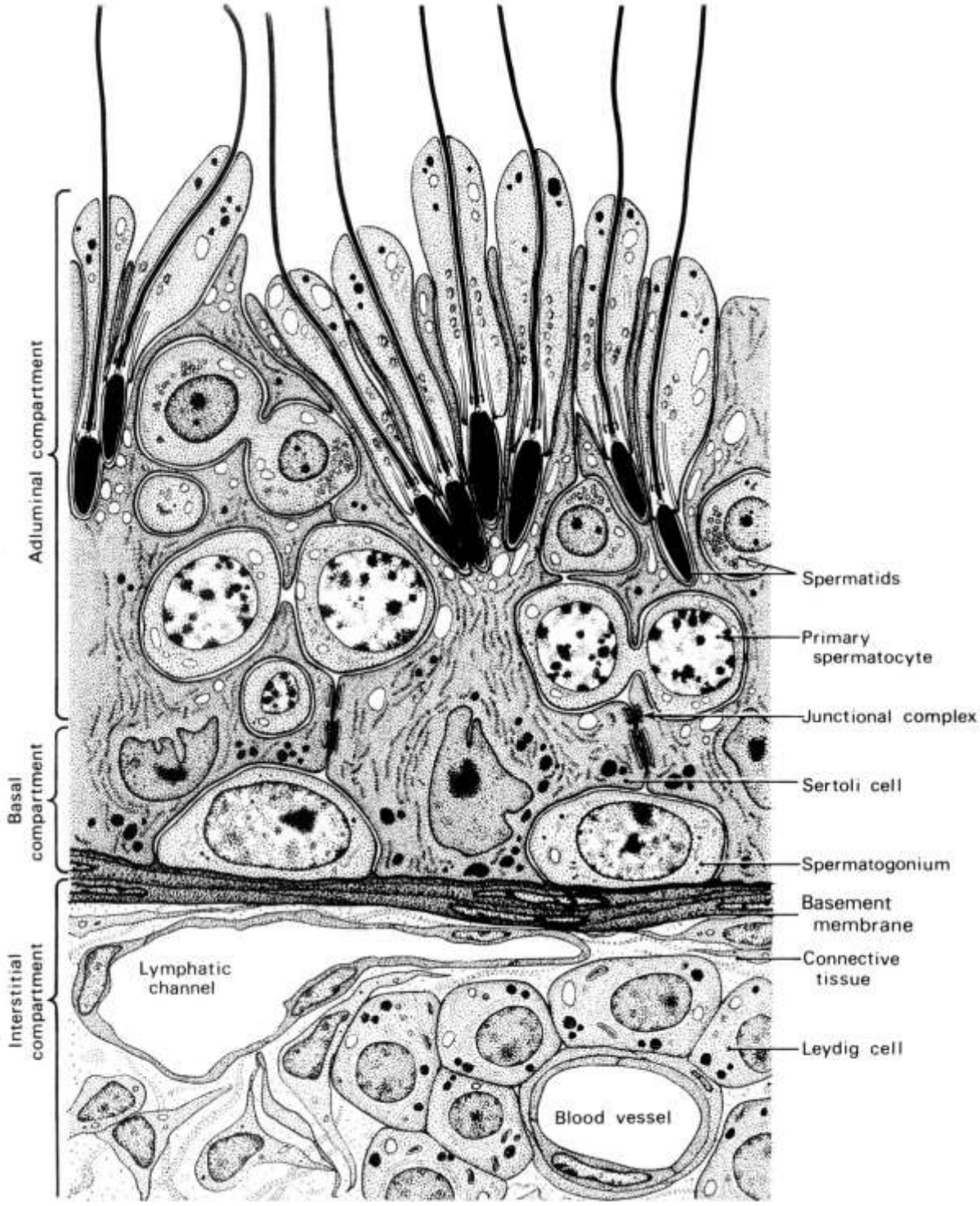

Figure 2.5 Cross-section of the seminiferous epithelium showing the relationship of the germ cells to the adjacent Sertoli cells (Amann, 1983).

2.4.2.5 Spermatogenic wave

In contrast to the cycle of the seminiferous epithelium, which is a histological phenomenon occurring at a given place in the seminiferous tubule in time, the spermatogenetic wave is used to describe the spatial arrangement of cell associations along the tubules. As early as 1871, Von Ebner reported in the rat that the various cell associations are distributed according to a regular order which corresponds to the numerical order of the stages of the cycle. Spermatogenesis progresses along the seminiferous tubule in the manner of a wave, and therefore, it is referred to as the spermatogenetic wave (Johnson, 1995).

Regaud (1901) gave a description of the spermatogenetic wave that remained accepted by scientists for more than half a century: 'The wave is to space what the cycle is to time.' A modulation consists of a series of tubule segments having a numerical order of increasing first, then decreasing, until the increasing general progression is seen again. Despite these irregularities, a portion of a tubule occupied by a given cell association (corresponding to a given stage of the cycle) is always bordered by segments that are in the immediately preceding or subsequent stage. This is called 'the continuity rule.' The wave is not a dynamic process but is a static way to describe in the spatial distribution of the associations along the tubule (Johnson, 1995).

2.4.3 Tubular transport system

Spermatozoa are transported from the testicles through a tubular system consisting of the convoluted seminiferous tubules, straight seminiferous tubules, rete testis, efferent ductules, epididymis, *ductus deferens*, and urethra. The tubular system allows for maturation and storage of spermatozoa and provides fluid to ease movement of the spermatozoa (Nabors and Linford, 2015).

The convoluted seminiferous tubules are the sites of the spermatogenic process: the development of spermatogonia to primary spermatocytes, to spermatids, and finally to spermatozoa (Nickel *et al*., 1973). This process occurs within the wall of the seminiferous tubule. Specific regions of the tubule are devoted to a particular stage of development, so that each stage can be identified by specific histological techniques (Mullins and Saacke, 2003).

Upon the completion of spermiogenesis, the spermatozoa are released into the lumen of the convoluted seminiferous tubule to begin transit through the straight seminiferous tubule. The straight seminiferous tubule is simply the connection between the convoluted seminiferous tubule and the rete testis. The rete testis is a "network of irregular labyrinth spaces and interconnected tubules" (Johnson *et al.*, 1970). The rete testes are located within the mediastinum testis connecting the seminiferous tubules to the efferent ducts that exit the testicle at the extremitas capitate (head). The efferent tubular system continues as the epididymis on the external surface of the testis (Nabors and Linford, 2015).

2.4.3.1 The Epididymis

The epididymis is an elongated, torturous duct extending from the rete testis along the medioposterior border of the testis. It comprises the head (caput), body (corpus) located on the medial surface, and tail (cauda) located at the distal extremitas caudate (Amann and Schanbacher, 1983). The epididymis is an extremely connected structure which is closely attached to the dorsal part of the lateral surface of the testes (Oyeyemi *et al*., 2000). As a duct leading from the testes, the epididymis serves to transport, concentrate, store and mature spermatozoa including the acquisition of progressive motility and fertility ability (Cornwall, 2009). All along the epididymal tubule, the lumen is bordered by an epithelium that is very active in protein synthesis and secretion (Hermo *et al*., 1991) under androgenic stimulation (Cuasnicu *et al*., 1984; Robaire and Viger, 1995). The pseudostratified epithelium is

composed mainly of principal (85%) and basal cells, accompanied by other specialized cells distributed in a segment-specific manner including apical, narrow, clear, and halo cells. The epithelium forms an epididymal barrier by the presence of tight junction between epithelial cells (Hermo *et al*., 2007). Fluid composition in each epididymal segment shows great variability from one epididymal segment to the other (Dacheux and Dacheux, 2002; Dacheux *et al*., 2006) as well as the pattern of gene expression (Kirchhoff, 1999; Rodr´ıguez *et al*., 2001).

The epididymal sperm maturation function involves the acquisition of the forward motility and the fertilizing ability (Cooper and Yeung, 2006). The latter is defined as the acquisition of the many physiological properties by the spermatozoon including the ability to bind the zona pellucida, an extracellular glycoprotein coat surrounding the oocytes, and to fuse with the egg plasma membrane. The fertilizing ability is acquired by the spermatozoa during the epididymal transit, since it has been demonstrated that spermatozoa collected from the proximal segments are unable to fertilize an oocyte in artificial insemination or *in vitro* fertilization procedures (Horan and Bedford, 1972) but, in the majority of species, they start to acquire fertilizing potential in the middle corpus (Dacheux and Paquignon, 1980).

When spermatozoa reach the elongated spermatid stage in the testis, their DNA starts to be progressively condensed, and as a consequence, the DNA transcription will be arrested. At the time the spermatozoa initiate the epididymal transit, the synthesis of new proteins is then at a very low range. Because of this, the sperm maturational process depends on the sequential interactions of sperm with different intraluminal fluids (Dacheux *et al*., 2003). During this transit, spermatozoa undergo many biochemical modifications including the nucleus chromatin condensation, increases in total surface negative charges and in disulfide bounds, changes in the plasma membrane protein and lipid composition (phospholipids

composition, cholesterol/phospholipids ratio), relocalization of surface antigens, elimination and modifications of surface proteins (Sullivan, 1999; Cuasnicu *et al*., 2002), structural modifications of the cytoplasmic perinuclear theca (Oko and Sutovsky, 2009), and the ability to respond to hypoosmotic stress (Sahin *et al*., 2009). Spermatozoa are transported through the caput and corpus epididymal regions by continuous peristaltic contractions originated in the smooth muscles present in the wall of the duct, whereas the cauda is maintained quiescent unless it can be stimulated to contract. The cauda is the main region responsible for the sperm storage and survival (Jones and Murdoch, 1996). In bulls and stallions, the number of sperms stored in the cauda is sufficient for ten successive ejaculates (Sullivan *et al*., 2007). The epididymal cauda environment also keeps spermatozoa in a metabolic quiescence by preventing premature activation. The epididymal gene expression and secretion are regulated by the intraluminal and circulating androgens (Holland and Nixon, 1998; Robaire and Henderson, 2006). These androgens come from the enzymatic reduction of the testicular testosterone in the epididymal proximal regions (Viger and Robaire, 1996). Estrogens synthesized by Leydig cells (Hess *et al*., 2001) and epididymal spermatozoa (Hess *et al*., 1995) also have a function in the epididymis, they are involved in water reabsorption especially in the proximal regions (Hess *et al*., 1997; Hess, 2000; Hess *et al*., 2001; Bilin´ska *et al*., 2006)

Hormones do not only influence the epididymal function but they also infleunce scrotal temperature (Bedford, 1991; Bedford and Yanagimachi, 1991; Jara *et al*., 2002; Reyes-Moreno *et al*., 2008), between 35 and 37ºC in the bull (Graves *et al*., 1970), and the presence of epididymal spermatozoa in close contact with the epididymal cells (Reyes-Moreno *et al*., 2008).

2.4.3.2 Ductus deferens

The *ductus deferens* is attached to the medial side of the testicle by the mesoductus (Ross *et al*., 2003). The *ductus deferens* is the continuation of the tail of the epididymis. The *ductus deferens* enters the abdominal cavity through the inguinal canal, crosses the lateral ligament of the bladder, and before it ends at the *colliculus seminalis* in the urethra, it widens into the ampulla.

2.4.4 Penis

The transfer of spermatozoa from the bull to the cow is achieved by the process of intromission, which requires erection of the penis and ejaculation of sperm. The pertinent anatomy for these processes to occur includes the penis, the musculature of the penis, the vasculature, and the innervations (Nabors and Linford, 2015).

The penis of the bull can be divided into a root, body, and glans penis. The root of the penis can be defined as the origin of the erectile tissue that comprises the penis as well as the origin of the muscles of the penis. The erectile tissue that makes up the bulk of the penis is the *corpus cavernosum*. The paired *corpora cavernosa* originate separately on each side of the ischiatic arch medial to the ischiatic tuberosity. These individual limbs are termed the crura of the penis (Amann and Schanbacher, 1983). The crura pass ventromedially until they join to form the body of the penis. The *corpus spongiosum* is the erectile tissue that surrounds the urethra. The origin of the *corpus spongiosum*, called the bulb of the penis, originates between the crura along the midline of the ischiatic arch (Amann and Schanbacher, 1983). Therefore, the root of the penis is composed of the crura (*corpus cavernosum*) and the bulb (*corpus spongiosum*).

