APPLICATION OF SOME BIOMETRICAL CORRELATES IN THE DETERMINATION OF OPTIMUM BREEDING AGE IN THE WEST AFRICAN DWARF BUCK.

BY

LUKMAN OLADIMEJI RAJI

DVM, M.VSc. (Ibadan)

MATRIC NUMBER: 86897

A Thesis in the Department of Theriogenology

Submitted to the Faculty of Veterinary Medicine

in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

OCTOBER 2017

ABSTRACT

Sexual maturity of male animals begins at puberty and peaks at the Optimum Breeding Age (OBA), which marks attainment of maximum functionality of the gonads. Despite the high socio-economic importance of goats, the OBA of the West African Dwarf Buck (WADB) is yet to be determined. Therefore, the OBA in the WADB was investigated using biometrical correlates.

A field study involving 450 semi-intensively raised WADB was conducted from October, 2012 to October, 2013. The bucks were in 15 equal groups (1-15 months old) for the determination of Body Score (BS), Body Weight (BW) and Scrotal Circumference (SC). Six bucks were randomly selected per group for subsequent studies. The bucks were electroejaculated to evaluate semen volume, normal spermatozoa, spermatozoa motility, livability and concentration, using standard methods. The TV determined ultrasonographically using Prolate Ellipsoid Formula (TVPEF) and Prolate Spheroid Formula (TVPSF) were contrasted with TV obtained by conventional Water Displacement Method (TVWDM). Testicular morphometry [Seminiferous Tubular Diameter (STD) and Germinal Epithelial Height (GEH)] and testosterone profile were evaluated using Motic Images Plus software and ELISA technique, respectively. Data were analysed using descriptive statistics, Pearson correlation and ANOVAat $\alpha_{0.05}$.

The BS (r = 0.2), BW (r=0.9) and SC (r=0.8) correlated significantly with age. The BW significantly increased from 3.4±0.1 kg at one month to 13.6±0.1 kg at 15 months. The SC significantly increased from 7.9±0.1 cm at one month to 17.6±0.2 cm at 15 months. Azoospermia, first appearance of spermatozoa and best quality semen were observed in the bucks' ejaculate at 1-5, 6 and 8-15 months, respectively. Spermiogram showed that semen volume, normal spermatozoa, spermatozoa motility, livability and concentration increased significantly from 0.2±0.1 mL, 87±2.7%, 85±4.0%, 88±3.7% and 1.7±0.0 $\times$ 10^9Cells/mL, respectively at six months to 0.3±0.1 ml, 92±3.4%, 93±5.0%, 93±4.6% and 2.1±0.1 $\times$ 10^9Cells/mL, respectively at eight months. The TVPEF, TVPSF and TVWDM significantly increased from 16.1±0.1, 23.6±1.7 and 16.2±0.1cm^3, respectively at one month to 28.3±0.2, 40.0±0.3 and 28.5±0.3 cm^3, respectively at eight months. Comparison of TV procedures showed that TVPEF did not significantlydiffer from TVWDM, while TVPSF was significantly higher than TVWDM. The STD and GEH significantly increased from 83.1±0.2 µm and 28.4±0.2 µm, respectively at one month to 574.1±0.3µm and 197.5±0.3 µm, respectively at eight months. The

testosterone concentrations increased significantlyfrom 0.1±0.0 ng/ml at one month to 4.7±0.2 ng/mL at eight months. In the parameters studied (excluding BW and BS), there were no significant increases from eight months to 15 months.

An optimum breeding age of eight months in semi-intensively raised West African Dwarf buckswas established. Ultrasonographic evaluation of testicular volume using the Prolate Ellipsoid Formula is a valuable non-invasive method for the determinationof optimum breeding age in West African Dwarf bucks.

Keywords: Biometrical correlates, Optimum breeding age, Testicular ultrasonography, Semi- intensive, WestAfrican DwarfBucks.

Word count: 455

ACKNOWLEDGEMENTS

I give all Glory, Praises, Thanks and Adoration to ALMIGHTY GOD for making it possible for me to complete this research work.

My sincere and unquantifiable appreciation goes to my Supervisor, Dr. Oluwatoyin O. Ajala, who has been there for me since my undergraduate days, only God can reward you for what you have been doing for me. My prayers for you and your generations yet unborn is God's infinite Mercy and Support in this World and in the Hereafter. I cannot also help but thank tremendously Professor O. E. Fayemi who has been a "Rock" not just for me but many before me and definitely many more to come. Your advice, moral and prayer supports are more than appreciated Sir. My profound gratitude also goes to Professor M. O. Oyeyemi, Dr. O. O. Leigh, Professor J. Olopade and Professor Emikpe for their immeasurable academic advices that have indeed led to the success of this work. Also, not forgetting Dr. A. K. Raheem and Dr. I. U. Osuagwuh who have both been great Brothers and friends to me in all ramifications.

I want to thank God for giving me wonderful parents, Alhaji and Alhaja Raji. I pray to God to make you both live long to enjoy the fruits of your labor in this World and in the Hereafter. To my loving, caring, supporting sisters, Madinat and Mistura; Brother, Surajudeen and cousin, Temitope, I say a big thank you to all of you.

Truly, behind every successful man is a good woman. I cannot stop thanking God for blessing me with a wonderful wife, Rukayat Alabi Raji who bore me three destined to be Great children, Maryam, Hamad and Aishat Raji. My prayer to God is to take the love and blessings between us beyond this World.

It is often said that friends in need are friends indeed. Dr. (Mrs) A. T. Jagun has been more than a friend and a sister. Your endless help and supports morally, financially, academically cannot be quantified. You are key to my success; paying you back will be too little to what God has in stock for you and your family. To my "Professor" friends in the making Dr. Ismail Odetokun, Dr. Ishola, Dr. Jamiu and of course my big brothers, Dr. Ameen and Dr. Lawal, may Almighty God reward you all abundantly.

I must extend my appreciation and thanks to my Professional colleagues and non-academic staff both in the Universities of Ibadan, Umudike and Ilorin especially Mr. Ejiro who has been supporting me since my undergraduate days. Also, to my students, especially Christantus, for their supports. God will bless you all abundantly.

There is a Prophetic saying that in every Congregation lies a true Blessing. Indeed my Congregations at Akufo farm settlement, Ologuneru, Ido, Ibadan and Araromi, Tanke, Ilorin, have been Blessings to me and my family. I thank you all for your prayers and supports.

Finally, to all the good people the ones I know and the ones I do not know, who have contributed in one way or the other to the success of this Project Work, I say God will bless and reward you all in better ways. Thank you all.

TO GOD BE THE GLORY!!

CERTIFICATION

I certify that this work was carried out by Dr. Lukman Oladimeji, Raji in the Department of Theriogenology, Faculty of Veterinary Medicine,

University of Ibadan, Nigeria.

………………………………………………………………….

Supervisor

Dr. Oluwatoyin. O. Ajala

DVM, M.VSc., Ph.D (Ibadan), FCVSN

Associate Professor, Department of Theriogenology

University of Ibadan, Nigeria.

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ABBREVIATIONS

AV:	Artificial Vagina
ANOVA:	Analysis of Variance
ALLP:	Alkaline Phosphatase
ALT:	Alanine Amino Transferase
AST:	Aspartate Amino Transferase
BS:	Body Score
BSE:	Breeding Soundness Examination
BW:	Body Weight
cAMP:	Cyclic Adenosine Monophosphate
COX:	Cyclooxygenase
DNA:	Deoxyribonucleic Acid
EE:	Electro-Ejaculator
EDD:	Epididymal Ductal Diameter
EEH:	Epididymal Epithelial Height
ELD:	Epididymal Luminal Diameter
ELISA:	Enzyme Linked Immunosorbent Assay
FAO:	Food and Agricultural Organization
FSH:	Follicle Stimulating Hormone
GEH:	Germinal Epithelial Height
GnRH:	Gonadotropin Releasing Hormone
H:	Height
HSD:	Hydroxy Steroid Dehydrogenase
ICSH:	Interstitial Cell Stimulating Hormone

L:	Length
LF:	Lambert Formula
LH:	Luteinizing Hormone
LP:	Longitudinal Plane
LRPDP:	Laboratory of Reproductive Physiologyand Developmental Programming
MIPlus:	Motic Image Plus
MTT:	Minimal Transit Time
NDGA:	Nigerian Dwarf Goat Association
NNBS:	Nigerian National Bureau of Statistics
OBA:	Optimum Breeding Age
PEF:	Prolate Ellipsoid Formula
PEM:	Protein Energy Malnutrition
PPMC:	Pearson Product Moment Correlation
PG:	Prostaglandin
PSF:	Prolate Spheroid Formula
RNA:	Ribonucleic Acid
SC:	Scrotal Circumference
SD:	Standard Deviation
SE:	Seminiferous Epithelium
SEM:	Standard Error of Mean
SLD:	Seminiferous Luminal Diameter
ST:	Seminiferous Tubules
STD:	Seminiferous Tubular Diameter
TMB:	Tetramethylbenzidine

TP:	Transverse Plane
Trp:	Transient Receptor Potential
TTT:	Total Transit Time
TU:	Testicular Ultrasound
TV:	Testicular Volume
UG:	Ultrasound Gel
UM:	Ultrasound Machine
UN:	United Nations
W:	Width
WAD:	West African Dwarf
WADB:	West African Dwarf Buck
WDM:	Water Displacement Method

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Experts have predicted that the world population will be about 9.6 billion by 2050 with the less developed countries being the major contributors (FAO, 2009; Zlotnik, 2009). Feeding this growing population is a challenge of now and the future (Sadik, 2014). By the United Nations (UN) estimation, about 25% of this population is undernourished; the highest being is in the sub- Saharan African countries (Jha, 2014). The food is available but majority of the populace (especially in the rural areas), cannot afford the dietary energy and particularly protein requirements. This is because most of these people are below the poverty level (Foley, 2009).