The erectile tissue is enclosed in the dense outer covering of the *tunica albuginea*. The *tunica albuginea* is a dense covering that consists of an inner circular layer and outer longitudinal

layer of fibres. The inner circular layer sends trabecular scaffolds throughout the *corpus cavernosum* for the attachment of the cavernous endothelium. Located caudal to the root of the penis are the muscles of the penis: the ischiocavernosus, bulbospongiosus, and retractor penis muscles. The paired ischiocavernosus muscles originate on the medial surfaces of the ischiatic tuberosities overlying the crura; the muscle fibres pass ventromedially in a "V" fashion until ending a short distance on the body of the penis (Nabors and Linford, 2015). During erection the ischiocavernosus muscle contracts pushing blood from the cavernous spaces of the crura into the body of the penis (Nickel *et al*., 1970). The bulbospongiosus muscle lies caudal to the bulb of the penis, originating along the ischiatic arch and continuing until the junction of the crura. The bulbospongiosus muscle fibers run transversely across the bulb of the penis and contraction of this muscle results in propulsion of the ejaculate through the urethra (Nabors and Linford, 2015). The retractor penis muscle extends from the caudal vertebrae and internal anal sphincter to insert distal to the sigmoid flexure (Budras, 2003). These paired muscles relax during erection allowing the penis to extend from the prepuce and contract during quiescence, retracting the penis into the sheath (Budras, 2003). The body of the penis begins where the two crura meet distally to the ischiatic arch; it extends craniad, along the ventral body wall to become at the mid-ventral abdomen the free part of the penis. The body of the penis is bent in an "S" shape called the sigmoid flexure. The proximal bend of the sigmoid flexure opens caudally and is located near the scrotum. The distal bend is opened cranially and the short suspensory ligaments of the penis attach the penis to the ventral surface of the ischiatic arch (Nabors and Linford, 2015).

The glans penis is a small restricted region at the tip of the free part of the penis (Budras, 2003). The free part of the penis is the distal extent from the attachment of the internal lamina of the prepuce to the glans penis. The free end of the penis is twisted in a counter clockwise direction of the raphe of prepuce continued as the raphe of the penis to the urethral process.

The twist of the free end of the penis is due to the attachment of the apical ligament. The apical ligament of the penis is formed by the longitudinal fibres of the *tunica albuginea* leaving the body of the penis just distal to the sigmoid flexure and reattaching near the apex of the penis (Nabors and Linford, 2015).

The prepuce of the penis is composed of an external and internal fold or lamina. The external lamina is the haired outer fold of skin attached to the ventral abdomen. The haired skin terminates at the preputial orifice where the external fold turns inward to line the preputial cavity as the internal lamina. The internal lamina serves to attach the external lamina to the penile epithelium (Amann and Schanbacher, 1983).

2.4.5 Spermatic cord

The spermatic cord includes the *ductus deferens*, vasculature, lymphatic vessels, and nerves of the testicle and epididymis (Schaller and Constantinescu, 1992). Essentially, the spermatic cord consists of all the tissue within the vaginal tunic so it extends from the vaginal ring within the abdominal cavity to the testicle (Ross *et al*., 2003).

2.4.5.1 Blood supply

Before ejaculation can occur the testis must produce spermatozoa. This requires an adequate blood supply for the metabolic demands of cellular division for spermatogenesis and steroidogenesis. The arterial blood supply to each testis is provided by a testicular artery, a direct branch of the abdominal aorta arising caudal to the renal arteries.

The testicular artery crosses the lateral abdominal wall and then passes ventrally through the inguinal canal (Nabors and Linford, 2015). As the testicular artery approaches the testis it begins to spiral with the nearby tortuous pampiniform plexus of the testicular vein forming a vascular cone. This arterial/venous arrangement is an effective thermoregulatory apparatus (Brito *et al*., 2004). An adequate blood supply to the penis and associated muscles is required

for the processes of erection, ejaculation, and tissue maintenance. This comes by way of the internal iliac artery. The internal iliac artery is a direct continuation of the abdominal aorta at the entrance to the pelvic cavity. The umbilical artery, a branch of the internal iliac, supplies the *ductus deferens* and the bladder (Schaller and Constantinescu, 1992). The prostatic artery leaves the internal iliac and supplies the prostate, vesicular glands, *ductus deferens*, ureter, and urethra (Schummer *et al*., 1981). As the internal iliac continues through the pelvic cavity it divides into the caudal gluteal and internal pudendal. The internal pudendal gives off the ventral perineal artery, urethralis artery, and continues as the artery of the penis. The artery of the penis gives off the artery of the bulb of the penis, which supplies the bulbospongiosus muscle and the cavernous spaces of the corpus spongiosum (Schummer *et al*., 1981). The deep artery of the penis is another branch of the artery of the penis that enters the crus of the penis and supplies the erectile tissue, the corpus cavernosum (Ashdown *et al*., 1979).

After the deep artery branches off, the artery of the penis continues as the dorsal artery of the penis which passes along the dorsal aspect of the penis toward the glans penis and prepuce. It is responsible for maintenance of penile tissue during quiescence (Beckett *et al*., 1997).

2.4.5.2 Nervous supply

The innervation of the external genitalia of the bull consists of the pudendal nerve and its branches. The pudendal nerve carries motor, sensory, and parasympathetic nerve fibres. The pudendal nerve passes through the pelvic cavity medial to the sacrosciatic ligament and divides as it approaches the lesser ischiatic notch of the pelvis into proximal and distal cutaneous branches supplying the skin of the caudal hip and thigh (Budras, 2003). The pudendal nerve continues through the ischiorectal fossa, terminating in a preputial branch, a scrotal branch, and finally the dorsal nerve of the penis (Mullins and Saacke, 2003).

The pelvic nerve provides parasympathetic innervations from the sacral plexus. The hypogastric nerve contributes sympathetic fibres from the caudal mesenteric plexus to the genital system (Nickel *et al*., 1973).

2.4.6 Accessory glands

The accessory genital glands of the bull include the vesicular gland, ampulla of the *ductus deferens*, and the prostate and bulbourethral glands. The bilateral vesicular gland is the largest accessory gland in the bull and contributes the greatest volume to the ejaculate. It is a lobated gland of firm consistency. It lies dorsal to the bladder and lateral to the ureter and ampulla of the *ductus deferens* (Nickel *et al*., 1973). The body of the prostate lies dorsal to the urethra between and caudal to the vesicular glands. The disseminate part of the prostate is concealed in the wall of the urethra and covered by the urethral muscle (Nabors and Linford, 2015). The ampulla, vesicular glands, and prostate all empty their contents into the urethra through the *colliculus seminalis*. The bilateral bulbourethral gland lies on each side of the median plane dorsal to the urethra; it is mostly covered by the bulbospongiosus muscle. Its duct opens into the urethral recess. The urethral recess is a blind pouch that exits dorsally into the penile urethra at the level of the ishiatic arch. The presence of this structure makes it difficult to pass a catheter retrograde into the bladder (Nickel *et al*., 1973).

2.5 Puberty in Bulls

2.5.1 Overview of puberty in bulls

Puberty, defined as the age at which ejaculated semen contains at least 50 million spermatozoa with a minimum of 10% motility (Rekwot *et al*., 1987a), is a major determinant of optimum reproductive efficiency. The ejaculated semen is considered to be able to lead to a pregnancy, however, the number of sperm cells per ejaculate and the number of motility increase greatly beyond these values as the bull matures (Barth and Oko, 1989). Age at

puberty has ranged from as early as 11 months under a high protein diet in Bunaji and Friesian-Bunaji cross (Rekwot *et al*., 1987b) to as late as 17 months under range condition in Nigerian cattle (Oyedipe *et al*., 1981). The age for puberty for Bunaji and Bokoloji reared under natural conditions were found to be 17.5 months and 18.25 months respectively (Oyedipe *et al*., 1981).

The variability of onset of puberty, among and within breeds, has resulted in inconsistent reproductive performance of young bulls. To promote the successful use of yearling bulls, it is very important to understand pubertal changes and factors that affect pubertal development (Barth, 2004).

In the male suckling calf, the seminiferous cords of the testes contain primordial germ cells and Sertoli cells that generate the seminiferous epithelium (Barth, 2004). A lumen is established in the seminiferous cords by about 5 month of age and spermatogenesis is established by about 8 month of age (Curtis and Amann, 1981). The onset of puberty relies on the initiation of the GnRH pulse generator, which signals the anterior pituitary to secrete FSH and LH (Duittoz *et al*., 2016). These hormones are also important for Leydig and Sertoli cell proliferation and differentiation in the testes. The timing and intensity of the early transient LH rise, which typically occurs from approximately 8 to 20 wk of age, is an important determinant of age at puberty in the bull (Evans *et al*., 1995). An initial rise in FSH between 3 and 5 month of age results in proliferation of Sertoli cells, seminiferous tubule lengthening and an increase in tubule diameter. At the same time, there is a rise in LH secretion resulting in increased testosterone production by the Leydig cells. Between 5 and 8 month of age, FSH and LH remain low and then rise again with the onset of puberty. In well-fed bulls testis growth is almost linear between 7 and 12 month of age and scrotal circumference (SC) increases at a rate of 0.06 – 0.07 cm per day (Barth and Omiski, 2000).

The rate of testis growth declines after 12 month of age, but by 24 month of age, the testes will be approximately 90% of mature size (Coulter, 1986).

Semen quality improves and achieves adult characteristics over a period of 3 to 4 months after the onset of puberty (Lunstra and Echternkamp, 1982). Bulls within the same breed mature as much as 4 months later than the earliest maturing bulls (Almquist *et al*., 1976). Approximately 33% of beef bulls produce satisfactory quality semen (mature characteristics) at 12 month of age, about 60% at 14 month of age, and most have matured by 16 month of age (Cates, 1975; Arteaga *et al*., 2001).

2.5.2 Effect of nutrition on onset of puberty

High-energy diets with adequate protein, vitamins and minerals may hasten the onset of puberty and thus the age at maturity. This was demonstrated in an early study in which bull calves were raised on 60-75%, 100% and 140-160% of recommended energy intakes. The low energy diet tremendously delayed puberty (Bratton *et al*., 1956). Since later maturing bulls have a smaller rise in LH at 3 to 5 month of age than early maturing bulls (Evans *et al*., 1995) it would seem likely that nutritional restriction in the pre-weaning period is detrimental to early attainment of puberty despite good post weaning nutrition. In support of this, it has been shown that bulls raised to weaning age by heifer-mothers have smaller testes at 1 year of age than those raised by cow-mothers (Lunstra *et al*., 1988).

Different levels of nutrition after weaning appear to affect the rate of testicular growth, however, it is not clear whether age of onset of puberty is also affected. Ohl *et al*. (1996) examined the effects of rate of gain on scrotal circumference and testicular histology in 23 half-sibling beef bulls from 11.6 to 15.3 month of age. Rations were designed to result in maximum gains, or low gains (0.5 kg per day). At the end of the test period, the mean scrotal circumference was 34.0 and 31.7 cm for high- and low-gain rations, respectively; however,

there were no differences in testicular histology. Seidel *et al*. (1980), fed 133% and 95% of TDN requirements to Angus bulls from 7 to 11 month of age. At the end of the feeding period, he also found a larger mean scrotal circumference in bulls on the high-energy ration. In a study by Coulter *et al*. (1987), Angus and Hereford bulls fed 80% grain and 20% forage from weaning to 15 month of age, had a greater mean scrotal circumference at 12 month, but not at 15 month of age, than bulls fed 100% forage. The bulls on the grain-forage diet had significantly lower sperm outputs at 15 month of age than bulls on a medium-energy diet. There is evidence that excessive energy intake in young bulls may result in laminitis, abnormal bone and cartilage growth, and increases the risk of rumenitis which may lead to the development of vesicular gland infections (Dargatz *et al*., 1987; Greenough *et al*., 1990). Byrne *et al*. (2018) reported that Holstein-Friesian bulls feed with high-high plane of nutrition before and after 6 months of age influenced metabolic profiles which are important for promoting GnRH pulsatility in young bulls. Enhanced early-life nutrition increased testes size and weight and reduced age at puberty in beef and dairy bulls, compared to those fed 70% of dietary requirements (Dance *et al*., 2016).

2.5.3 Effect of breed on onset of puberty

Significant genetic variation exists between breeds of beef cattle for age at puberty (Cundiff *et al., 1986*). In general, the faster-gaining breeds of larger mature size reach puberty at a greater weight than slower-gaining breeds of smaller mature size. Breeds, historically selected for milk production, (e.g., Braunvieh, Gelbvieh, Red Poll, Pinzgauer and Simmental) reach puberty at significantly younger ages than do breeds not selected for milk production (e.g., Charolais, Limousin and Hereford). There are great differences between breeds of bulls and testicular size at any given age (Barth, 2000). In double muscled bulls, testes weight at 12 months of age was 14% less than in normally muscled bulls; however, the effect of double muscling on age at maturity has not been reported (Michaux and Hanset, 1981). There is

considerable evidence that SC measurement between 1 and 2 years of age is moderately to highly heritable (Coulter *et al*., 1976). Therefore, breeders could make rapid progress in selection for increased testes size and consequently earlier maturity.

2.5.4 Effect of season on onset of puberty

Although cattle do not have distinctly seasonal reproductive activity, there is evidence of a seasonal basis in bovine reproduction. For example, return to oestrous cyclicity is longer in cows that calf in winter than for those calving in summer (Hansen, 1985). In Wisconsin, heifers born in September were younger at puberty than those born in March. Photoperiod most likely played a role in these responses since heifers given supplemental light in autumn were younger at puberty than those exposed to natural light (Hansen *et al*., 1983). Season of birth also may influence age at puberty of bull calves. Aravindakshan *et al*. (2000), in his study reported that LH pulse amplitude was significantly lower from 4 to 24 weeks of age in bull calves born in autumn than those born in spring and age at puberty was more variable in the fall-born calves. In 2110 mature western Canadian range bulls, semen quality was lowest in fall and winter and highest in spring and summer (Barth and Waldner, 2002). Since semen quality in these bulls improved in the spring while bulls remained on the same winter feed supplies, differences in semen quality might have been due to milder weather conditions and/or increasing photoperiod.

Seasonal variation in LH and testosterone has been reported in other studies as well (Sundby and Tollman, 1974). In four Norwegian Red bulls, blood testosterone levels were significantly lower in October and December than in February, June and August. However, there is some conflict in the literature regarding the relationship of season and testosterone concentrations in bulls. Studies on seasonal effects on hormone secretion and spermatogenesis must be interpreted carefully, since latitude, housing, climatic heat, or cold

stress could influence testosterone levels. In addition, there is diurnal variation in LH and testosterone levels and, therefore, frequent blood sampling throughout a large portion of the day is necessary as a basis for comparison of hormone levels at different times of the year.

2.6 Reproductive Endocrinology

The hypothalamo-pituitary-gonadal axis (HPG axis) controls the pre-pubertal maturation and the post-pubertal function of the male reproductive tract and spermatogenesis.

2.6.1 GnRH, LH, Leydig Cells, and Testosterone

The hypothalamus major role in males is to secrete the decapeptide hormone, Gonadotropin-releasing hormone (GnRH). GnRH is synthesized within the arcuate nuclei of the hypothalamus and released in a pulsatile manner from specific GnRH-releasing neurons in mature males. These GnRH-releasing neuron's axons originate in the surge and tonic centres of the hypothalamus and release GnRH where they terminate upon blood vessels of the hypothalamo-hypophyseal portal system in the median eminence of the hypothalamus. Once in the hypothalamo-hypophyseal portal system, GnRH's target receptors lie in close proximity, in the anterior pituitary, and secreted GnRH travels through the hypophyseal portal system to reach this tissue (Schwanzel-Fukuda and Pfaff, 1989). Upon release from the portal vasculature, GnRH reaches its cellular target tissue, the gonadotropes of the anterior pituitary. Here, the binding of GnRH to GnRH receptors (GnRH-R) in the anterior pituitary stimulates these cells to produce and release the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Campbell *et al*., 2009).

The steps involving the release of LH from the anterior pituitary in response to GnRH are dynamic (Schanbacher and Echternkamp, 1978), and the time it takes for a GnRH pulse to elicit an LH pulse has been reported.

The main target tissue of LH in the reproductive axis is the testicular Leydig cells, which produces testosterone upon LH binding to LH-R. Testosterone is then able to regulate hypothalamic hormone release as well as spermatogenesis in mature bulls most notably by feeding back negatively on the anterior pituitary and hypothalamus (Schanbacher, 1982). However, testosterone's major role in the adult involves the maintenance of spermatogenesis. Testosterone is required for the production of sperm and their subsequent maturation in the epididymis, for the function of the accessory sex glands and for the development of masculine secondary sexual characteristics. Within the testis, androgen receptors are present in Leydig, Sertoli and myoid cells (Holdcraft and Braun 2004) but not in germ cells. Testosterone is converted by 5α-reduction into 5α-dihydrotestosterone (DHT) in the Sertoli cells and in accessory sex glands (Bardin *et al*., l994). DHT, which is not susceptible to aromatization and is a more potent androgen than testosterone itself, appears to be the primary androgen controlling accessory sex gland activity, whereas testosterone is the primary androgen involved in spermatogenesis (Walker and Cheng 2005). Both testosterone and DHT are bound within the tubule lumen by the secretory product of the Sertoli cells, androgen-binding protein (ABP). The role of ABP therefore appears to be to maintain high androgen concentrations in the lumina of the seminiferous tubule and epididymis (Walker and Cheng 2005).

Testosterone concentrations will begin to reach levels similar to those seen in adulthood around 6 months of age in bulls (Miyamoto *et al*., 1989), and concentrations within the testes will also increase drastically at this time. The concentration of testosterone within the testis is high due to binding with androgen binding protein produced by mature Sertoli cells (Dadoune and Demoulin, 1993). These high concentrations are believed to be crucial for prevention of degradation of maturing spermatocytes (Walker and Cheng, 2005).

2.6.2 FSH and Sertoli Cells

GnRH controls another 'branch' of the reproductive axis which consists of the production of the hormone FSH and its target, the Sertoli cells, which are needed in order to facilitate spermatogenesis. In similar fashion to LH, FSH is produced by the anterior pituitary in response to GnRH binding GnRH-R, although most likely not in a pulsatile manner (Dunkel *et al*., 1992). FSH has target receptors on the Sertoli cells of the testis and it is necessary for the general viability of Sertoli cells (Bagu *et al*., 2006).

2.6.3 Inhibin and Activin

Inhibins and activins are dimeric glycoproteins that fit dynamically into the above mentioned processes, essentially working as brakes and accelerators to the system by controlling FSH production and secretion by the anterior pituitary (Vale *et al*., 1988). Both of these proteins are formed from combinations of the same dimers that are either designated as α-subunits or β-subunits. Furthermore, β-subunits have one of five varieties that are designated βA through βE, but only the βA and βB subunits have physiological effects in animals (Mason, 1987).

Inhibins are comprised of heterologous pairings of α- and β-subunits, with the two main types of inhibin being inhibin A and inhibin B, nomenclatured αβA and αβB. Activins are homologous dimers of two β-subunits. The pairs of dimers form one of three forms of activin: Activin A (βAβA), activin AB (βAβB), and activin B (βBβB; Mason, 1987). Inhibin plays a negative regulatory role with respects to FSH production. It is produced by Sertoli cells upon the binding of FSH to the FSH-R located on the cell membranes and negatively feeds back on the gonadotrophs of the anterior pituitary to prevent further FSH production and release. In essence, circulating levels of inhibin relate inversely to circulating FSH in the male (de Kretser and Robertson, 1989). It was first observed that inhibin decreases the production of FSH from the anterior pituitary by injecting charcoal-extracted bovine

follicular fluid (containing inhibin) into bulls aged 1-2 years of age and observing the decrease in circulating FSH (McGowan *et al*., 1988). Inhibin also positively correlates with the number of Sertoli cells present within the testes of rats, and this relationship hints at the possibility for the estimation of Sertoli cell number noninvasively via the systemic inhibin levels in other species (Sharpe *et al*., 1999). Additionally, since it is known Sertoli cell number in humans is correlated with mature sperm production, the level of systemic inhibin could possibly even be used to estimate the mature sperm production in other animals such as bulls (Johnson *et al*., 1984).

The role of activins in the mature male are less evident, but it is known that activin is produced and secreted by the adult Sertoli cells and has nourishing autocrine effects on the cells (Buzzard *et al*., 2004). However, the main source of activin is from outside the tubules, probably from peritubular myoid cells (Buzzard *et al*., 2003).

2.7 Breeding Soundness Examination

Breeding soundness refers to a bull's ability to get cows pregnant. Although 20–40% of bulls may have reduced fertility, few are completely sterile (Kastelic *et al*., 2000). Examinations of male animals are made for two main purposes: either to ascertain whether normal fertility can be expected from the animal, or for the diagnosis of infertility (Oaks, 2001). In either situation, the requirements are a history of the animal, a general examination, a detailed examination of the genital tract, observation of copulation, and collection and evaluation of semen (Oaks, 2001). Sub-fertility in bulls delay pregnancy, prolongs calving interval, reduces calf weaning weight and increases culling rate of females. Multiple sire breeding groups and low breeding pressure may mask sub-fertility, but single-sire mating groups and artificial insemination (AI) increase the importance of bull fertility (Shaha *et al*., 2008).

Bulls can dramatically affect the reproductive efficiency of breeding herds, therefore, it is essential to have bulls examined at least 60 days before the breeding season in order to identify animals with low fertility (Chacon *et al*., 1999). Breeding soundness evaluation (BSE) of bulls has been extensively used for evaluation of male fertility prior to the breeding period over the past 50 years (Hoflack *et al*., 2008). BSE is a useful tool in identifying bulls with reduced fertility or physical problems which lower their ability to sire calves. Thus, eliminating the bulls with physical problems or reduced fertility from the breeding herd improves the overall reproductive efficiency of the herd (Bagley and Chapman, 2005).

According to the Society for Theriogenology (SFT), bull BSE comprises general and reproductive physical examination, scrotal circumference indexed for age, semen motility and sperm morphology examinations (Alexander, 2008). Additional parameters, such as trans-scrotal ultrasonography, have been reported recently to enhance the routine BSE in bulls (Chapwanya *et al*., 2008). Trans-scrotal scanning is a convenient, non-invasive technique which allows an examination of both palpable and non-palpable testicular lesions in goats and rams (Ahmad and Noakes, 1995), making its use ideal in farm situations (Chapwanya *et al*., 2008). However, the application and interpretation of its findings in determining fertility status of the bulls in relation to the routine BSE technique still require further research.

Despite the potential effect of the bulls' fertility on the overall reproductive efficiency of cattle herd, their role in the breeding programmes is often underestimated and the use of standard BSE for bulls before use for breeding has been overlooked. This allows poor performing bulls to be used for breeding and hence leads to reduction in an overall reproductive performance of the herd (Yimer *et al*., 2011).

In tropical breeds of bulls, the readily measurable reproductive parameters have been reported to include body weight (BW), scrotal circumference (SC), testicular size and sperm

morphology (Lunstra and Cundiff, 2003). Scrotal circumference and testicular measurements are superior of all testicular parameters in estimating testicular size and sperm output (Brito *et al.,* 2004; Ahmad *et al.,* 2005). Scrotal circumference is an indirect but reliable indicator of onset of puberty, semen quality, total semen production, testicular pathology, testicular weight and fertility of bulls (Ahmad *et al.,* 2005); whereas, testicular measurements provide reproductive and spermatogenic ability in post pubertal period of bulls (Siddiqui *et al.,* 2008). Likewise, age and BW of the breeding bulls are significantly correlated with each other and with SC as well (Raji *et al.,* 2008). Some researchers suggest paired testicular volume (PTV) as a more adequate selection parameter for reproductive performance in Zebu cattle as compared to SC (Unanian *et al.,* 2000).

2.7.1 Steps for routine breeding soundness evaluation in the bull according to McGowan, (2004)

2.7.1.1 Recording the husbandry and history of the bull.

Clinical illness, lameness and significant body condition changes in the past 2 to 4 months should be noted. The levels of concentrate feeding in the past 4 months and details of the bull's previous reproductive performance should be noted.

2.7.1.2. General physical examination including assessment of gait.

The bull should be adequately developed for its age and breed and be in good general health condition; free from conformational abnormalities likely to affect reproductive function, e.g. excessively straight legs. The posture and gait should be observed with the bull standing, walking and trotting freely in a yard, and particular note should be taken to the legs including the hooves.

2.7.1.3. Collection and evaluation of semen

2.7.1.4. Examination of the external and internal genitalia

After a visual observation, the prepuce, penis, scrotum, testicles, epididymides and spermatic cord should be carefully palpated. Measurement of the scrotal circumference (SC) is an accurate predictor of both testicular weight and sperm output, and for yearling bulls SC should be at least 30 cm. The internal genitalia are examined by rectal palpation.

2.7.1.5. Assessment of mating ability and libido.

A serving and mating ability test requires observation of the bull repeatedly attempt to mount and serve restrained females. This procedure is not in use in Sweden due to ethical matters. Instead the farmers are instructed to carefully observe the bull during the first 3 weeks of mating. Today no complete BSE protocol is routinely used in Sweden. Measurement of the SC and a clinical examination of the genital organs are performed routinely at the performance testing station, but still not routinely by practitioners in the field. Some bulls with normal SC and normal testicular tone may still have unsatisfactory semen pictures, reemphasizing the importance of including the microscopic examination, and especially the morphological examination of semen (Holroyd *et al.,* 2002), as part of the BBSE (McGowan *et al.,* 2002). However, no satisfactory methods are, at present, available that facilitate collection and evaluation of semen of potential beef sires in Sweden.