Taking Nigeria as a case study, according to data by the Nigerian National Bureau of Statistics (NNBS, 2014), Nigerian citizens were estimated to be 178.5 million people. With a population growth rate of about 2.47%, Nigeria is expected to contribute about 20% of the increasing world population by 2050 (Ogunbiyi, 2012). It has been estimated that approximately 70% of this population are living below poverty level. Hence, the purchasing power of most of these Nigerian citizens is low. As a result, many cannot afford the average dietary energy and especially protein requirement (Kanayo, 2014; Kolawole and Omobitan, 2014). The effect of this situation is significantly undermining the potentials and development of this great Nation. Hence, there is need to tackle these problems to prevent further damage (Ubesie and Ibeziakor, 2012; Ubesie *et al.* 2012).

Nutrition is one of the keys to the survival of the human race (Vodovotz *et al*., 2000; Elmadfa and Leitzmann, 2004; Hoffman and Falvo, 2004). Man depends on plants and animals for its nutritional supply, essential components of which include vitamins, carbohydrates and most importantly, protein (Elmadfa and Leitzmann, 2004; Guoya, 2016). Many of the body tissues are made up of proteins without which man and animals cannot survive. It is only next to water in terms of abundance in the human body tissues. It is the

building block of life and very vital for tissue growth, maintenance and repair (Guoya, 2016). Deficiency or total lack of nutrition results in malnutrition or undernourishment. The devastating effect of which depending on the severity can result in high morbidity and mortality rates especially in the young ones (Muller and Krawinkel, 2005).

Protein-Energy Malnutrition (PEM) has been reported to be the major cause of death and many debilitating diseases in the less developed countries (Odebode and Odebode, 2005; Ubesie *et al*., 2012). In early or non-severe cases, clinical manifestations are few. However, features in severe cases which occur majorly in children include low birth weight, poor growth, infectious diarrhea, severe wasting malnutrition (Marasmus), Kwashiorkor (due to lack of breast milk), Marasmic-kwashiokor, impaired mental, motor and behavioral development and death (West, 2006). As a rule, PEM is often associated with some vitamin deficiency related disease conditions such as angular stomatitis of vitamin B deficiency, xerophthalmia, rickets, anaemia, amongst many others (Blossner *et al*., 2005). In women or mothers, common features include obstetric morbidity, nutritional abortion, poor lactation, anaemia and death (West, 2006). There is a popular saying that a hungry man is an angry man as malnutrition has been associated with mental stress and frustration in adult men. Other common features in men include, adult wasting and underweight (Muller and Krawinkel, 2005).

The West African Dwarf (WAD) goat is an excellent source of protein in terms of meat and milk (Devendra, 1999). They are small ruminants endowed with great breeding potentials. They have high multiple birth rates ranging from twins, triplets and quadruplets (Chukwuka *et al*., 2010). They are trypano-tolerant (Chiejina *et al*., 2009). They can resist intestinal nematodes and can adapt to harsh climatic conditions better than other ruminants (Chiejina *et al*., 2015). They are of great socio-economic values especially to the small-holder livestock owners in the rural areas of West Africa. They are commonly reared under the semi-intensive system of production (SISP) where they have thrived best so far (AdebayoandChineke,2011). Considering the rapidly increasing level of animal protein deficiency in Nigeria and most developing countries, improving the production of these WAD goats, will definitely play significant roles in the challenges of protein deficiency and

undernourishment which is fast threatening the survival especially of the poor Nigerian populace (Oppong-Anane*et al*., 2008; Chukwuka *et al*., 2010; Chiejina *et al*., 2015).

In WAD goat production, the bucks play a very important role like any other male animal in livestock production. They can sire many females at least at a ratio of one buck to five does (Nolte, 2012). They can also detect female goats on heat. Reproductively sound or proven bucks can produce offsprings having the same quality genetic traits like the sire even up to the second generation (Ugwu, 2009). However, the proficiency of this performance largely depends on the pubertal and breeding age.

Puberty is the age at which matured sperm cells are first noticed in the ejaculate. It is the age of sexual maturity at which the buck becomes capable of reproduction (Osinowo and Williams, 2008). However, during early puberty, most of the energy is directed towards the enhancement of the functionality of the testis, thereby affecting growth and productivity (Henkel, 2015).

Optimum Breeding age (OBA) commences when the buck has fully attained reproductive maturity and most importantly, the testes and epididymides are fully developed and functional (Abebe, 2008; Osinowo and Williams, 2008). The OBA represents the best time to start using the buck for breeding. This is because at OBA, energy is directed basically towards spermatogenesis (Henkel, 2015). Also, the rate of matured sperm cell replacement after depletion is faster at OBA (Kinne, 2001). There is increase in the number of does the buck can serve and fertilize successfully (Noble, 2004). There is also increase in litter sizes (Suckow *et al*., 2005). Also, the bucks are less prone to health problems (Henkel, 2015).

An invaluable tool that is used in determining or assessing reproductive indices and organs in breeding goat bucks (and male animals) is the Breeding Soundness Examination (BSE) protocol (Chapwanya*et al*., 2008; Ford *et al*., 2009; Ridler *et al*., 2012; Menegassi *et al*., 2014; Stewart and Shipley, 2014). For instance, an important parameter taken during BSE in goat bucks is scrotal circumference (SC). Research has shown that SC is a significant correlate of fertility in goat bucks. Bucks with bigger testicles have been reported to sire does at relatively younger ages (Raji *et al*., 2008). Increase in testicular size indicates onset of spermatogenesis and SC is highly related to semen quality and reproductive soundness

(Bongso *et al.,* 1982; Bezerra *et al.,* 2009). Also, there is only a dearth of information on WAD bucks testes and epididymides, which are usually given priority during BSE.

The testis and epididymis are important reproductive organs of the WAD goat bucks (Oyeyemi and Babalola, 2006; Ugwu, 2009; Archana *et al*., 2014). The testis produces sperm cells and testosterone. The sperm cells are important for the fertilization of the ova from the doe while testosterone is the major hormone of reproduction in the goat bucks. Testosterone is responsible for the production of sperm cells and other major WAD goat buck characteristics required for efficient reproduction (Daramola *et al*., 2006; Ugwu, 2009). The epididymis is the site of full storage, maturation and passage of these spermatozoa during ejaculation (Joseph *et al*., 2009; Archana *et al*., 2014). Hence, considering the importance and potentials of these bucks in WAD goat production, it is important to carry out studies and researches on how to improve the function, diagnose, treat and control normal and abnormal conditions of their testes and epididymides using modern, safe and economic methods and equipment.

Testicular Ultrasound (TU) is a modern diagnostic tool that is used in Human and Veterinary Medicine practices. It is a safe, painless and non-invasive procedure. Researches and studies on TU have been performed on the foreign breeds of goats and small ruminants (Ahmad and Noakes, 1995; Ahmad *et al*., 1999; Ulker *et al*., 2005; Ali *et al*., 2011; Carazo *et al*., 2014). It has also been documented to be valuable in taking important BSE parameters such as Testicular Volume (TV) which is an index of spermatogenesis (Kollin *et al*., 2006; Sotos and Tokar, 2012). Establishing and encouraging the use of TU in these animals may reduce the practice of the more invasive methods of studying the gross, histology and histomorphometric studies of the testes for diagnostic purposes.

During BSE, it is not uncommon to come across pathologic conditions such as orchitis and epididymitis (Costa *et al*., 2007; Bousmaha and Khoudija, 2012; Abba *et al*., 2014; Guerra, 2014). These may be observed physically or may require further examination using invasive methods such as blood sample collection for hormonal analysis, morphological and morphometric studies (Holstein, 1969; Cohen *et al*., 1996;Nawar*et al*., 2008; Shittu *et al*., 2008) and non-invasive methods such as TU (Gouletsou*et al*., 2005; Ulker *et al*., 2005;

Anderson and Alanko, 2009). Of these, TU have been observed to be very valuable. However, this is yet to be standardized in the WAD goat buck.

Morphological and morphometric studies of the WAD bucks' testes and epididymides are important for the better understanding of the gross and micro anatomy and how these organs functions (Nicander and Glover, 1973; Ugwu, 2009; Awobajo *et al.*, 2010; Etim, 2015). Therefore, carrying out researches in this area (Cooper and Hamilton, 1977; Cosentino *et al.*, 1984; Oke, 1988; Bordooi and Dhingra, 1990; Caussanel*et al.*, 1996; Abd-El- Maksoud, 2005; Jahan *et al.*, 2009) will lead to exploring new areas for improved WAD goat production.

The endocrinology of reproduction of male animals shows that hormones such as testosterone, Follicle Stimulating Hormones (FSH) and Luteinizing Hormones (LH) play significant roles to ensure reproductive performance is established and maintained adequately (Sanford*et al.*, 1977; Grasselli *et al.*, 1992; Castro *et al.*, 2000; Babu *et al.*, 2004; Millar *et al.*, 2004; Fattahi*et al.*, 2009; Amrane *et al.*, 2013; Ángel-Garcíaa *et al.*, 2015). In this regard, testosterone plays key roles in the development of the testes, prostate and production of spermatozoa. Apart from these major roles, it also increases libido and frequency of erection as well as secondary sexual characteristics amongst other numerous roles in the goat bucks (Daramola *et al*., 2006; Leite-Browning, 2009). The LH also known as Interstitial Cell Stimulating Hormone (ICSH) is produced by gonadotroph cells in the anterior pituitary and is responsible majorly for the stimulation of the production of testosterone by the leydig cell acting synergistically with the FSH. Its production is under the influence of Gonadotropin Releasing Hormone (GnRH) which is produced from the hypothalamus (Mckeown *et al.*, 1997; Flanagan*et al.*, 2007; Ochiogu *et al.*, 2015). The FSH also synthesized and secreted by the anterior pituitary, stimulates the proliferation and secretory activities of the sertoli cells. Sertoli cells function majorly to aid spermatogenesis, move developing sperm cells to the lumen of the seminiferous tubules and to reduce motility and capacitation of sperm cells to maintain viability (Babu *et al.*, 2004; Hafez and Hafez, 2000; Mullen, *et al.*, 2013).

Considering these highlighted values and importance of WAD goat bucks in WAD goat production, this study was carried out to determine the OBA in WAD bucks under the SISP

using non-invasive BSE correlates such as SC and TV. Also, to determine the morphological and morphometric characteristics of directly dependent organs particularly the tesis and epididymis in relation to the OBA for diagnostic, research and improved production purposes. Lastly, to determine testosterone, LH and FSH profiles in relation to OBA in WAD buck.