2.7.2 Scoring of a breeding soundness examination

The scoring criteria for a breeding soundness examination should have a 30% minimal sperm motility, a 70% minimum sperm normality and a minimum scrotal circumference by age should be as mentioned in Table 2.3

Table 2.3: Minimum scrotal circumference by age

Age (months)	Circumference (cm)
15 or younger	31
16-18	32
19-21	33
22-24	34
25 or older	35

During physical examination, the bulls must have adequate body condition score, sound feet, legs and eyes; also the bulls must not have abnormalities in seminal vesicle, ampullae, prostate, bulbourethral gland, inguinal rings, penis, prepuce, testicles, spermatic cord, scrotum (shape and content) and epididymis (Adapted from Society of Theriogenology, 1992).

2.8 Bull Fertility

A bull is said to be fertile if it has the ability to produce sperm to fertilize and activate the egg and sustain development. Bull fertility is crucial for efficient production of cattle and it is measured by the number of viable animals that have been sired by a specific bull (Peddinti *et al*., 2008).

2.8.1 Physiological determinants of bull fertility

Spermatozoa develop within the walls of the seminiferous tubules of the testes by the complex process of spermatogenesis. In the bull, spermatogenesis occurs over a 65-day process in which the spermatogonial stem cells undergo first mitosis, then meiosis, and subsequent physiological and morphological changes to produce mature spermatozoa in the epididymis. Both cellular and molecular integrity of sperm are essential for the sperm to fertilize and activate the ovum, and then sustain early embryonic and subsequent foetal development. Because these processes are influenced by a number of intrinsic and extrinsic factors, the heritability of fertility is low (Parisi *et al*., 2014). If the fertility phenotype is not identified in animal selection, major economic losses can occur due to expenses associated with housing of non-pregnant females as well as bulls that are not of value as they are unable to impregnate females.

In general, a bull can produce 4 to 5 billion sperm in a single ejaculation. If the ejaculate contains a large number of sperm afflicted with any of a variety of abnormalities, fertility of

the bull will be expected to be low (Kaya *et al*., 2014). This low fertility can be improved by increasing the number of sperm used in artificial insemination if it is a compensable phenotype, but not in the case of uncompensable fertility characterized by molecular defects in the sperm (Oliveira *et al*., 2013). In general, success of a mating depends on both quality and quantity of semen delivered to the female. However, no correlation exists between quantity of sperm per artificial insemination dose and maximum fertility of a bull (Parisi *et al*., 2014). Males differ not only in fertility but also in the minimum number of sperm per insemination required to reach maximum fertility (Parisi *et al*., 2014).

Production of1 viable sperm is influenced by many systems within the whole animal. The primary organs that produce the necessary hormones and regulators involved in sperm production are the hypothalamus, pituitary gland, and testes. The endocrine system maintains the critical balance between cellular requirements and concentrations of hormones. Key hormones involved in sperm production include GnRH, FSH, LH, testosterone, activin and inhibin. The male reproductive system must maintain the proper balance of these hormones. If the balance is threatened or altered, normal sperm production is then changed and infertility could become an issue (Gilbert *et al*., 2012). Endocrine disruptors are chemicals that act as agonists or antagonists to hormones and interfere with hormonal balance. Endocrine-disrupting compounds include dichlorodiphenyltrichloroethane, glycol ethers, dibromochloropropane, and methoxychlor. Other systems such as the digestive, immune, and cardio vascular systems are important for bull health and sperm production. For example, diseases, especially testicular diseases, can alter sperm quality and cause low fertility or infertility. Seminal vesiculitis and epididymitis are common diseases of the secondary sex organs of the bull, in which collected sperm must be discarded if these diseases are present (Parisi *et al*., 2014). Hence, study of systems physiology is essential in order to examine the reproductive health of the sires to identify changes that could affect bull fertility. Economic

savings from eliminating low fertility bulls will have a huge impact on a producer's herd (Feugang *et al*., 2009).

Overcoming environmental challenges (nutrition, climate, and management) is paramount for producers to maximize reproductive efficiency and genetic improvement. A combination of short photoperiod, cold stress, and reduced feed quality will have detrimental effects on semen quality and spermatogenesis in bulls. A well-managed nutrition program should meet nutrient requirements to ensure that animals are not under- or overfed. Overfeeding can have negative effects on reproductive performance as increased scrotal temperatures reduce sperm production and the quality of stored sperm (Parisi *et al*., 2014).

The three periods on which to focus on nutrition are pre-weaning nutrition, post-weaning nutrition, conditioning prior to breeding season, breeding season, and post-breeding season (Walker *et al*., 2009). Nutrition may have a major impact on secretion of gonadotropins and consequently sexual development in bulls (Brito *et al*., 2007; Barth *et al*., 2008).

Climate (heat, cold, wind, humidity) can affect sperm number, morphology, and physiology. Ambient temperature between 5 °C and 15 °C is optimal for semen production (Fuerst-Waltl *et al*., 2006). Paying close attention to body condition and providing bedding such as hay and shelter from wind and weather during the winter months helps prevent losses of bull breeding capability.

2.8.2 Cellular determinants of bull fertility

Several methods can be used to examine quality of spermatozoa: microscopy, computer-assisted sperm analysis, and flow cytometry (Lorton, 2014). These 3 methods help analyse different sperm characteristics including motility, membrane integrity, viability, and morphology. Abnormal spermatozoa lack the morphometric shape and/or size of the sperm

characteristic of the species. Abnormal sperm often are associated with subfertility or sterility, depending on the type or frequency of the morphological abnormalities.

Origins of abnormal sperm morphology can be determined by looking at the localization of the abnormality, reactive oxygen species (ROS), environment, DNA methylation, and chromatin structure (Kaya *et al*., 2014). Prediction of fertilizing ability is still largely a mystery owing to the fact that abnormal sperm coexist along with normal sperm cells.

Economic impact of this is so important that conducting sperm evaluations and breeding soundness exams (BSEs) is essential for establishing fertility levels in advance (Walker *et al*., 2009).

2.8.3 Molecular determinants of bull fertility

Sperm head which contains the sperm DNA is a feature vital for sperm function during the sperm's progress through the female reproductive tract and subsequent fertilization and activation of the zygotic/embryonic genome. Integrity of sperm DNA is critical for reproduction because, depending on the correct functions of a damaged region, embryonic gene expression or chromatin structure might be disrupted (Bochenek *et al*., 2001). A change in the conformation of the sperm chromatin can cause a decrease in fertility, so stable and correct chromatin structure is essential for sperm function (Evenson *et al*., 1980). Sperm DNA is under the constant pressure of oxidative stress because of excessive generation of ROS, which oxidize DNA and interfere with capacitation, hyper-activation, and sperm–oocyte fusion (Kaya *et al*., 2014). They are produced internally within the cell as well as exogenously by atmospheric oxygen and other environmental factors including pollution and radiation (Agarwal *et al*., 2005). Methods of measuring ROS and sperm chromatin structure assay can be used to assess molecular and cellular characteristics of sperm. However, caution should be taken because the feasibility and reliability of these approaches have limitations

(Agarwal and Said, 2003). Researchers screened DNA from high versus low fertility bulls using high density single nucleotide polymorphism (SNP) microarrays and demonstrated specific SNPs associated with bull fertility (Blaschek *et al*., 2011). In another genomics study, an SNP in the *itgb5* gene was shown to be associated with bull fertility (Feugang *et al*., 2009). Using a proteomics approach, Peddinti *et al*. (2008) showed 125 proteins differentially expressed in sperm from bulls with varied fertility.

Researchers have demonstrated that sperm contain mRNAs as well as small noncoding RNAs. Although sperm are transcriptionally silent, the significance and functions of these small noncoding RNAs are not totally clear (Gilbert *et al*., 2007). Possibly, sperm transcripts can be a mirror of spermatogenesis and, thus, can reveal the health of the sperm. For example, Govindaraju *et al*. (2012) showed that top mRNAs from bull sperm are involved in the expression of genes. These include highly important genes such as *DALRD3*, which is essential to ATP and nucleotide binding, and *IFT80*, which is required by the cell to maintain functional cilia. Sperm transcripts might also be transferred into the oocyte during fertilization and play important roles in zygotic and embryonic gene expression. Feugang *et al*. (2010) demonstrated differentially expressed sperm transcripts that exhibited functions for biological processes critical for embryonic development. Some of these biological processes include transport, signalling pathways, and cell protein modifications. Liu *et al*. (2012) discovered that the first embryonic cleavage in mice is directly dependent on the presence of mRNA-34c, which is found mostly in mammalian sperm. Sperm transcripts can be detected using RNA sequencing (also known as deep sequencing or next generation sequencing) and by real-time reverse-transcriptase polymerase chain reaction. Special attention needs to be given to RNA isolation because sperm contain large amounts of DNA and only minute amounts of RNA. Thus, it is essential to use techniques to ensure isolation of RNA, free from DNA.

Spermatozoa contain diverse proteins that are essential for sperm structure and function. For example, AQP7, an integral membrane aquaporin protein essential for aqueous movement, is found in the sperm tails, providing motility, and is often found lacking in sperm from patients or animals suffering from infertility (Govindaraju *et al*., 2012). Due to the important nature of these sperm proteins, their expression can/should be monitored to determine male infertility. Additionally, some sperm proteins play important roles in fertilization and embryonic development. As an example, PLC Zeta and PAWP regulate egg activation and embryonic development, respectively (Wu *et al*., 2007). Still, some other sperm proteins regulate sperm chromatin structure; these are protamines and histones. Concentrations of sperm protamines are associated with male fertility (Oliveira, 2006). Oliveira *et al*. (2013) demonstrated that bull sperm contain histones that may play important roles in sperm chromatin structure associated with bull fertility. Two of the histones involved in the structure of chromatin, specifically H2B and H3, are often associated with gene activation. Another histone, H4, is important for proper chromatin remodelling during spermatogenesis and is necessary for the zygote to inherit the correct chromosomal structure. These examples show that paternal histones play important roles in early embryonic development and can be analysed to evaluate male fertility (Oliveira *et al*., 2013).

Other macromolecules contained in the membranous sperm are polyunsaturated fatty acids, which are highly susceptible to oxidative damage and can interfere with the ability of the sperm to fertilize the ovum (Kasimanickam *et al*., 2007). Any toxic lipid peroxides cause membrane damage and reduce motility. Bulls with lower sperm lipid peroxidation have higher chances of siring calves. This is attributed to the deleterious effects of lipid peroxidation on sperm plasma membrane integrity and sperm DNA, which may reduce fertilizing potential of spermatozoa (Kasimanickam *et al*., 2007). The lipid content of a spermatozoon is very precise and provides functions for maintaining proper cell structure and

physiology. Spermatozoa contain polyunsaturated fatty acids, sterols, plasmalogens, and sphingomyelins in great quantity. Lipid composition supplies a necessary flexibility to the cell. Lipids regulate other cellular functions, such as spermatogenesis and capacitation (Sanocka and Kurpisz, 2004). However, high levels of lipids cause the sperm cell to be susceptible to damage by reactive oxygen species. Amounts of sperm lipids can be measured using lipidomics approaches (Petcoff *et al*., 2008).

2.9 Conception Rates and Scrotal Circumference

Research in *Bos taurus* (British breed type) bulls, in Victoria, indicates that in normal testicles, scrotal circumference, a measure of testicle size, is a relatively accurate indicator of bull fertility. According to Schoenian (2012) scrotal circumference (SC) measurement can be taken along with the palpation of the testicle area as part of breeding soundness evaluation. Neary (2014) found that conception rates in cows mated to bulls with a scrotal circumference of less than 30 cm were low, but were satisfactory in cows mated to bulls with a scrotal circumference of 30 cm or greater.

Research in Queensland has shown that this cut-off point of 30 cm in scrotal circumference can also be used in *Bos taurus indicus* (Brahman type*)* bulls and crossbreds of *Bos taurus indicus* and *Bos taurus taurus.* Most beef bulls that are older than two years and which have a scrotal circumference of less than normal range will have lower fertility due to insufficient sperm per ejaculate.

However, better fed bulls will have larger scrotal circumferences at a given age. For example, a 24-month old Brahman bull under feedlot conditions will have a scrotal circumference of 32 cm compared to 28 cm for a similar bull raised on native pasture (Jayawardhana, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Samples were collected at the Zango-Zaria and Kano Central abattoirs and the samples were analysed at Biotechnology Laboratory Research Unit of the National Animal Production Research Institute, Ahmadu Bello University, Shika-Zaria. Shika is located in the Northern Guinea Savannah zone of Nigeria which is within latitude 11°8'19.56" and longitude 7°45'51.22" and at an elevation of 650 m above sea level. Average annual maximum and minimum temperature of 38.8 ± 3.2°C and 18.0 ± 3.7°C respectively characterising the climate of the area. The monthly average relative humidity is 70.1 ± 9.7%. There are two main seasons in the zone which are rainy season (April - October) and dry season (November – March).

3.2 Experimental Bulls

Sixty (60), apparently, healthy post-pubertal bulls comprising 20 Bunaji (White Fulani), 20 Bokoloji (Sokoto Gudali) and 20 Rahaji (Red Bororo) breeds were used for the experiment.

3.3 Experimental Design

The different breeds were divided into three groups: Group Bu (20 Bunaji), Group Bo (20 Bokoloji) and Group Ra (20 Rahaji) bulls. Apparently healthy Bunaji and Bokoloji bulls of between the ages of 3 and 6 years were randomly selected as they entered the Zango-Zaria abattoir, and also, apparently healthy Rahaji bulls of between the ages of 3 and 6 years were randomly selected as they entered the Kano Central abattoir. These bulls were from different background. The Body condition score was noted for each selected bull, the estimated body weights and the scrotal circumference were taken and recorded for each selected bull. Testes (comprising the testicles and epididymides) were collected after slaughter of each selected

bull, preserved in an ice packed insulated container and transported directly to the laboratory for testicular dimension measurements, gonadal and epididymal sperm reserves evaluation.

3.4 Age Estimation, Body Weight Measurement and Body Condition Score of the Experimental Bulls

The age of each selected bull was determined using their dentition according to Lasisi *et al*. (2002) as described on table 3.4.

The body weight of each bull was estimated using the chest girth tape which was in kilogram (kg).

Body condition score of a five-class scale ranging from 1 (extremely thin) to 5 (extremely fat) as described by Moraes *et al*. (2007) was taken before the bulls were slaughtered. The scoring as described by Moraes *et al*. (2007) is described below:

Score 1 (Extremely Thin)

Individual short ribs have a thin covering of flesh. Bones of the chine, loin and rump region are prominent. Hook and pin bones protrude sharply, with a very thin covering of flesh and deep depressions between bones. Bony structure protrude sharply and ligament prominent.

Score 2 (Thin)

Individual short ribs can be felt but are not prominent. Each rib is sharp to touch but have a thicker covering of flesh. Short ribs do not have as distinct an over-hanging shelf effect. Individual bone is the chine, loin and rump regions are not visually distinct but easily distinguishable by touch. Hook and pin bones are prominent but the depression between them is less severe. Area below tail head and between pin bones is somewhat depressed but the bony structure has some covering of flesh.

Score 3 (Moderate)

Short ribs can be felt by applying slight pressure. Altogether, short ribs appear smooth and the over-hanging effect is not so noticeable. The backbone appears as a rounded ridge, firm pressure is necessary to feel individual bones. Hook and pin bones are rounded and smooth. Area between pin bone and around tail head appears smooth without sign of fat deposit

Score 4 (Fat)

Individual short rib is distinguishable only by firm palpation. Short ribs appear flat or rounded, with no overhanging shelf effect.

Ridge formed by backbone in chine region is rounded and smooth. Loin and rump region appear flat. Hooks are rounded and the space between them is flat. Area of tail head and pin bones is rounded with evidence of fat deposit.

Score 5 (Extremely fat)

Bony structures of backbone, short ribs and hook and pin bones are not apparent; subcutaneous fat deposit very evident. Tail head appears to be buried in fat tissue.

Table 3.1: Permanent Teeth Eruption in Cattle

Permanent Tooth	Age at Eruption	Level and Neck Emerged from Gum
Central/ pincher/ I_1)	1.5-2 years old	6 years old
1st intermediate incisor/ lateral/ I_2	2-2.5 years old	7 years old
2nd intermediate/lateral incisor/ I_3	3 years old	8 years old
Corners / I_4	3.5-4 years old	9 years old
Full mouth and in wear	5 years old	

(Adapted from Lasisi *et al.*, 2002)

3.5 Scrotal Circumference Measurement

Scrotal circumference was measured with a flexible tape after restraining the bull (Perumal, 2014). The two testicles were pushed firmly into the ventral part of the scrotum by placing the thumb and fingers laterally on the side of the neck of the scrotum and pushing ventrally. A flexible cloth tape formed into a loop and slipped over the scrotum and scrotal circumference was measured in centimetres by pulling the tape snugly around its greatest diameter (Perumal, 2014).

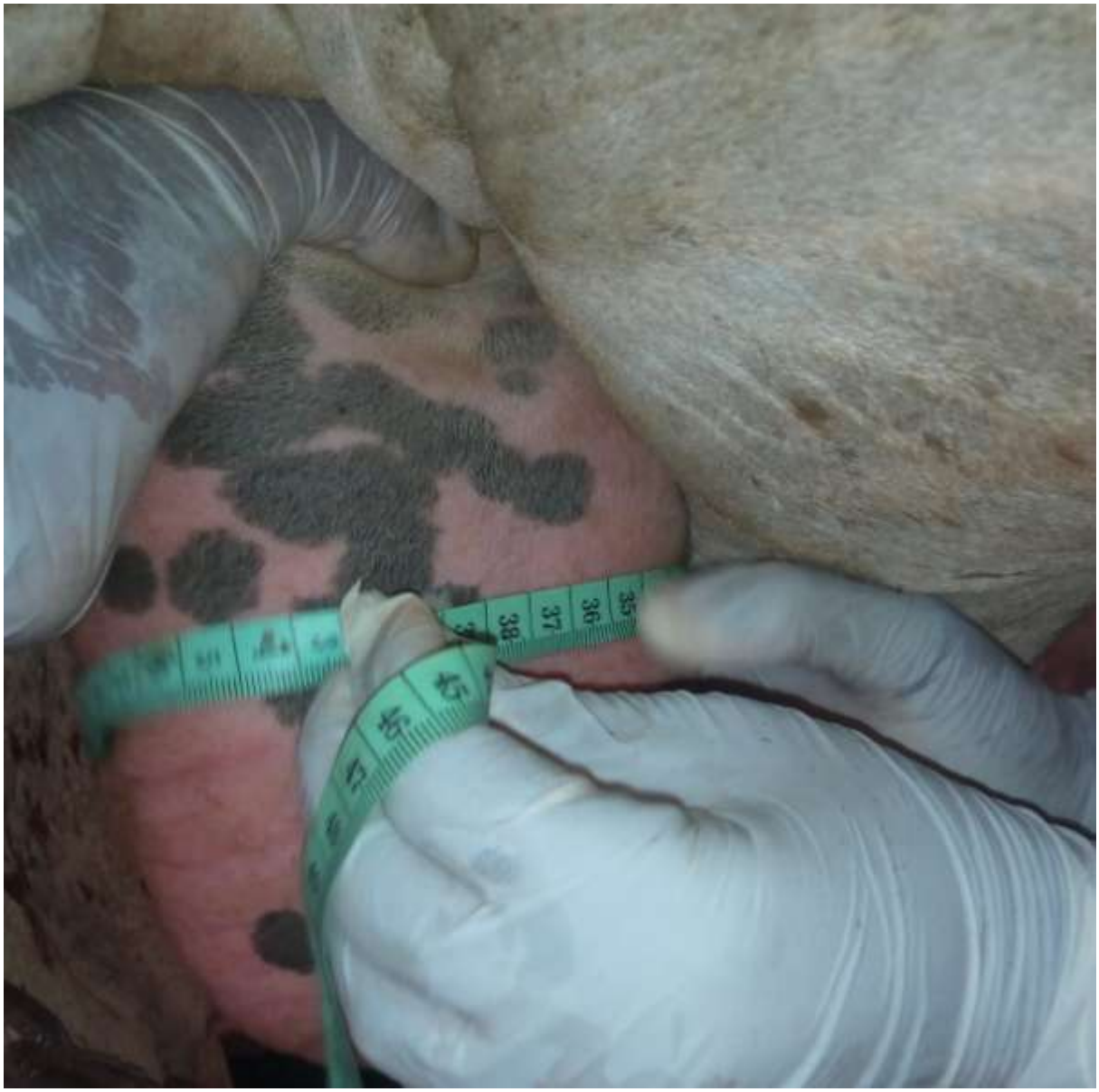

Plate IV: Scrotal Circumference measurement in centimetres of a Bunaji bull

Plate V: Removal of epididymis from a testis of a Rahaji bull

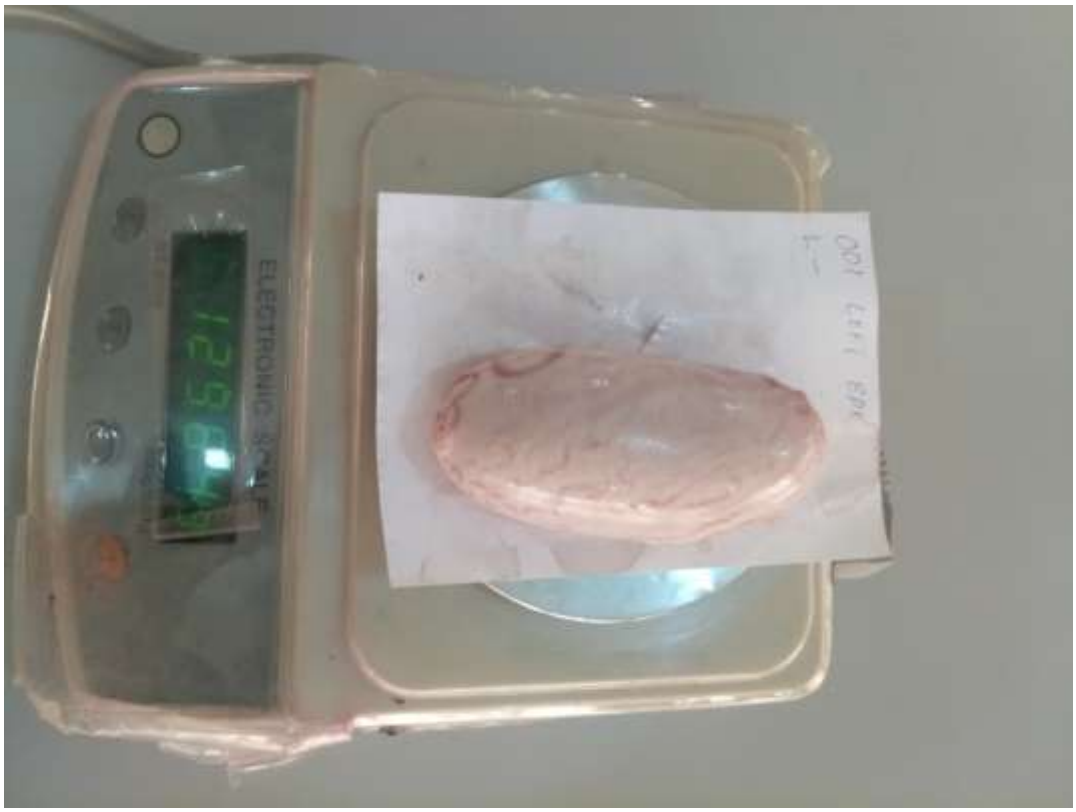

Plate VI: Weighing of a testis of a Bokoloji bull

Plate VII: Counting of sperm of Bokoloji bulls

3.6 Gonadal and Epididymal Sperm Reserves

Gonadal and epididymal spermatozoa reserves were determined by the method of Addass (2011b). The epididymis was separated from each testis with a scalpel, then, the volume, length and weight of each testis from each of the slaughtered bulls were determined using water displacement method, a measuring tape and a digital weighing scale respectively. The *tunica albuginea* was then removed from each testis. The lengths and weights of the epididymides of each bull slaughtered were determined using measuring tape and a digital weighing balance, respectively. The left and right caput, left and right corpus and left and right cauda of each testes of each bull slaughtered were separated using sharp scissors. Records of the left and right testes and the different segments of the left and right epididymides of each bull slaughtered were taken. Thereafter, the testicular and epididymal spermatozoa count were determined by homogenisation (Addass, 2011b).