1.1 JUSTIFICATION FOR THIS STUDY

The WAD goats are endowed with great reproductive potentials as they have high twinning, triplets and quadruplets birth rates. Thus, they can increase their own population very rapidly especially if well managed. Furthermore (and most importantly), they are excellent sources of animal protein in terms of meat and milk. Infact, their milk is very palatable and nutritious containing a comparable amount of protein as that of the cow and human milk; as well as minerals and vitamins (especially vitamin A) that are required for human growth and development. However, with the increasing human population, coupled with the prevailing animal protein deficiency (particularly amongst the ever increasing population in the developing countries including Nigeria), there is a need to increase protein supply to meet up with the challenges of the present and the equally potentially threatening future protein deficiency.Considering the socio-economic valuesof the WAD goat highlighted (especially in terms of potential protein supply), increasing WAD goat production will play major roles in alleviating or solving these challenges of protein deficiency. Thus, by extension, there is a need to improve the buck's reproductive performances being an integral part of goat production. The buck detects estrus in does and most importantly serves these does at least a ratio of 1 buck to 5 does; and even very much higher when its semen is collected, extended and used to inseminate many does (depending on the dose and concentration). Bucks' semen can even be stored to be used in future even after the death of the buck. However, maximizing these potentials largely depends on the age at which the WAD buck attains puberty and most importantly the OBA when he will be fully capable of reproducing offsprings while maintaining and maximizing its reproductive potentials and health status. Therefore,determining the OBA of WAD bucks will reduce wasting estrus in does, post breeding interval and lengths of reproductive cycles. This will also increase possibilities of multiple birthsthereby leading to increase WAD goat production and ultimately, increase in animal protein that is required for human survival.

1.2 OBJECTIVES OF THIS STUDY

The objectives of this study were to determine:

1. The value of SC (in correlation with semen production) as a determinant of the OBA in WAD bucks under the SISP.
2. The value of TV (using TU) as a determinant ofthe OBA in WAD bucks.
3. The morphological and morphometric characteristics of the testes and epididymides in relation to OBA in WAD bucks.
4. The testosterone, LH and FSH profiles in relation to the OBA in WAD bucks.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The West African Dwarf goat.

The West African Dwarf (WAD) goats constitute a major proportion of the modern domestic goats (*Capra hircus*), which are believed to have originated from the wild Bezoar goat(*Capra aegagrus*)of the mountains of Asia Minor, across the Middle East (Porter and Mason, 2002). Historically, they are commonly referred to as Pigmy goats but today, the common names includes, Nigerian Dwarf, Cameroonian Dwarf, Guinean, Guinean Dwarf, Diougry, Tibetana, Forest goat, Grassland Dwarf, Djallonke, Fouta Djallon Dwarf, Chevrede Casamance, Chevre naine de Savanea, and Chevre naine de l'est Kosi; relating to the countries and regions (West and Central Africa) where they are mainly distributed (Gifford-Gonzalez and Hanotte, 2011).

The Nigerian WAD goat has the largest population of WAD goats with about 11 million distributedin the humid zone of Eastern Nigeria alone. There are two major ecotypes namely, the humid and the savanna WAD goats (Chiejina *et al*., 2009). They are of varying colors including black, brown, white, pied, red, cream, mottled and mixed (Bitto and Egbunike, 2006; Yakubu *et al*., 2010; Adedeji *et al*., 2012; Chiejina *et al*., 2015). According to the specification of the Nigerian Dwarf Goat Association (NDGA), the height at withers of a WAD doe is between 43 – 48 cm, with a maximum allowed height of 53 cm, while that of the buck is between 48 – 53 cm, with a maximum allowed height of 58 cm (Oseni and Ajayi, 2014). They also express varying genetic traits such as presence or absence of wattles which may be unilateral or bilateral and supra-numerary teats in the does which could be two, three or four in number. They have characteristically long neck, erect ears and a straight to slightly dished face (Yakubu *et al*., 2010; Adedeji *et al*., 2012). Uniquely, they can resist trypanosome and intestinal infections more effectively than any other known breed of goat (Chiejina *et al*., 2015). They can also survive and thrive under

extreme harsh weather conditions. They are typically scavengers and are commonly referred to as “stubborn goats” eating any available food within their reach (Oyeyemi *et al*., 2002; Oppong-Anane*et al*., 2008). The male, female and young goats are referred to as the buck, doe and buckling respectively (Porter and Mason, 2002; Yakubu *et al*., 2010; Adedeji *et al*., 2012; Oseni and Ajayi, 2014).

2.2 Nutritional value and economic importance of West African Dwarf goats.

The WAD goat has been reported to be the commonest and most important breed of goat in the West and Central Africa (Chiejina *et al*., 2009; Yakubu *et al*., 2010; Adedeji *et al*., 2012;Chiejina *et al*., 2015). The Nigerian WAD has a population of about 34.5 million constituting about 40% of the total African WAD buck population (Aina, 2012). The WAD goat is endowed with diverse nutritional and socio-economic values. They are excellent sources of protein in terms of milk and meat (Devendra, 1999). The milk production capacity of the Nigerian WAD goat has been put at 1 - 4 kg with an average of 1.2 kg per day. It has high butterfat content compared with other full-sized dairy goats, making its milk excellent for cheese and soap making. WAD goats generally provide their owners with a broad range of products and socio-economic services such as protein and (emergency) cash income (in terms of meat and milk), ordinary friendly gifts (skin), wedding gift (whole goat) and manure for the crops. They are commonly reared by the poor people in the rural areas, hence, they are popularly referred to as the “poor peoples’ cattle” (Aina, 2012; Chiejina *et al*., 2015).

2.3 The Functional Anatomy of the Reproductive system of the goat buck.

The goat buck reproductive system consists of the testes, scrotum, epididymis, penis, prepuce, spermatic cords, vas deferens, urethra external and the accessory glands including the vesicular glands, ampulae, prostate glands and bulbo-urethral glands (North, 2004; Leite-Browning, 2009; Gofur, 2015) as shown in Figure 2.1.

2.3.1 The testes

These are the primary organs of reproduction of the goat buck (Abebe, 2008; Leite-Browning, 2009). They are paired and usually descend during the mid-pregnancy to lie in the scrotum (Saxena, 2012). They could be split or undivided; symmetrical or asymmetrical (Ford *et al*., 2009). They produce the male gametes (spermatozoa) andsecrete the male sex hormone, testosterone (and other androgens).Testosterone is essential for the development of male sexual characteristics and sperm production (Daramola *et al*., 2006; Abebe, 2008). For optimum production of spermatozoa, the temperature of the testis is usually maintained at 2 to 5°C below that of the buck's body temperature. In cold weather, the testes rise near theabdominal cavity; in hot weather, themuscular relaxation permits testes toswing and hang down from the body. This helps in the normal thermoregulation within the testis. The structures responsible forthermoregulation are the cremaster muscle, dartos and the pampiniform plexus (Figure 2.2). This relaxed statemaintains optimum temperature for thespermatogenesis to process and thespermatozoa to survive. A buck's low fertility rate could be attributed toenvironmental conditions and theincapacity to regulate the optimumtesticular temperature (Skinner, 1991; Weinbauer *et al*., 2010, Saxena, 2012). Considering the importance of this organ to WAD production, there is need to carry out researches in this area for improved production purposes.

2.3.1.1 Testicular descent

Testicular descent occurs as a result of the shortening of a ligament that extends from the inguinal region and attaches to the tail of the epididymis called gubernaculums (Saxena, 2012). This is becausethe growth of the gubernaculum is slower compared to the body wall. The intra-abdominal pressure also aidspassage of the testes through the inguinal canals into the scrotum. These activities are regulated by gonadotropic hormones and androgens

(Ackland *et al*., 1992; Saxena, 2012; Paul, 2014). Occasionally one or both testes may fail to descend due to a defect in development. In cases where both testes fail to descend, the buck is termed abilateral cryptorchid. Such bucks are sterile and cannot be used for breeding. When only one testis descends, the buck is referred to as a unilateral cryptochid. A unilateral cryptochid buck may be fertile if the descendedtestis is functional (Amann and Veeramachaneni, 2006; Igbokwe *et al.*, 2009; Ozegbe, 2012). This condition can be corrected surgically. But it is not advisable to use such bucks for breeding because the condition may be passed down to the next generations that will emerge from the buck (Amann and Veeramachaneni, 2006; Igbowke *et al*., 2009). During testicular descent, the testis becomes surrounded by a serous cavity which pushes the visceral layer of the *tunica vaginalis* against the *tunica albuginea* and the testis. This then makes the outer layer of the newly formed cavity to become theparietal layer of the *tunica vaginalis*. This descent or migration of the testis occurs through theinguinal canal into the scrotal sac (Saxena, 2012; Paul, 2014).

2.3.1.2 Testicular blood supply and lymphatic drainage:

Blood supply to the testes ismainly through the gonadal artery. But there are other collateral arteries that supply blood to the testes and these include the cremasteric artery (abranch of the inferior epigastric artery, which is a branch of the externaliliac artery) and ductus deferens artery (a branch ofthe inferior vesical artery, which is a branch of the internal iliac artery).The right testicular veindrains directly into the inferior vena cava while the left testicular vein drains intothe left renal vein. The pudendal artery supplies the scrotum (which encloses the testes) and the remaining parts of the external genitalia (Herwig *et al*., 2004; Paul, 2014).