Each fraction of the testis was homogenised in 50 mL of physiological saline solution using a mortar and pestle. Streptomycin sulphate (1 mg/mL) and Penicillin G (10000 IU/mL) were added to the saline solution. The homogenate volume was measured after rinsing the mortar with 20 ml of the saline solution and the effluent was added. Exactly 5 mL of the homogenate was transferred into a conical flask and further diluted with 80 ml of the physiological saline solution. This was stored over night at about 5ºC in a refrigerator to allow for separation of spermatids and spermatozoa from other cells. Samples were then introduced into the Neubeur haemocytometer counting chamber. The cells were allowed to stand for 2 minutes to settle down (Addass, 2011b). The determination of spermatozoa and spermatid reserves was done according to the standard method of Zemjanis (1970).

The left and right caput, left and right corpus and left and right cauda of each testes of each bull slaughtered were minced (using a sharp-sharp scissors) separately in 40 ml of physiological saline solution. The mixture was stored for 24 hours at 5°C, each mixture was

filtered using gauze; thereafter, the filtrate volume was measured. Spermatozoa/spermatid concentration was determined using a haemocytometer according to the method of Kwari and Waziri (2001).

3.7 Data Analysis

Statistical analysis was conducted using the Graph Pad Prism version 5.0. Values obtained were expressed as mean ± standard error of mean (±SEM). Mean values (± SEM) for each parameter were computed. Data were analysed using One-way Analysis of Variance (ANOVA) and Tukey's post hoc test to compare between the groups. Significant of differences between groups were estimated as $P < 0.05$. Correlation coefficients using Pearson's correlation were estimated among the age, body condition score, body weight, scrotal circumference, testicular dimensions, gonadal sperm reserves and epididymal sperm reserves of the bulls. Differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered to be statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Ages, Body Measurements and Testicular Morphometric Characteristics of Bunaji, Bokoloji and Rahaji breeds of cattle

The mean values of the body weights of Bunaji, Bokoloji and Rahaji bulls ranged from 329.50 ± 11.28 kg to 396.8 ± 13.15 in kg, with Bunaji breed having the significantly ($P < 0.05$) least body weight, while the Rahaji breed had the relatively highest body weight.

The mean body condition score ranged from 3.05 ± 0.02 to 3.30 ± 0.06, the mean values were not significantly ($P > 0.05$) difference from one another.

The mean values of the scrotal circumferences ranged from 28.33 ± 0.69 cm to 33.70 ± 1.47 cm, with the Bunaji breed having the significantly lowest ($P < 0.05$) value and Bokoloji breed recorded significantly the highest ($P < 0.05$) value as seen in Table 4.1

The Bokoloji breed had significantly the lowest ($P < 0.05$) values of testicular weight and testicular volume, when compared to the corresponding values recorded in either the Bunaji or Rahaji breed. The testicular length of Rahaji breeds did not differ significantly among the breeds as seen in Table 4.1.

Table 4.1: Mean (± SEM) Values of the Ages, Body Measurements and Testicular Morphometric Characteristics of Bunaji, Bokoloji and Rahaji Breeds of Cattle

Parameters	Bunaji n=20	Bokoloji n=20	Rahaji n=20
Age (years)	3.50 ± 0.12	4.10 ± 0.23	5.4 ± 0.17
Body measurements			
Body weight (kg)	329.50 ± 11.28[a]	392.90 ± 18.59[b]	396.8 ±13.15[b]
Body condition score	3.05 ± 0.20	3.00 ± 0.11	3.30 ± 0.06
Scrotal circumference (cm)	28.33 ± 0.69[a]	33.70 ± 1.47[b]	30.90 ± 0.78[a]
Testicular Weight (g)			
Right	176.55 ± 13.93[a]	147.70 ± 8.08[b]	166.10 ± 7.15[a]
Left	170.86 ± 11.73[a]	144.82 ± 8.29[b]	165.30 ± 7.73[a]
Paired	347.41 ± 25.37[a]	292.52 ± 15.89[b]	331.38 ± 14.77[a]
Testicular Volume (mL)			
Right	179.50 ± 17.07[a]	144.50 ± 10.46[b]	174.00 ± 6.74[a]
Left	175.00 ± 14.05[a]	144.00 ± 8.77[b]	174.50 ± 7.78[a]
Paired	354.50 ± 30.52[a]	288.50 ± 18.59[b]	348.50 ± 14.31[a]
Testicular Length (cm)			
Right	10.63 ± 0.42	10.17 ± 0.23	10.87 ± 0.25
Left	10.82 ± 0.32	10.05 ± 0.20	10.91 ± 0.20
Average	10.72 ± 0.30	10.11 ± 0.20	10.89 ± 0.22

abc =Values with different superscripts across the same rows differ significantly ($P < 0.05$), n=20

4.2 Epididymal Weights and Lengths of Bunaji, Bokoloji and Rahaji breeds of cattle.

The epididymal weights of Bunaji, Bokoloji and Rahaji breeds did not differ significantly. The mean values of the caput weights, corpus weights and cauda weights were statistically ($P > 0.05$) similar among the breeds as seen in Table 4.2.

The right and left epididymal length did not differ significantly among the breeds. Bunaji breed had the lowest value of the average epididymal length compared to the Bokoloji or Rahaji breed. Rahaji breed had significantly ($P < 0.05$) the highest value of average caput length compared to that of the Bunaji or Bokoloji breed.

The Bokoloji breed had the significantly ($P < 0.05$) the highest mean values for the right, left and average corpus length, when compared to the corresponding mean values recorded in either the Bunaji or Rahaji breed.

The right, left and average values of the cauda length were significantly higher ($P < 0.05$) in both the Bunaji and Bokoloji bulls than in the Rahaji breed as seen in table 4.2.

Table 4.2: Mean (± SEM) Values of the Epididymal Weight and Length of Bunaji, Bokoloji and Rahaji Breeds of Cattle

Parameter	Bunaji n=20	Bokoloji n=20	Rahaji n=20
Epididymal weight (g)			
Right	22.37 ± 1.60	22.73 ± 1.35	21.57 ± 1.19
Left	21.95 ± 1.53	21.56 ± 1.35	22.18 ± 1.25
Paired	44.33 ± 3.05	44.29 ± 2.66	43.75 ± 2.38
Caput weight (g)			
Right	11.05 ± 0.82	10.84 ± 0.65	10.97 ± 0.69
Left	11.10 ± 1.01	10.29 ± 0.71	11.18 ± 0.69
Paired	22.15 ± 1.71	21.12 ± 1.33	22.15 ± 1.34
Corpus weight (g)			
Right	2.19 ± 0.21	2.39 ± 0.26	1.91 ± 0.10
Left	2.09 ± 0.15	2.28 ± 0.20	2.04 ± 0.15
Paired	4.28 ± 0.34	4.67 ± 0.44	3.95 ± 0.22
Cauda weight (g)			
Right	8.25 ± 0.62	8.16 ± 0.44	7.71 ± 0.50
Left	8.01 ± 0.64	7.79 ± 0.51	8.10 ± 0.50
Paired	16.25 ± 1.25	15.95 ± 0.93	15.81 ± 0.98
Epididymal length (cm)			
Right	16.15 ± 0.57	17.39 ± 0.41	16.48 ± 0.44
Left	15.82 ± 0.46	16.46 ± 0.37	16.47 ± 0.22
Average	15.98 ± 0.47^{a}	16.93 ± 0.33^{b}	16.47 ± 0.25^{b}
Caput length (cm)			
Right	5.89 ± 0.28	5.80 ± 0.17	6.57 ± 0.18
Left	5.87 ± 0.33	5.58 ± 0.20	6.38 ± 0.18
Average	5.88 ± 0.28^{a}	5.69 ± 0.16^{a}	6.44 ± 0.16^{b}
Corpus length (cm)			
Right	6.22 ± 0.40^{a}	7.16 ± 0.28^{b}	6.59 ± 0.15^{a}
Left	5.89 ± 0.29^{a}	6.76 ± 0.19^{b}	6.08 ± 0.11^{a}
Average	6.05 ± 0.30^{a}	6.96 ± 0.20^{b}	6.33 ± 0.11^{a}
Cauda length (cm)			
Right	4.84 ± 0.33^{a}	4.75 ± 0.21^{a}	4.13 ± 0.14^{b}
Left	4.62 ± 0.23^{a}	4.49 ± 0.15^{a}	4.07 ± 0.16^{b}
Average	4.73 ± 0.22^{a}	4.62 ± 0.16^{a}	4.10 ± 0.14^{b}

ab =Values with different superscripts across the same rows differ significantly ($P < 0.05$), n=20

4.3 Gonadal and Epididymal Sperm Reserves of Bunaji, Bokoloji and Rahaji breeds of cattle

The mean values of the gonadal sperm reserve in Bunaji, Bokoloji and Rahaji bulls did not differ significantly. The Rahaji breed had the highest mean value for the right testis but Bunaji breed had the highest in the left testis and the paired testes among the breeds.

The mean counts obtained in the paired epididymal sperm reserve was significantly highest ($P < 0.0001$) in the Bunaji breed, compared to the Bokoloji and Rahaji breed.

In the right caput, Bunaji and Bokoloji breeds had significantly higher mean ($P < 0.01$) counts for the sperm reserve than Rahaji breed. In the left caput, the count in Bunaji breed was significantly higher ($P < 0.01$) than the Bokoloji breed.

The count recorded in the right and left corpus epididymides were insignificant ($P > 0.05$).

In the right and left cauda epididymis, the count recorded for the Bunaji breed was significantly higher ($P < 0.0001$) than the counts obtained in Bokoloji or Rahaji breeds as seen in table 4.3.

Table 4.3: Mean (± SEM) values of Gonadal and Epididymal Sperm Reserves of Bunaji, Bokoloji and Rahaji breeds of Cattle

Parameter	Bunaji n=20	Bokoloji n=20	Rahaji n=20
Gonad (x 10^9/g testis)			
Right	30.15 ±3.27	28.48 ±8.98	31.09 ±3.81
Left	25.60 ±3.90	17.43 ±4.46	22.88 ±2.78
Paired	55.75 ±6.19	45.90 ±13.31	53.97 ±6.33
Caput (x 10^9/g)			
Right	8.38 ±2.15^a	6.58 ±1.39^a	0.66 ±0.27^b
Left	10.92 ±4.93^a	2.61 ±0.45^b	0.00 ±0.00^c
Paired	19.30 ±6.76^a	9.19 ±1.54^b	0.66 ±0.27^c
Corpus (x 10^9 /g)			
Right	0.85 ±0.45	0.17 ±0.06	0.00 ±0.00
Left	0.77 ±0.42	0.38 ±0.13	0.20 ±0.10
Paired	1.63 ±0.73	0.55 ±0.14	0.20 ±0.10
Cauda (x 10^9/g)			
Right	91.71 ±19.46^a	8.14 ±1.56^b	7.12 ±1.20^b
Left	65.88 ±14.27^a	6.35 ±0.85^b	5.45 ±0.99^b
Paired	157.60 ±32.97^a	14.49 ±1.90^b	12.58 ±2.13^b
Epididymal (x 10^9/g)			
Right	101.00 ±21.02^a	14.89 ± 2.53^b	7.79 ± 1.30^b
Left	77.57 ± 16.39^a	9.34 ± 0.87^b	5.65 ± 0.98^b
Pair	178.50 ±39.22^a	24.23 ± 2.77^b	13.44 ± 2.24^b

abc =Values with different superscripts across the rows differ significantly ($P < 0.05$), n=20

4.4 Correlation coefficients between age, body weight, body condition score, scrotal circumference, testicular dimensions, gonadal and epididymal sperm reserves of Bunaji, Bokoloji and Rahaji breeds of cattle.

The relationship between age and scrotal circumference was significant (r = 0.603, $P < 0.01$) in the Bunaji bulls when compared to the other breeds ($P > 0.05$) which were insignificant, though, Bokoloji bulls showed negative (r = -0.030) correlation. The relationships between age and PTV, age and PTW, age and ATL; and, age and PEW in all the three breeds were all positively correlated but insignificant ($P > 0.05$). The relationship between age and GSR in the Bunaji bulls was positive and significant (r = 0.815, $P < 0.001$) when compared to the other breeds that predicted negative correlation that were not significant ($P > 0.05$). The Relationship between age and ESR in the Bokoloji bulls was positive and insignificant (r = 0.029, $P > 0.05$) when compared to the other breeds that predicted a significant ($P < 0.05$) and positive correlation.