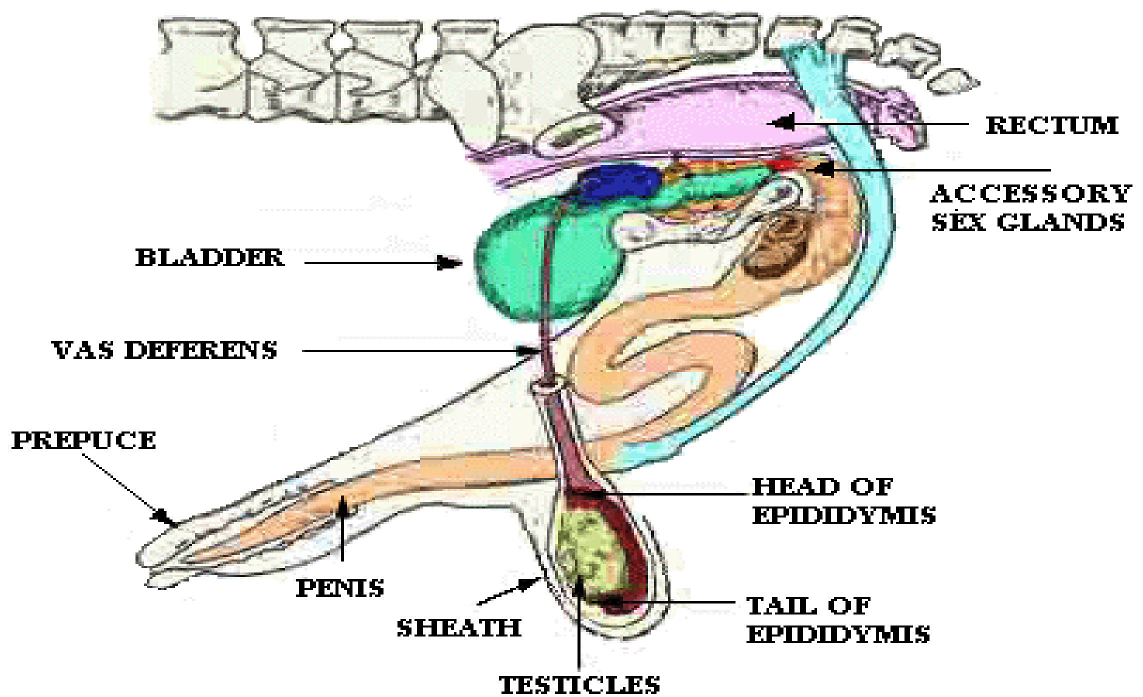


Figure 2.1: Schematic diagram of the anatomy of the reproductive system of the goat buck.

Source: Reproductive management of meat goat operations – slide presentation by Fred Hopkins (UTK) and Kyle Rozeboom (UTM).

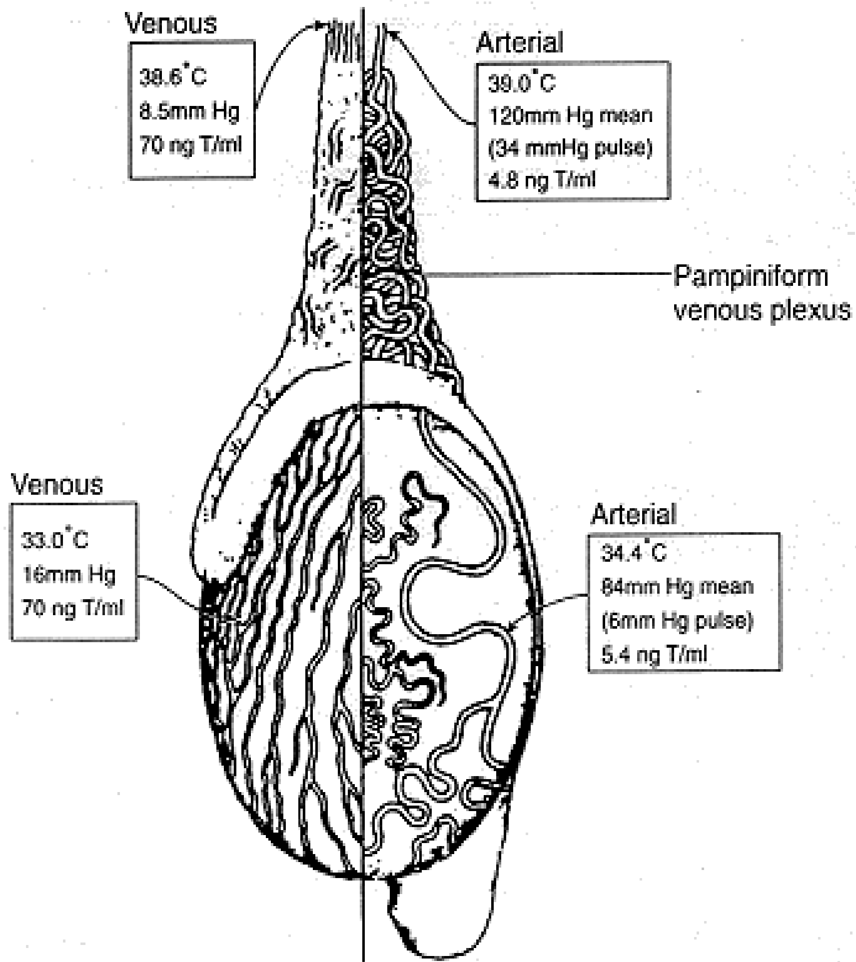


Figure 2.2: Cooling of the testis by heat exchange through the circulatory system.(Setchell, 1977. Reproductionin Domestic Animals.(3rd ed) ed. Cole and Cupps.

Academic Press

2.3.1.3 The functional histology of the testes

A multilayer tunica covers the testis. These layers serve to facilitate blood supply to the testis and also to create a partition between sperm producing regions of the testis. These *tunica* layers are three namely *tunica vasculosa, tunica albuginea and tunica vaginalis* (Nishimura, 2009; Mohammed *et al*., 2011).

2.3.1.3.1 *Tunica vasculosa*

This is the inner layer of the tunica and comprises of a plexus of blood vessels and connective tissues. Its function is to facilitate blood supply to the testis. It forms clothing for the inner surface of the *tunica albuginea* and an internal investment to all the spaces of which the testis is made up (Archana *et al*., 2014).

2.3.1.3.2 *Tunica albuginea*

The outermost layer of the testis is covered by *tunica albuginea* which is made of a dense blue-grey membrane packed with collagen fibres amongst which many elastic fibres are present. It encloses the testis and forms a connection to the layers of fibres surrounding the epididymis. The *tunica albuginea* extends into the testes creating partitions (septae) between the seminiferous tubules. It connects to the *tunica vasculosa* at the glandular surface of the testes. It is reflected into the interior and at the posterior end of the testis and forms an incomplete vertical septum known as mediastinum testis (Khan *et al*., 2016).

2.3.1.3.3 *Tunica vaginalis*

The *tunica vaginalis* has two layers namely the visceral *tunica vaginalis* and parietal *tunica vaginalis*. The visceral layer overlies the *tunica albuginea* (which is the middle *tunica*). The parietal layer on the other hand lines the scrotal cavity. In between these two layers is the vaginal cavity. This cavity contains a thin fluid that separates these two layers of *tunica vaginalis* which functions to reduce friction between the testis and the scrotum. However, any excessive increase in this fluid can result in what is referred to as hydrocoele (Nimase, *et al*., 2008).

2.3.1.3.4 Seminiferous tubules

The Seminiferous Tubules (ST) are highly convoluted tubules that form coiled loops terminating at both ends into the rete testis located within the mediastinum. Each of these tubules is lined by Seminiferous Epithelium (SE) which constitutes the germinal epithelium where spermatogenesis takes place. The SE forms a basal lamina that is attached to a thin wall of connective tissues (containing myofibroelastic cells), the *lamina propia* (consisting of collagens type I and IV), fibrocytes and occasionally blood vessels and nerve fibres. The fibrocytes lie more peripherally while the myofibroblasts lie next to the germinal epithelium (Sarma and Devi, 2012). Contractile actions cause narrowing of lumen of the ST resulting in the movement of formed spermatozoa into the adjacent epididymis where maturation takes place and then eventual subsequent release during ejaculation. But as aging occurs (especially in stallions), atrophy of the ST occurs. As this takes place, the *lamina propria* also becomes thickened with increase in collagen type IV and elastic fibers resulting in interstitial fibrosis (Mohammed *et al*., 2011; Mohammadzadeh *et al*., 2013).

2.3.1.3.4 Sertoli cells

The sertoli cells (supporting cells) are located within the ST. They serve to nourish the spermatozoa and to create a haemato-testicular barrier. They are tall or elongated cells sited on a basement membrane. Latero-medially, they stand side by side of each other binding together with the germ cells through tight junctions. This results into the formation of two compartments namely basal and adluminal compartments. The basal compartment comprises of the early germ cells including the spermatogonia while the adluminal compartment contains the spermatocytes, spermatids and spermatozoa. The haemato--testicular barrier functions to block the spermatozoa from entering into the blood circulations of the lymphatic systems. The significance of this is to prevent auto-immune orchitis which could lead to sterility (Leal, *et al*., 2004; Sarma and Devi, 2012).

2.3.1.3.5 Leydig cells

Leydig cells (interstitial cells) are the cells located in-between adjacent ST that functions primarily to produce testosterone (which is the primary male sex hormones). Two types of leydig cells have been identified in mammals namely fetal leydig cells and pubertal or adult

leydig cells. The fetal leydig cells are responsible for the masculinization of the male urogenital system during fetal life. Thereafter, these cells regress except in the male rat where some remain till puberty. The pubertal leydig cells are responsible for the production of testosterone (and other androgens) required for the onset of spermatogenesis and the maintenance of reproductive activities or functions. During the transition from fetal leydig cells to adult Leydig cells, the events that take place includes increase in cell size, volume of smooth endoplasmic reticulum and decline in cytoplasmic lipid droplets. By comparison, adult leydig cells produce more testosterone compared to fetal leydig cells. However, the activity and the expression of the two testosterone metabolizing enzymes, 5ἀ-reductase and 3ἀ - Hydroxysteroid-Dehydrogenase (HSD), markedly decline in adult Leydig cells than in fetal leydig cells (Lejeune *et al*., 1998; Elsayed *et al*., 2007). In general, the regulation of leydig cell function includes endocrine control, sertoli-leydig cell interaction, interaction of leydig cell with other testicular cells and regulation of leydig cell functions by other locally produced factors. (Shan *et al*., 1993; Viger and Robaire, 1995)

2.3.1.3.5.1 Endocrine regulation of leydig cell functions

Under physiological conditions, the regulation of leydig cell function is under the influence of LH via its specific receptor which is coupled to both adenylate cyclase and phospholipase C pathways (Segaloff and Ascoli, 1993). There are two major responses of leydig cells to the effect of LH and these include; the first response occurs in the first minute and involves a sharp increase in Cyclic Adenosine Monophosphate (cAMP) and steroid production. This effect is sensitive to protein synthesis inhibitors, but does not require Ribonucleic Acid (RNA) synthesis and involves translocation of cholesterol from the cytosol to the inner mitochondrial membrane. The second type of response is a long-term trophic effect of LH on the structure and function of leydig cells (Abney and Carswell, 1986; Cooke, 1996; Lin, 1996).