The relationship between the BW and SC was positive and significant (r = 0.601, $P < 0.001$) in the Bunaji bulls when compared to the others that predicted insignificant relationships ($P > 0.05$) though the Rahaji bulls predicted a negative correlation (r = -0.043). The relationships between BW and PTV; and BW and PTW in Rahaji bulls were negative and insignificant (r = -0.068, $P > 0.05$; r = -0.059, $P > 0.05$ respectively), when compared with Bunaji ($P < 0.001$) or Rahaji ($P < 0.05$) bulls which predicted positive and significant ($P < 0.05$) correlations. The relationship between BW and PEW; and BW and AEL, were positive and insignificant (r = 0.153, $P > 0.05$; r = 0.073, $P > 0.05$ respectively) in the Rahaji breed when compared to the others which were positive and significant ($P < 0.05$). The relationship between BW and GSR was significant ($P < 0.05$) in Bunaji (r = 0.743, $P < 0.001$) and Rahaji bulls, though positive in Bunaji and negative in Rahaji (r = -0.454, $P < 0.5$) bulls. Bokoloji (r = 0.435, $P > 0.5$) bulls predicted a positive but insignificant correlation. There was significant and positive

(r = 0.835, $P < 0.001$) in the relationship between BW and ESR in the Bunaji bulls when compared to the Bokoloji or Rahaji bulls which predicted an insignificant correlation.

The relationships between BCS with SC (r = 0.518, P $P < 0.05$), BCS with PTV (r = 0.765, $P < 0.001$), BCS with PTW (r = 0704, $P < 0.001$), BCS with ATL (r = 0.483, $P < 0.05$), BCS with PEW (r = 0.573, $P < 0.01$); BCS and GSR (r = 0.754, $P < 0.001$ and BCS with ESR (r = 0.814, $P < 0.001$) in Bunaji bulls were all positive and significant when compared to the other breeds which were all insignificant ($P > 0.05$). The relationships between BCS with AEL were insignificant ($P > 0.05$), though, positive in all the breeds.

The relationship between SC with PTV, and SC with PTW were significant ($P < 0.05$) and positive in all the three breeds. Bunaji bulls predicted a positive and insignificant relationship between SC and ATL ($P > 0.05$) when compared with Bokoloji (r = 0.540, $P < 0.05$) or Rahaji breed (r = 0.697, $P < 0.001$) which were significant. Bokoloji breed predicted insignificant ($P > 0.05$) relationships between SC and PEW; and, SC and AEL from those observed in the other breeds which were significant ($P < 0.05$) and positive. The relationship between SC and GSR were positive in all the breeds but significant ($P < 0.01$) in Bunaji bulls. The relationship between SC and ESR were positive and significant ($P < 0.01$) in Bunaji and Rahaji bulls when compared to Bokoloji bulls which predicted a negative and insignificant ($P >0.05$) correlation.

Bunaji and Bokoloji breeds predicted significant ($P < 0.01$) and positive relationships between GSR and PTV; and, GSR and PTW when compared with the Rahaji breed which were insignificant ($P > 0.05$) but also positive. The relationship between GSR and ATL were positive in all the breeds but only significant ($P < 0.01$) in the Bokoloji breed.

The relationship between ESR and PEW; and, ESR and AEL were positive and significant ($P < 0.05$) in Bunaji and Rahaji bulls when compared to those predicted in Bokoloji bulls which were negative and insignificant ($P > 0.05$) statistically.

Table 4.4: Correlation coefficients between age, body weight, body condition score, scrotal circumference, testicular dimensions, gonadal and epididymal sperm reserves of Bunaji, Bokoloji and Rahaji breeds of cattle.

Cp	Gp	Sc	Ptv	Ptw	Atl	Pew	Ael	Gsr	Esr
Age	Bu	0.605**	0.378	0.422	0.226	0.322	0.188	0.815***	0.689***
	Bo	-0.030	0.108	0.094	0.147	0.016	0.017	-0.064	0.011
	Ra	0.293	0.036	0.079	0.073	0.118	0.241	-0.169	0.523*
Bw	Bu	0.601**	0.802***	0.767***	0.437	0.782***	0.522*	0.743***	0.835***
	Bo	0.102	0.516*	0.520*	0.400	0.665**	0.478*	0.435	-0.250
	Ra	-0.043	-0.068	-0.059	-0.135	-0.153	-0.073	-0.452*	0.014
Bcs	Bu	0.518*	0.765***	0.704***	0.483*	0.573**	0.412	0.754***	0.814***
	Bo	-0.062	0.241	0.268	0.228	0.345	0.320	0.278	-0.132
	Ra	0.081	-0.020	-0.011	0.277	-0.260	0.146	-0.240	0.106
Sc	Bu	-	0.468*	0.585**	0.303	0.592**	0.477*	0.608**	0.587**
	Bo	-	0.675**	0.567**	0.504*	0.422	-0.055	0.178	-0.263
	Ra	-	0.753***	0.775***	0.697***	0.528*	0.836***	0.137	0.613**
Gsr	Bu	-	0.639**	0.588**	0.354	-	-	-	-
	Bo	-	0.622**	0.754***	0.582**	-	-	-	-
	Ra	-	0.342	0.346	0.115	-	-	-	-
Esr	Bu	-	-	-	-	0.758***	0.554*	-	-
	Bo	-	-	-	-	-0.341	-0.414	-	-
	Ra	-	-	-	-	0.482*	0.620**	-	-

*= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$ (Bw= body weight, Bcs= body condition score, Sc= scrotal circumference, Ptv= paired testicular volume, Ptw=paired testicular weight, Atl=average testicular length, Pew= paired epididymal weight, Ael=average epididymal length, Gsr= gonadal sperm reserve, Esr= epididymal sperm reserve, Bu= Bunaji breeds of bull cattle, Bo= Bokoloji breeds of bull cattle, Ra= Rahaji breeds of bull cattle, Cp= correlated parameter and Gp= group)

CHAPTER FIVE

5.0 DISCUSSION

Bokoloji bulls showed a higher mean value of scrotal circumference measurement than Rahaji and Bunaji bulls which were similar to findings observed by Ahmad *et al.* (2005) in Sahiwal bulls (32.14 ± 1.02 cm) of above 5 years of age, Brito *et al.* (2004) in Nelore *(B. indicus)* bulls (28.50 ± 0.90 cm) of 2 years of age, Perumal (2014) in Tho Tho (*B. indicus*) bulls (31.28 ± 0.98 cm and 32.27 ± 1.22 cm) of above 3 years of age, Nwakalor and Obasi (1991) in Bunaji bulls (30.80 ± 0.90 cm) and Addass (2011) in Nigerian indigenous breeds (30.37 ± 0.12 cm) of $3^1/_2$- 4 years of age. Mahmood *et al*. (2014) reported a higher value of 36.02±2.25 cm for Cholistani bulls of 7 years of age and also Daudu and Shoyinka (1983) recorded higher values (34.12 ± 0.42 cm and 34.54 ± 0.65) of scrotal circumference measurement in Nigerian indigenous bulls of over 4 years of age than those observed in this study.

The variations in SC observed in this study may be due to the differences in body weight, breed, scrotal shape and husbandry practices of the experimental animals.

Bokoloji bulls showed the lowest testicular volume than Bunaji and Rahaji bulls in this study which were similar to the findings reported by Osinowo *et al.* (1977) in West African bulls (142 - 620 cm^3) of 1 to 5 years of age, Nwakalor and Obasi (1991) in Bunaji breed of bulls, Perumal (2014) in Tho Tho breed (338.19 ± 6.59 cm^3) of bulls of $1^1/_2$ - 2 years of age, by Akosman *et al.* (2013) in Holstein (285 cm^3) and Simmental (295 cm^3) bulls of 1-1.5 year of age. Higher values of 874.37 ± 137.07 cm^3 in Cholistani breed of bulls of 7 years of age reported by Mahmood *et al*. (2014), 835.29 ± 52.39 cm^3 in yak bulls reported by Das *et al*. (2005), 618 ± 24 cm^3 in Angus bulls and 550 ± 18 cm^3 in Brahman bulls reported by Lunstra

and Cundiff (2003) than those observed in this study. The differences observed in this study may be attributed to the variations in their ages, breed and management practices.

In this study, significant ($P < 0.05$) paired testicular weight and paired testicular volume variations were observed in the breeds. Significantly higher values were recorded in Bunaji and Rahaji bulls when compared to Bokoloji bulls. The values in this study are similar to those reported by Osinowo *et al*. (1977) in West African bulls (a range of 145.2 – 645.6 g), and Coulter and Keller (1982) in 1 - 2 year old Hereford and Angus bulls (a range of 128 – 952 g). Lower values were reported by Igboeli and Rakha (1977) in Africander (190.8 ± 13.4 g), Angoni (200.3 ± 25.5 g) and Barotse (140.4 ± 15.2 g) bulls of Central Africa than observed in this study. Daudu and Shoyinka (1983) reported higher values of paired testicular weights in Bunaji (398·71 ± 12.55 g) and Bokoloji (412·24 ± 14·94 g) bulls of Nigeria than those observed in this study. There were no significant variations in the caput, corpus and cauda weight recorded in this study. The values obtained in this study were similar to the values reported by Daudu and Shoyinka (1983) in Nigerian Bunaji and Bokoloji bulls. Lower values were reported by Igboeli and Rakha (1977) in Africander (12.90 ± 1.79 g, 3.80 ± 0.65 g and 7.84 ± 2.61 g in paired caput, corpus and cauda weight, respectively), Angoni (11.50 ± 2.17 g, 2.89 ± 0.50 g and 8.18 ± 2.65 g in paired caput, corpus and cauda weight, respectively) and Barotse (9.79 ± 0.80 g, 2.71 ± 0.38 g and 6.59 ± 0.72 g in paired caput, corpus and cauda weight, respectively) indigenous Central African bulls than recorded in this study.

Average testicular length in this study showed no significant ($P > 0.05$) breed variation and these values were lower than those recorded by Mahmood *et al*. (2014) in Cholistani breeding bulls (16.91 ± 0.70 cm) of average age of 7.5 ± 2.5 years and Akosman *et al.* (2013) in 1-1.5-year-old Simmental and Holstein bulls (12.1 cm). The differences in the values may be

attributed to age, breed, species variation, Husbandry practices, genetic variations and environmental factors.

The gonadal sperm reserve showed no significant ($P > 0.05$) breed variation but a significant ($P < 0.05$) breed variation was shown in the epididymal sperm reserve in this study. Lower mean counts were reported by Igboeli and Rakha (1977) in Africander, Angoni and Barotse breeds of bulls; Almquist (1961) in dairy breeds; Osinowo (1979) in Bunaji and Bokoloji breeds of Nigeria indigenous cattle; Tegegne (1992) in Boran breeds of cattle in Ethiopia; and Daudu and Shoyinka (1983) in Nigerian indigenous bulls (Bunaji and Bokoloji breeds) Addass (2011) reported higher mean count of both testicular and epididymal sperm reserves in Nigerian indigenous breeds than observed in this study. Variations observed in the sperm reserves can be associated with the differences in their testicular weights which could also be as a result of age, breed, husbandry practices and environmental factors.

A positive and significant correlation between age and SC was shown in Bunaji bulls but not in Bokoloji or Rahaji bulls in this study which was similar to the positive and significant correlation recorded by Devkota *et al*. (2008) in Holstein pubertal and post-pubertal bulls; Vásquez *et al*. (2003) in Brahman bulls; and Perumal (2014) in Tho Tho bulls. However, the relationships between age and ATL; age and PTV; age and PTW in Tho Tho bulls were not similar with those shown in this study. Mahmood *et al*. (2014) reported a positive and significant correlation between age and SC, which was in line with that of the Bunaji breed observed in this study; a positive but not significant correlation between age and PTW which agreed with those predicted in this study; a positive but insignificant correlation between age and PTV was in agreement with the result obtained in all the breeds in this study; the negative but insignificant correlation between age and ATL predicted was not in line with that obtained in this study. Vásquez *et al*. (2003) also reported very high significant ($P <$

0.001) positive correlations between age and PTW, age and ATL, which disagree with the findings of the breeds in this study. A positive but insignificant correlation between age and PTV in Cholistan bulls was obtained by Mahmood *et al*. (2014) which is similar to those obtained in the three breeds in this study. Mahmood *et al*. (2014) also recorded a negative and insignificant correlation between age and ATL which was not in agreement with those predicted in this study. This result clearly shows that age is not a useful parameter for determining the reproductive efficacy in a bull. Studies have shown that testicular parameters changes little with age in matured (post pubertal) bulls unlike younger (peri-pubertal) bulls, this could also explain the insignificant correlations with age obtained.