2.3.1.3.5.2 Sertoli cell interaction

Studies have shown that sertoli cells have paracrine effect on leydig cell numbers and functions. Johnson and Ewing (1971) were the first to postulate that FSH enhanced testosterone production significantly in an experiment where in rabbit testes was

perfusedwith maximal concentrations of LH, but had no effect alone. Several other studies involving both in vivo and in vitro models, have also confirmed that indeed FSH, indirectly through Sertoli cells, modulates Leydig cell function (Odell and Swerdloff, 1976; Mason *et al*. 1986; Saez *et al*., 1989;Sharpe, 1993 and Saez, 1994).

2.3.1.3.5.3 Interaction of Leydig cells with othertesticular cells

Some studies have shown that leydig cells functions can be influenced directly or indirectly by peritubular myoid cells and macrophages. Also, peritubularcells are capable of secreting proteins in particular testicular paracrine factor(P-Mod-S) which plays active roles in regulating Sertoli cell functions (Lejeune *et al*., 1998). Testicular macrophages are required for the initial phases of precursor proliferation and for the proliferative activity of immature Leydig cells, however, not for the maintenance of mature Leydig cell functions (Gaytan *et al*., 1994; Cohen *et al*., 1996; Hales, 1996).

2.3.1.3.5 Spermatogonia

Spermatogonia are the undifferentiated male germ cells that originate from the ST. They lie next to the basal lamina of ST and further develop to become the spermatocytes which directly lie above them on the germinal cell epithelium layer. There are three cell types namely type A (d), type A (p) and type B spermatogonia. The type A (d) spermatogonia have dark oval nuclei and replicate to ensure a continuous supply of spermatogonia that are required for spermatogenesis. The type A (p) spermatogonia have pale oval nuclei and divide by mitosis to produce the type B spermatogonia. The type B spermatogonia are similar to the type A (p) spermatogonia but with their nuclei round instead of being oval and divide to give rise to the spermatocytes. However, all the three types of spermatogonia form a syncytium because they are connected by intercellular bridges (Sarma and Devi, 2012; Abba and Igbokwe, 2015).

2.3.1.3.6 Spermatocytes

Spermatocytes are male gametocytes that derive from the division of type B spermatogonia. They are found at the adluminal space of ST of the testes. There are two types, namely, primary and secondary spermatocytes. The major function of spermatocytes is to produce

immature sperm cells called spermatids. The difference between the primary spermatocytes and the secondary spermatocytes is that the primary spermatocytes are diploid cells and contains 46 chromosomes while the secondary spermatocytes are haploid cells and contains 23 chromosomes (Mohammadzadeh *et al*., 2013; Abba and Igbokwe, 2015).

2.3.1.3.7 Spermatids

Spermatids are immature sperm cells formed from secondary spermatocytes. The initially formed spermatids are round in shape but become elongated as they develop. They are found within the adluminal space of the S.T. Spermatids also form syncytium by connecting cytoplasmic bridges. Spermatids develop to form spermatozoa by the process of spermatogenesis (Leal *et* al., 2004; Mohammadzadeh*et al*., 2013).

2.3.1.3.8 Spermatozoa

Spermatozoa (sperm cells) are the matured motile sex cells of male animals being the final product of spermatogenesis. They are required for the fertilization of the ova from female animals. The non-motile sperm cells are referred to as spermatia. There ae three main regions of the sperm cell namely the head, midpiece and the tail. The head contains the nucleus and the acrosome contains genetic materials (that are transferred to the offsprings) and enzymes that are required for the penetration of the ovum from the female animal. The energy for motility is derived from the metabolism of fructose (in the seminal fluid) which takes place in the mitochondria of the midpiece. The tail is used for the forward movement of the spermatozoon towards the ovum (Daskin *et al*., 2011; Soares*et al*., 2015).

2.3.2 The scrotum

This is a muscular sac containing the testes. It supports and protects the testes and alsoplays a major role in temperature regulation. It helps in maintaining the temperature of the testis 2 to 5°C below body temperaturefor optimal functioning. In terms of appearance or shape, the scrotum could be split, partially split or undivided. The different layers of tissues present between the scrotal skin and the testis include the *tunica dartos*, loose connective tissue, vaginal process and the *tunica albuginea*. The *tunica dartos* is under the skin and is composed of smooth muscle fibers with fibrous and elastic connective tissues which

surrounds both testes and forms a medial septum in between them. Next to this layer is the loose connective tissue layer, after which is found the vaginal process. The vaginal process is an extension of the peritoneum passing through the abdominal wall at the inguinal canal. This layer is further divided into the superficial layer called *tunica vaginalis communis*, which corresponds to the parietal peritoneum of the abdominal cavity; and the deeper layer called *tunica vaginalis propria*, which corresponds to the visceral layer of the peritoneum of the abdominal cavity (Leite-Browning, 2009; Saxena, 2012; Gofur, 2015).

2.3.3 The epididymis (epididymides)

The epididymides are paired, long, convoluted tubes situated caudo-lateral to the testes. They serve as the site of maturation, storage and passage for the sperm cells produced by the testes. They also serve to dispose of old sperm cells. At the embryonic and early postnatal development, the mammalian epididymis structurally appear as a straight tube before transforming to a highly coiled, complex duct that links the efferent ducts to the vas deferens (Djakiew and Jones, 1982; Turner *et al*., 1990; Robaire *et al*., 2006; Archana *et al.*, 2011; Turner, 2011).

2.3.3.1 Structural organization of the epididymis

The epididymis of the goat buck is majorly divided into three gross anatomical regions comprising of the head (caput), body (corpus), and tail (cauda) regions (Benoit,1926). The epididymis can be further divided into different zones or segments including the initial segment, caput, corpus, proximal caudal epididymis and distal caudal epididymis (Nicander and Glover, 1973; Hoffer and Karnovsky, 1981; Holstein, 1969; Reid and Cleland, 1957; Hermo,1995). However, the most commonly used nomenclatures are the three regions described earlier. Generally, each of these regions of the epididymis is further organized into lobules separated by connective tissue septa (similar to the organization of the ST of the testis). These septa serve to provide a functional separation between lobules hence allowing for selective expression of genes and proteins within individual lobules. They also serve as internal support for these regions and the epididymis as a whole (Turner *et al*., 2003). The epididymis extends into a slender straight tube called the vas deferens (which is surrounded by a very thick muscular layer) thatconnects with the urethra and

ultimatelyempties to the exterior of the body (Holtz, 1972; Hoffer and Karnovsky, 1981; Robaire *et al*., 2006; Joseph *et al*., 2009).

2.3.3.2 Epididymal Cell Types and Specific Markers

There are different cell types that appear on the appropriate regions of the epididymis of mammals generally. However, these cells share similar structural features, functions and show very similar patterns in the manner they express some of the secretory proteins (Yeung *etal*., 1991;Yeung, *etal*., 1993). These cell types include the principal cells, basal cells, narrow cells, apical cells, hallow cells and clear cells (Hermo and Robaire, 2002; Robaire *etal*., 2006).

2.3.3.2.1 Principal cells

The principal cell is the main cell type of the mammalian epididymis. They are present in all the ducts of each region but with structural variations (Hamilton, 1975; Robaire and Hermo, 1988). They are the largest of all cell types of the epididymis. They are typically characterized of highly developed secretory and endocytic machinery with basally aligned nuclei. They occupy approximately 65% to 80% volume of the ducts depending on the region of the epididymis they are situated (Robaire and Hermo, 1988). Studies have shown that the principal cells vary structurally and functionally in terms of appearance and organization of their secretory apparatus (endoplasmic reticulum, golgi apparatus, and secretory granules) and endocytic apparatus (coated pits, endosomes,multivesicular bodies, and lysosomes) (Hamilton, 1975; Robaire and Hermo, 1988). Previous researches revealed the precise characterization of these secretory granules and golgi apparatus of the principal cells. Also, the organelles and the mechanism of uptake of luminal substances have been well documented (Robaire and Hermo, 1988). A major observation has been the abundance of lipid droplets that are peculiarly characteristicof the corpus epididymidis. However, the exact significance of this feature is still not fully understood. Principal cells are known to synthesize a large number of proteins which are either retained in the cells or actively secreted into the luminal compartment (Hamilton, 1975; Lea, 1978; Vierula *etal*., 1992). They also play active roles in endocytosing proteins found in the luminal compartment of the epididymis (Hermo and Robaire, 2002).

2.3.3.2.2 Apical cells

The apical cells are found primarily in the epitheliumof the caput and corpus regions of the epididymis (Sun and Flikinger, 1980; Adamali and Hermo, 1996). However, some studies have suggested that the apical cells can occasionally be observed in other regions of epididymis especially in aging rats (Hermo and Robaire, 2002). The apical cells' nuclei are characteristically spherical, apically located and do not have any contact with the basement membrane. Their protein expression profiles are unique and differ clearly from adjacent narrow and principal cells (Adamali and Hermo, 1996). Studies by the examination of β-hexosaminidase A in a knockout mouse revealed that the apical cells functions basaically to endocytosesubstances from the lumen of the epididymal ducts (Adamali *etal.*, 1999), and that they contain many proteolytic enzymes (Adamali and Hermo, 1996).

2.3.3.2.3 Narrow cells

The narrow cells are attenuated, relatively narrower than the adjacent principal cellsand possess thin processes of cytoplasm to reach the basement membrane. Typically they appear pencil-like and are commonly located at the caput and corpus regions of the epididymis (Sun and Flikinger, 1980; Adamali and Hermo, 1996). They are characterized by numerous apically locatedcup-shaped vesicles which function in secreting H^+ions into the lumen byrecycling to and from the apicalplasma membrane and in endocytosis (Hermo *etal.*, 2000). By distribution, morphological appearance and expression of proteins, the narrow cells relatively differ from apical cells. Similarly, they also differ from neighboring principal cells by displaying region-specific expression of proteins such as the glutathione S-transferases and lysosomal enzymes (Adamali and Hermo, 1996). Narrow cells have been documented to be present(in the sameregions) in other species such as bovine, hamster, echidna, and human (Flickinger *etal.*, 1978; Djakiew and Jones, 1982; Goyal, 1985; Adamali and Hermo, 1996; Abou-Haila and Fan-Maurel, 1984).

2.3.3.2.4 Clear cells

The clear cells comprise of two distinct regions namely the apical and basal regions. The apical region of the clear cells are characterized by numerous coated pits, vesicles, endosomes, multivesicular bodies, and lysosomes; while the basal regionis made up of

thenucleus anda variable amount of lipid droplets large (Abou-Haila and Fan-Maurel, 1984; Robaire and Hermo., 1988; Hermo *etal*., 1988). The clear cells are active in endocytosis and are present in the three major regions of the epididymis (i.e the caput, corpus, and cauda regions) in many species of animals and humans (Hamilton, 1975; Robaire and Hermo, 1988; Adambali *etal*., 1999). In comparison with the principal cells, the endocytic activity of the clear cells is much greater than in the adjacent principal cells, especially in the cauda epididymidis (Hermo *etal*., 1988). Normally, clear cells function to endocytose the contents of cytoplasmic dropletsreleased by spermatozoa during transit in the epididymal ducts (Robaire and Hermo, 1988;Hermo *etal*., 1988). The cytoplasmic droplets contain golgi saccular elements that are often involved in modification of the plasma membrane of spermatozoa. They also endocytose a number of different proteins, but often in a region-specific manner (Lea *etal*., 1978;Hermo *etal*., 1992; Flickinger *etal*., 1988; Vierula *etal*., 1995). Under experimental conditions in which normal testicular and epididymal functions were disrupted, the clear cells were observed to be exceptionally large and filled with lysosomes (Hermo*etal*., 1988).

2.3.3.2.5 Basal cells

The basal cells are hemispherical in appearance adhering to the basement membrane but with no direct contact with the lumen of the duct. They have thin attenuated processes that extend from their main hemispherical cell body along the basement membrane majorly covering a large proportion of the circumference of the epididymal tubule and occasionallytowards the lumen. They have been observed to be presentin all animal and human species studied so far (Robaire and Hermo, 1988; Flickinger *etal*., 1988; Hermo *etal*., 1994; Veri *etal*., 1993). Similar to the observations in principal cells, basal cells do not divide in adults nor act as stem cells to replenish expired cells (Robaire and Hermo, 1988). Basal cells also have coated pits which are found on the plasma membrane which face oppose the basement membrane and overly the principal cells, suggesting the receptor-mediated endocytosis of factors derived from the blood or principal cells. Occasionally, basal cells may also be observed to have accumulations of secretory materials in golgi saccules which function to regulate principal cells activities and possess distinct secretory granules that appear next to the Golgi apparatus (Robaire and Hermo, 1988). Basal cells

have been reported also to express apolipoprotein E and alcohol dehydrogenases (Robaire and Hermo, 1988). Studies have also suggested that basal cells play immune cells role because of their ability to respond in numbers as well as macrophage sperm autoantigen expressions that may be present in the lumen and that they may be extratubular in origin (Robaire *et al.*, 2006). Basal cells may have a role in regulating electrolyte and water transport by principal cells. This process was suggested to be mediated by the local formation of Prostaglandins (PGs) and therefore, require the participation of the Transient Receptor Potential (Trp) proteins. The Trp serve as transmembrane pathways for $Ca2^{+}$influx, whereas Cyclooxygenase-1 (COX-1) is a key enzyme in the formation of PGs. These two proteins are uniquely and exclusively expressed by the basal cells (Hermo *et al.*, 2000; Robaire *et al*., 2006).

2.3.3.2.5 Halo cells

The halo cells are small cells with a narrow rim of clear cytoplasm which are found throughout the epididymal epithelium. They are usually located at the base of the epithelium containing variable numbers of dense core granules. Recent researches (Flickinger *etal*., 1978; Flickinger *etal*., 1988) suggest that, in young adult animals, halo cells do not have B lymphocytes but possesshelper T lymphocytes, cytotoxic T lymphocytes, and monocytes. However, with age, the numbers of these immune cell types increase in aregion-specific pattern (Robaire *et al*., 2006).

2.3.3.2.6 Stereocilia

The stereocilia are long non-motile apically modified cytoplasmic projections of the epididymal epithelium that function majorly to reabsorb the large volume of fluid (secreted in the testis for the movement of sperm cells into the epididymis) as well as to aid the transport of the sperm cells through the epididymal ducts. They are numerous and are most commonly found at the caput and corpus epididymis. They are usually absent at the cauda epididymis (Archana *et al*., 2011; Sharma*et al*., 2014).

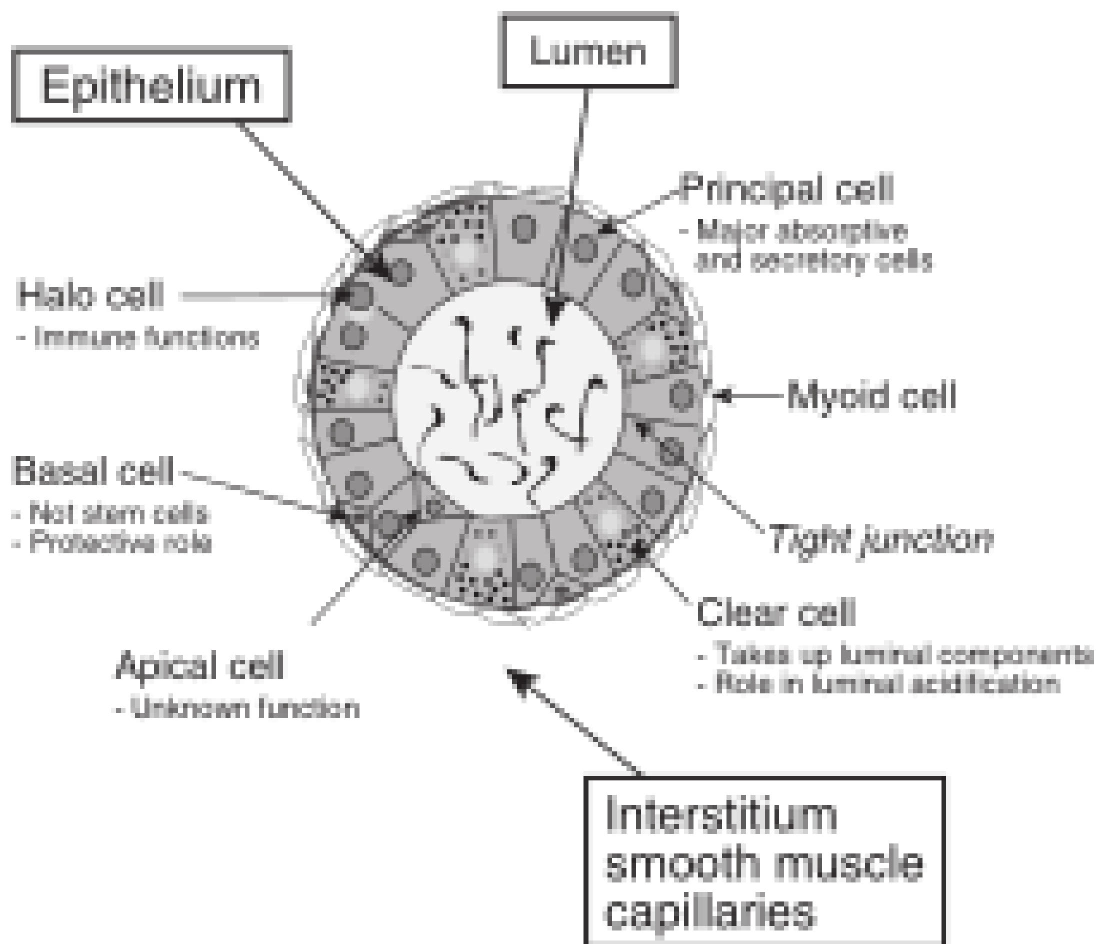


Figure 2.3: Schematic organization of the major cell types in theepididymis as observed at the light microscope. The three epididymalcompartments as well as the relative position and distribution ofeach of the main cell types are illustrated. The major functionsassociated with each cell type are also identified.

Source: The epididymis – chapter 22, pp 1077; *Knobil and Neill's Physiology of Reproduction, Third Edition* edited by Jimmy D. Neill,Elsevier © 2006

2.3.4 The Blood-Epididymis barrier

Different studies have revealed that beyond the blood-testis barrier, there exists also the blood-epididymis barrier which functions basically to block the proteins in spermatozoa which the body recognizes as foreign bodies (Flickinger *etal*., 1988)

2.3.4.1 The structure

It has been documented that the junctional complex between adjacent epididymalprincipal cells is composed of apically located gap, adherens, and tight junctions, functions of which include forming of the blood–epididymis barrier by the tight junctions betweenadjacent principal epithelial cells at their luminal surface. The gap junctions, on the other hand, allow communication betweenadjacent principal cells. These tight junctions formedseals the spaces between the epithelial cells by forming a continuous zonule around the cell. By this, two separate compartments are formed namely the luminalcompartment and the intercellular spaces physiological compartments. The formation of these tight junctions starts as early as the embryonic life specifically during the differentiation of the Wolffian duct (Robaire *et al.*, 2006).

2.3.5 Functions of the epididymis

The epididymis perform four major functions and these includes transport of spermatozoa, development of sperm motility, development of sperm fertilizing ability, and the creation of a specialized luminal environment that is conducive for the maturation process of the sperm cells through the absorptive and secretory activities of the epididymal epithelium (Robaire *et al*., 2006; Saxena, 2012).