A significant ($P < 0.01$) positive correlation was reported by Coulter and Foote (1977) and Kupferschmied *et al*. (1974) between BW and SC which is in line with that observed in Bunaji ($P < 0.05$), but not with those observed in Bokoloji, which showed positive but insignificant correlation; and Rahaji, which recorded negative insignificant correlation. Osinowo (1979) and Tegegne *et al*. (1992) also reported highly significant ($P <0.001$) and positive correlation between BW and SC which is in line with the relationship obtained in Bunaji, but not Bokoloji and Rahaji bulls in this study. Tegegne *et al*. (1992) recorded very high significant ($P < 0.001$) positive correlation between BW and PTW; BW and PEW; and, BW and ESR in Boran and Boran Cross bulls. Mahmood *et al*. (2014) reported positive and significant correlation between BW and ATL; and BW and PTV (which were similar to the result obtained in this study in Bunaji and Bokoloji breeds, for the Rahaji breed, the correlation coefficients between BW and PTV differed). An insignificant positive correlation was recorded between BW and SC, which was similar to that predicted in Bokoloji bulls, but not in Bunaji and Rahaji bulls. Perumal (2014) reported significant ($P < 0.01$) positive correlation between BW and SC (similar to that predicted in the Bunaji bulls in this study); BW and PTW (similar to the predictions in Bunaji and Bokoloji bulls in this study); BW and

PTV (similar to Bunaji and Bokoloji breeds in this study); and, BW and ATL (which were insignificant in this study for all the three breeds). Body weight were positively correlated with paired testicular weight and paired testicular volume of Bunaji bulls (P<0.001) and Bokoloji bulls (P<0.05) but not with Rahaji bulls and this indicates that larger faster growing bulls did not necessarily have larger testes than did the smaller bulls of the same age. Though, this result also shows that body weight is more closely related to testicular parameters and sperm output than age. Furthermore, this relationship observed shows that adequate prediction of reproductive status of the bull is possible in the absent of birth records as long as body weight is known.

A positive significant correlation between SC and PTW was predicted in all three breeds of this study which was similar to those reported by Coulter and Foote (1976) and Palasz (1994) ($r = 0.91$, $P < 0.001$). There was a positive correlation between SC and ESR in Bunaji and Rahaji bulls, but different from that of the Bokoloji bulls in the present study. Tegegne *et al*. (1992) reported a positive correlation between SC and PTW ($P < 0.01$), SC and PEW ($P < 0.05$), SC and GSR ($P < 0.01$), and SC and ESR ($P < 0.05$), which were similar to those observed in Bunaji and Rahaji bulls in this work, except for SC and GSR in Rahaji which showed a positive but insignificant ($P > 0.05$) correlation. Bokoloji bulls in this study only had positive and significant relationship between SC with PTV and PTW. Perumal (2014) reported positive and significant correlations between SC and PTV; SC and PTW; and SC and ATL; which were all similar to those observed in this study, except for the correlation between SC and ATL in Bunaji bulls. The result of this study reveals that SC is significantly positively correlated with testicular parameters and the sperm reserves, thus measurement of scrotal circumference in bulls is very useful to predict testicular parameters and can be used in breeding centres to select suitable breeding males for breeding purposes.

Bunaji and Rahaji bulls in this study had positive and significant correlation between GSR and PTW, which were similar to the findings of Tegegne *et al*. (1992) unlike that of Bokoloji bulls which had a positive but insignificant correlation. Gonadal sperm production has been reported to be dependent on the amount of sperm producing testicular parenchymal tissue which is mainly influenced by nutrition and breed (Tegegne *et al.*, 1992). Increased sperm production is associated with increased testicular weight. Animals with larger testes tends to produce more sperm hence this shows that testicular size is a good indicator of the present and future capacity of sperm production and breeding quality of bulls.

Bokoloji bulls recorded a negative and insignificant correlation when compared to Bunaji and Rahaji bulls, which showed positive and significant correlations similar to the findings of Tegegne *et al*. (1992) in Boran and Boran cross bulls.

The results of this study revealed that scrotal circumference of all three breeds of bulls have been correlated with other testicular parameters, thus measurement of scrotal circumference in all the three breeds of bulls is useful to predict the testicular parameters and can be used in breeding centres to select suitable breeding males for breeding purposes. This also shows its role as a reliable parameter for prediction of reproductive efficacy. The presence of positive and significant correlations observed between scrotal circumference with gonadal sperm reserves in Bunaji bulls and with epididymal sperm reserves in Bunaji and Rahaji bulls in this study may indicate the importance of scrotal circumference measurement in determination of sperm producing ability of bulls after puberty. Also, the positive and significant correlations seen between scrotal circumference with paired testicular volume and paired testicular weight in all the three breeds, and, the positive and significant relationship between gonadal sperm reserves with paired testicular volume and paired testicular weight in Bunaji and Bokoloji but

not significant in Rahaji bulls shows their usefulness in predicting the reproductive efficacy of bulls.

The variations observed in this study may be attributed to age, compounding breed effects, season and husbandry practices (Ha *et al.*, 2012). This is because the studies of the other investigators were mostly carried out in controlled environments. Negative or lower positive correlations observed between scrotal circumferences and other parameter in this study could also be associated with factors like differences in testicular shape and scrotal skin thickness.

The results of this study clearly demonstrate that these parameters are still reliable and readily measurable indicators of reproductive potential in breeding bulls. Furthermore, it verifies that quantitative aspects of these parameters coupled with estimates of correlations among them can strengthen the reliability of breeding soundness examination of bulls.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Bunaji bulls were having the smallest body weight and scrotal circumference with similar BCS with the others. Rahaji bulls were having similar body weight with Bokoloji bulls and similar scrotal circumference with Bunaji bulls.

Bunaji and Rahaji bulls had higher testicular weight, testicular volume and testicular length when compared with the Bokoloji bulls. The epididymal, caput and caudal weights were similar in all the three breeds, but the corpus weight recorded a lower value in the Rahaji bulls when compared with the other breeds.

Bokoloji and Rahaji bulls recorded a higher value in the epididymal length than the Bunaji bulls, while the Rahaji breed recorded a higher value in the caput length when compared with the others. Bokoloji bulls had a higher value of corpus length than the others, but the Bunaji and Bokoloji bulls had higher values of caudal length.

The testicular sperm reserves had similar values in all the three breeds, although the Bunaji bulls had relatively higher values of epididymal sperm reserves when compared to the other breeds.

Age and SC in Bunaji bulls, age and GSR in Bunaji, age and ESR in Bunaji; and, age and ESR in Rahaji predicted significant and positive correlations. The BW and SC in Bunaji, BW and PTV in Bunaji, BW and PTW in Bunaji, BW and PEW in Bunaji, BW and AEL in Bunaji, BW and GSR in Bunaji, BW and ESR in Bunaji, BW and PTV in Bokoloji, BW and PTW in Bokoloji, BW and PEW in Bokoloji; and BW and AEL in Bokoloji predicted positive and significant correlation, while BW and GSR in Rahaji predicted negative and significant correlation. Positive and significant correlations were observed between BCS with

SC, PTV, PTW, ATL, PEW, GSR, and ESR in Bunaji bulls. SC and PTW in the three breeds, SC and PTV in the three breeds, SC and ATL in Bokoloji and Rahaji breeds; SC and PEW in Bunaji and Rahaji; SC and AEL in Bunaji and Rahaji bulls, SC and GSR in Bunaji, SC and ESR in Bunaji and Rahaji breeds predicted positive and significant correlation. The GSR and PTV in Bunaji and Bokoloji breeds, GSR and PTW in Bunaji and Bokoloji breeds; and GSR and ATL in Bokoloji bulls predicted positive and significant correlations. The ESR and PEW in Bunaji and Rahaji bulls; and ESR and AEL in Bunaji and Rahaji bulls predicted positive and significant correlations.

6.2 Conclusion

Based on the findings in this study, the following conclusions were drawn:

1. Bunaji bulls, having the least mean value of body weight (329.50 ± 11.28 kg) and lower mean value of scrotal circumference (28.33 ± 0.69 cm), together with the Rahaji bulls exhibited:
    i. Higher mean value of testicular weight (347.41 ± 25.37 g) and testicular volume (354.50 ± 30.52 cm^3) together with Rahaji bulls when compared with Bokoloji bulls, and similar testicular length with the other breeds.
    ii. Similar mean values of epididymal weight, caput weight, corpus weight and cauda weight with the other breeds.
    iii. Lowest mean value of epididymal length (15.98 ± 0.47 cm), lower mean value of caput length (5.88 ± 0.28 cm) together with Bokoloji bulls as compared with Rahaji bulls, lower mean value of corpus length (6.05 ± 0.30 cm) together with the Rahaji bulls as compared with Bokoloji bulls and higher mean values of cauda length (4.73 ± 0.22 cm) together with the Bokoloji bulls as compared to Rahaji bulls.

iv. Similar mean value of testicular sperm reserves with the other breeds.

v. Highest mean value of caput sperm reserves (19.30 ±6.76/gm) as compared to the other breeds, similar mean value of corpus sperm reserve with the other breeds, highest mean values of cauda sperm reserves (157.60 ±32.97/gm) and epididymal sperm reserve (178.50 ±39.22/gm) when compared to the others.

2. Bokoloji bulls, having similar mean value of body weight (392.90 ± 18.59 kg) with Rahaji bulls and the highest mean value of scrotal circumference (33.70 ± 1.47 cm), exhibited:

   i. Lowest mean values of testicular weight (292.52 ± 15.89 g) and testicular volume (288.50 ± 18.59 cm^3) when compared to the other breeds, and similar testicular length with the other breeds.

   ii. Similar mean values of the epididymal weight, caput weight, corpus weight and cauda weight with the other breeds.

   iii. Higher mean values of epididymal length (16.93 ± 0.33 cm) together with the Rahaji bulls when compared with Bunaji bulls, lower mean value of caput length (5.69 ± 0.16 cm) together with the Bunaji bulls when compared with Rahaji bulls, highest mean value of corpus length (6.96 ± 0.20 cm) when compared to the other breeds and higher mean value of cauda length (4.62 ± 0.16 cm) together with the Bunaji breeds when compared with Rahaji bulls.

   iv. Similar testicular sperm reserves with the other breeds of bulls.

   v. Lower mean value of caput sperm reserve (9.19 ±1.54/gm) when compared with Bunaji bulls and a higher value when compared to the Rahaji bulls; similar mean values of the corpus sperm reserve with the other breeds, lower

mean values of the cauda sperm reserves (14.49 ±1.90/gm) together with Rahaji bulls when compared with the Bunaji bulls; and, lower mean values of the epididymal sperm reserves (24.23 ± 2.77/gm) together with Rahaji bulls when compared with the Bunaji bulls.

3. Rahaji bulls, having a higher mean body weight (396.8 ±13.15 kg) together with the Bokoloji bulls when compared to the Bunaji bulls, and, lower mean value of scrotal circumference (30.90 ± 0.78 cm) together with Bunaji bulls when compared with the Bokoloji bulls exhibited:

   i. Higher mean values of the testicular weight (331.38 ± 14.77 g) and testicular volume (348.50 ± 14.31 cm^3) together with the Bunaji bulls when compared with the Bokoloji bulls, and similar mean values of testicular length with the other breeds.

   ii. Similar mean values of the epididymal weight, caput weight, corpus weight and cauda weight with the other breeds.

   iii. Higher mean values of epididymal length (16.47 ± 0.25 cm) together with the Bokoloji bulls when compared with the Bunaji bulls, highest mean value of caput length (6.44 ± 0.16 cm) when compared to the other breeds, lower mean value of corpus length (6.33 ± 0.11 cm) together with Bunaji bulls when compared with Bokoloji bulls, and lowest mean value of the cauda length (4.10 ± 0.14 cm) when compared to the other breeds.

   iv. Similar testicular sperm reserves with the other breeds of bulls.

   v. Lowest mean value of caput sperm reserve (0.66 ±0.27/gm) when compared with the other breeds, similar mean values of the corpus sperm reserve with

the other breeds, lower mean values of the cauda sperm reserves (12.58 ±2.13/gm) together with Bokoloji bulls when compared with the Bunaji bulls; and lower mean values of the epididymal sperm reserves (13.44 ± 2.24/gm) together with Bokoloji bulls when compared with the Bunaji bulls.

6.3 Recommendations

Based on the results of this study, it is recommended that:

i. Comparative study should be carried out on the relationship between testosterone and testicular dimension of these three breeds of cattle.
ii. Comparative study should be carried out on the relationship between testosterone and gonadal sperm reserve of these three breeds of cattle.
iii. Comparative study should be carried out on the relationship between testosterone and epididymal sperm reserve.
iv. Comparative study should be carried out to check the seasonality effect on testicular dimension, gonadal and epididymal sperm reserve of these three breeds of cattle.
v. Future semen and seminal parameters should be correlated with testicular and scrotal parameter to confirm the present findings.
vi. Bunaji breed of bulls should be selected as the breeding sire because of their optimal breeding potentials and possible higher fertility followed by Rahaji breed of bulls and Bokoloji breed of bulls should be the last choice.

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