2.3.5.1 Transport of spermatozoa

Following the release of spermatozoa into the lumen of seminiferous tubules, large volumes of fluids are also secreted to aid the transport of these spermatozoa through the efferent ducts into the epididymis. The transit time for spermatozoa varies from species to species. The methods of estimation also vary. A popular method for estimating Total Transit Time

(TTT) of spermatozoa is by using the formula stating that TTT is the ratio of epididymal sperm reserves and daily testicular resorption. However, a more direct approach is by the incorporation of a labeled isotope into theDeoxyribonucleic Acid (DNA) of germ cells at the spermatogonia and preleptotene spermatocyte stages and then following the progressionof the first wave of labeled spermatozoa down theepididymis. This method estimates the Minimal Transit Time (MTT) required for the spermatozoa to pass through the epididymis (Orgebin-Crist, 1998). The variation in transit time in most species takes place at the cauda epididymis (Amir and Ortavant, 1968). The transport of spermatozoa before entering the epididymis is by the testicular fluid and probably by the beat of the ciliated cells of the efferent ducts. At the caput epididymis, the transit of spermatozoa is by the immotile stereocilia on the epithelium and the massive fluid intake which the initial segment of the epididymis reduces by reabsortion. The transport of spermatozoa from the testis to the cauda epididymis occurs against an increasing hydrostatic pressure gradient (Jaakkola, 1983). In a study in which the *ductuli efferentes* was ligated in order to prevent this fluid flow, this movement of spermatozoa by the hydrostatic pressure gradient still occurred. This suggests that these mechanisms alone are responsible for sperm transport (Macmillan and Aukland, 1960). Movement of the spermatozoa through the epididymis is also by the rhythymitic contraction of smooth muscle layer which increases (with its adrenergic innervations) from the caput to the cauda regions (Talo *etal*., 1979; Jaakkola and Talo, 1982, Jaakkola and Talo, 1983; Jaakkola, 1983). This can be influenced by the hormonal and neural factors. The hormones that have been revealed to influence spermatozoa within the epididymis include the testosterone (Hib and Oscar, 1978), PGF2 alpha (Cosentino *etal*., 1984), neurohypophysial peptides including oxytocin and vasopressin (Whittinton *etal*., 2001). Neuronal influence may be through the inferior mesenteric ganglion that provides sympathetic innervations to the cauda epididymidis (Cosentino *etal*., 1984). Also, adrenergic and cholinergic drugs have been documented to influence contractility of the epididymis both in vitro and in vivo (Yamamoto *etal*., 1995). Studies have also shown that temperature has effect on epididymal contractility. Increase in temperature (for instance, from scrotal to body temperature) can increase the frequency and spread of electrical activity of the smooth muscle of the epididymis (Jaakola and Talo, 1983). This can as well lead to a significant speeding up of sperm transport through the

epididymis (Bedford, 1978; Bedford, 1978) which could result in a potential deleterious effect on sperm maturation and fertility (Macmillan and Aukland, 1960; Harayama*et al*., 1993; Robaire *et al*., 2006).

2.3.5.2 Maturation of spermatozoa

Sperm maturation takes place in the epididymis. A sperm cell is matured when it attains fertilizing ability (Robaire *et al*., 2006; Saxena, 2012).

2.3.5.2.1 Fertilizing ability

In higher vertebrates (such as bulls, rams, goats etc), sperm cells only attain full fertilizing ability after passing through the epididymis. This is in contrast with what occurs in lower vertebrates where the sperm cells are already matured as they are released in the seminiferous tubules of the testis. The specific site in the epididymis where the full fertilization occurs in the higher vertebrates varies from one species to the other (Jindal and Panda, 1980; Robaire *et al*., 2006).

2.3.5.2.2 Motility

Motility is an integral part of maturation characteristics that spermatozoa have to acquire. The spermatozoa need to swim through the female animal reproductive tract to fertilize the ova. The maturation of sperm motility potential involves both a quantitative increase in the percentage of motile spermatozoa and a qualitative difference in motility pattern. Testicular spermatozoa are either immotile or show only a slight twitch of the flagellum. Spermatozoa released from the caput epididymidis swim in a circular pattern. Fully motile spermatozoa are released from the cauda which move progressively and vigorously forward (Jindal and Panda, 1980; Robaire *et al*., 2006).

2.3.5.2.3 Other maturational changes

Apart from the capacity for progressive motility, epididymal spermatozoa also develop other capacities as part of the maturation process; these capacities include to undergo the acrosome reaction [mouse (Lakoski *etal*., 1988), ram (Williams *etal*., 1991), pig (Burkin and Miller, 2000), dog (Sirivaidyapong *etal*., 2001), monkey (Yeung *etal*., 1996), and

human (Yeung *etal*., 1997)], recognize and bind to the zona pellucida [mouse (Saling, 1982), pig (Burkin and Miller, 2000)], and fuse with the vitelline membrane as tested with zona-free hamster eggs [pig (Harayama *etal*., 1993), human (Moore *etal*., 1983)]. Along with these functional changes, spermatozoa also undergo structural changes during epididymal transit; these include migration of the cytoplasmic droplet along the sperm flagellum, acrosomal reshaping, changes in the sperm nuclear chromatin and some tail organelles, and changes in the sperm plasma membrane (Bedford, 1973; Bedford, 1978). These structural and functional changes collectively enable the testicular spermatozoa to attain full maturation and fertilizing ability during epididymal transit (Yeung *etal*., 1991; Hermo *et al.*, 1994; Sirivaidyapong*et al*., 2001; Robaire *et al*., 2006).

2.3.5.2.4 Storage of spermatozoa

In the mammalian species, spermatozoa are stored in the cauda epididymis. Although, the major function of the caudal epididymis is to store mature, live spermatozoa, other functions include recognition of abnormal-appearing or dead spermatozoa and the ability to develop a mechanism to neutralize or destroy them (Cooper and Hamilton, 1977; Weigsenberg *etal*., 1995).

2.3.6 Vas deferens

This is the duct that rises from the tail of the epididymis and runs into the abdomen, whereit joins the urethra at the neck of the bladder. It is often regarded to be part of the spermatic cord. Removal of asection of the vas deferens in each testis is known as vasectomy, preventing passage of sperm from theepididymis (Amir and Ortavant, 1968; Hamilton, 1975; Saxena, 2012; Khan *et al*., 2015).

2.3.7 Spermatic cord

The spermatic cord is composed ofmuscles and fibre tissues and a portionof the vas deferens. The cord connectsthe testicles to veins and arteries thatirrigate the testicles in conjunction withthe scrotum to position the testiclesoutside the body, and to help regulate the temperature of the testis (Saxena, 2012).

2.3.8 Accessory sex glands

The accessory sex glands include the bulbo-urethral, prostate, and seminal vesicleglands and the ampulla. They secrete additional fluids, which mix with the spermcells and other secretions from the epididymis to form the semen. Some of these secretions contain nutrients likefructose while others produce alkali secretion to raise the pH of the ejaculate. These secretions are addedquickly and forcibly during the mating and semen collection (for Artificial Insemination) to propel sperm cells into the urethra (Saxena, 2012, Gofur, 2015).

2.3.9 Penis

This is the final part of the goat buck reproductive tract and its function is to deposit semen into the vaginal tract of the doe. Extending about 2 -3 cm beyond the end of the penis is a narrow tube called the urethral process (or ‘worm')that sprays the semen in and around the cervix of the doe. The preputial sheath protects the penis, exceptduring mating. The goat buck penis is sigmoid shaped (S- flexure) whichallows it to lengthen during mating (Saxena, 2012; Kibiria, *et al*., 2016).

2.3.10 Urethra

This serves as a common passage for urine and transport of semen. It has three distinct parts namely, the pelvic, bulb of urethra and the penile part. The pelvic urethra is enclosed by heavy urethral muscle. The bulb of the urethra is the extra pelvic part situated at the ischial arch and is bending ventral to the penis. The penile urethra runs inside the penis proper. It carries the urethral process which is whip-like extending from the head of the urethra. This is quite distinct in the goat buck (Saxena, 2012; Kibiria, *et al*., 2016).

2.3.11 Prepuce

This is the invaginated fold of skin surrounding the free end of the penis. The prepucial orifice forms behind the umbilicus and is surrounded by a tuft of hair. It is similar to that of the ram and bull but relatively shorter (Saxena, 2012; Reece, 2013).

2.4 The Male Reproductive Hormones.

Reproductive hormones (RH) are hormones that affect growth or function of reproductive organs and the development of secondary sexual characteristics. They include GnRH**,** FSH, LH and testosterone (Abecia *et al*., 2012; Daramola*et al*., 2006).

2.4.1 Gonadotropin Releasing Hormone (GnRH)

GnRH is a neuropeptide (decapeptide) that is produced by the hypothalamus and functions primarily to regulate reproduction in the male and female animals. It acts on the anterior pituitary gland (particularly the gonadotroph cells) to cause the release of FSH and LH which control gametogenesis and sex steroid production. GnRH has also been reported to cause the releaseof growth hormone from the pituitary (Marchant *et al*., 1989), regulate prolactin (Weber *et al*., 1997)and somatolactin secretions (Kakizawa*et al*., 1997). The release of GnRH is regulated by a negative feedback mechanism relating to the quantity or concentration of FSH and LH in the system at a particular point in time. Alterations in the external environment or internal conditions can also affect the release or production of GnRH. For instance, in some foreign rams, there are seasonal variations in sexual activities which affect GnRH release. However, on the average in the male animals, there are 4 – 12 GnRH peaks per day. Going by molecular phylogeny, three types of GnRH have been identified namely GnRH 1, GnRH 2 and GnRH 3 (Fernald and White, 1999). However, most commonly observed amongst vertebrates and mammals are the GnRH 1 and GnRH 2 (Yoo *et al*., 2000); GnRH 3 has only been identified in teleosts so far (Mohamed and Khan, 2006). GnRH 1, GnRH 2 and GnRH 3 neurons are located in the preoptic area (Amano *et al*., 1991; White *et al*., 1995; Carolsfeld*et al*., 2000); midbrain and hindbrain having terminals in the third ventricle directly (Yoo *et al*., 2000; Gonzalez-Martinez *et al*., 2002; Steven *et al*., 2003); and terminal nerve ganglion near the olfactory bulb projecting primarily into the telencephalon but also widely into the whole brain,including the retina and olfactory epitheliumrespectively. Studies have shown that the roles GnRH 1, GnRH 2 and GnRH 3 play varies from one species of animals to the other. GnRH 1 was reported to influence reproductive behavior in musk shrew *Suncus murinus* (Schiml and Rissman, 2000). GnRH 2 on the other hand has been shown to have influence on the regulation of female reproductive behavior in birds (Maney *et al*., 1997), teleosts (Volkoff and Peter,

1999) and mammals (Kauffman and Rissman, 2004; Barnett *et al*., 2006). Other studies also suggested that GnRH 2 can mediate a balance between survival and reproduction by regulating food intake and energy balance (Soga*et al*., 2005; Kauffman and Rissman, 2004). Reports also suggest that GnRH 3 play important roles of regulating sexual behavior, such as nest building as observed by Yamamoto *et al*., 1997; Ogawa *et al*., 2006), aggressive behavior (Ogawa*et al*., 2006) and spawning behaviour (Volkoff and Peter, 1999). The GnRH 3 has influence on the signal processing in sensory systems in relation to reproductive status. Phylogenetic and structural analyses suggested that GnRH receptors can be classified into four subfamilies including a1, a2, b1 and b2 (Troskie *et al*., 2000; Mohamed and Khan, 2006; Flanagan *et al*., 2007).

2.4.2 Follicle Stimulating Hormone

The FSH is a glycoprotein produced by the gonadotroph cells at the anterior pituitary of the brain. The release of FSH is under the influence of GnRH production by the hypothalamus.It stimulates the secretion of inhibin which acts to maintain a relatively stable concentration of FSH (compared with the LH) within the system via a regulatory feedback mechanism to the anterior pituitary. Its action is on cells expressing the FSH receptor (FSH-R). When FSH binds to its receptor, dissociation of the ἀ-subunit of the receptor-associated Gs protein occurs. This in turn, results to the activation of adenylyl cyclase and production of cAMP. The cAMP releases the catalytic subunit of Protein Kinase (PKA), allowing for phosphorylation of numerous intracellular proteins including the transcriptional activator, cAMP response element bindingprotein. Several studies have shown that the target tissues for FSH are the sertoli cells which are located within the testes of male animals. The invaluable effect of FSH is fully expressed in the significant roles the sertoli cells play in male animal reproduction (Johnson and Ewing, 1971; Hafez and Hafez, 2000; Daramola *et al*., 2006).

2.4.3 Luteinising Hormone

Luteinising Hormone (LH) is produced and stored in basophilic cells called gonadotrophs inthe anterior pituitary gland. It is present in male and female vertebrates from fishes to mammals.In the male animal, the major effect of LH is on the leydig cell to stimulate

testosterone production. The LH secretion is regulated by the secretion of GnRH (Hafez and Hafez, 2000; Daramola *et al*., 2006).

2.4.4 Testosterone

Testosterone is the male hormone responsible for spermatogenesis and secondary male sexual characteristics. It is produced in the leydig cells in the testis and is circulated around the body system by diffusion through the spermatic cord. Its production is regulated via a feedback system that operates within the male sex hormone system (Shan *et al*., 1993; Hafez and Hafez, 2000; Daramola *et al*., 2006).

2.5 The endocrinology of reproduction in the goat buck.

The goat buck reproductive functions are dependent on the actions of hormones. (Hormones are chemical substancesproduced by endocrine (ductless) glandslocated in different regions of the body whichtravel (through blood, lymph, extracellular fluids) totarget tissues where they have their effect(Parraguez*et al.*, 2012). This hormonal control starts as early as the embryonic stage and testicular descent which begins at mid-pregnancy and completed at birth or soon after in the goat buck (Paul, 2014). The testis secretes the androgens that cause the testicular descent. After birth, prior to puberty and at puberty, the hormonal interrelationships in the goat buck starts from the higher centers where the hypothalamus producesGnRH which causes the anteriorpituitary gland to release the two majorgonadotrophins, LH andFSH. The actionsof LH are primarily upon the leydig cell, where,acting through adenyl cyclase, promotessteroidogenesis by regulating the rate-limitingstep of steroidogenesis; namely, conversion ofcholesterol into the testosterone precursor, pregnenolone and ultimately testosterone.Testosterone is required for the production ofsperm cells in the testis and their subsequent maturation in the epididymis. It is important for the normal function of the accessory sex glands and the development ofsecondary sexual characteristics. The LH release is regulated by the negative feedback mechanism initiated by testosterone peak in circulation. The main target of FSH is the Sertoli cells (nurse cells) which play the important roles of support and maintenance of spermatogenesis. Some evidence suggests that theproduction of pyruvate and lactate, which act as energy substrates for germ cells, may be the key factor in the FSH-stimulated activity of the Sertoli cell in maintaining spermatogenesis.The FSH secretion is regulated by gonadal steroids and inhibin, the regulatory protein secreted by sertoli cells (Adashi *et al*., 1987; Daramola*et al*., 2007; Parraguez*et al.*, 2012; Saxena, 2012).

2.5.1 Spermatogenesis in the goat buck.

Spermatogenesis is the basic process of malereproduction, resulting in the production of sper-matozoa. It takes place in the seminiferous tubule of thebuck's testis. It comprises of two major stages namely Spermatocytogenesis and Spermiogenesis. Spermatocytogenesis is the male form of gametocytogenesis and involves stem cells dividing to replace themselves and to produce a population of cells destined to be sperm cells. At this stage, initially, the relatively undifferentiated spermatogonia undergo a period of mitotic,multiplication, divisions, followed by the meioticreduction of the diploid to haploid genome. Spermiogenesis is the final stage of spermatogenesis and it involves postmeiotic cells undergoing the morphological transformation that results in the release of formed spermatozoainto the lumen of the tubule. These processes of spermatogenesis arereflected in the functional morphology of the seminiferous tubule. All spermatogonia remain in contact with the basement membrane but as the final meiotic division of spermatogonia give rise to the primary spermatocytes,the first meiotic division then proceeds through the highly sensitive zygotene and pachytene stages.The pachytenestage is particularly sensitive to noxious damage,such as by high testicular temperature and inadequate maintenance of spermatogenesis by inappropriate gonadotropin levels. Theprogeny of the first meiotic division, the secondary spermatocytes, move from the basal to theapical compartment of the seminiferous epithelium and are thereafter separated from the generaltissue fluid compartment. The second meioticdivision produces spermatids, which do not dividefurther. The spermatids thereafter differentiateinto spermatozoa (Cupps, 1991).The duration of spermatogenesis, i.e. the timebetween spermatogonial divisions and the releaseof the spermatozoan, is approximately 60 days in goat bucks. Epididymal transit takes afurther 8–14 days.Thus, the interval between themost sensitive stage of spermatogenesis, meioticprophase, and ejaculation, is approximately 30 days.Hence, the interval between damage to the testis and the appearance of abnormal spermatozoa inthe ejaculate is generally between 30 and 60 days,depending upon the site of damage (Franca*et al.*, 1999; Machado Júnior*et al.*, 2008; Berndtson, 2014).

2.6 Puberty in goat bucks

Puberty in the male animal is defined as the first appearance of mature sperm cells in the ejaculate. It marks the first stage of complete spermatogenesis. Onset of puberty in goat bucks varies from breed to breeds. Generally, an average adult buck can weigh anywhere between 100 to 350 pounds, depending on their breed, health and nutritional status (Souza *et al*., 2011). Although some reports have it that goat bucks reach puberty and breed does as early as 2 months of age. But it is highly recommended to wait until they are about a year old before allowing them to start breeding (Nolte, 2012). The number of does a buck can breed during the breeding season is often referred to as “Buck Power” (Noble, 2004). At one year of age, the buck can service up 10 does at a time (in one month). This can increase up to 25 does in two to threeyear old. Older bucks can breed up to 40 does at one time, as long as his health and nutritional status are okay. The number of does a buck can service at one time also depends on individual sex drive of the buck, the terrain of the land and if it is managed by a hand- or pasture- mating system. The buck has the greatest genetic impact on the herd and should be well taken care of at all times (Noble, 2004; Maha*et al.*, 2012; Maha*et al.*, 2013; Nolte, 2012).

2.7 Factors affecting Puberty in goat bucks

Factors that can affect puberty in goat buck include seasonal/climatic, genetic/environmental, nutritional and hormonal factors (Saxena, 2012; Maha*et al*., 2013),

2.7.1 Seasonal/Climatic factors: Photoperiod (day length) has effect on the some breeds of goats bucks and they have been observed to have the highest libido (sex drive), fertility, and semen quality and volume in summer and autumn. As the day length increases, less sperm cells are produced and more abnormal sperm cells are found in the semen. During the fall, the endocrine system also increases levels of the sex hormones, testosterone and luteinizing hormone. The climatic status of an environment determines the type of rainfall, sunlight and vegetation or availability of plants and food materials for animals and these have significant effect on puberty in goat bucks (Saxena, 2012).

2.7.2 Genetic factors/environmental factors: Pubertal age in bucks is largely determined by the interaction between genetic and environmental factors. The variations in breeds,

strains and individual buck goats have shown that genetic factors have influence on the age at puberty in buck goats. For instance, bucks with larger scrotal circumference have been documented to service does successfully at earlier age and produce offsprings with the same genetic trait even up to the second generation (Maha *et al*., 2013).

2.7.3 Nutritional factors: Nutrition plays a key role in age at puberty in buck goats. Studies have shown that well fed bucks reach puberty earlier than the underfed ones. The nutrition of the buck should be maintained at optimum level starting from birth. Age at puberty is highly related to body weight which is influenced by the level of nutrition. Deficiency in vitamin A, phosphorus and calcium may also cause delay in the occurrence of puberty due to slow body growth rate. In addition, deficiency of vitamin A may lead to irreversible degeneration of the seminiferous tubules where sperm cells production takes place (Saxena, 2012, Maha *et al*., 2013).

2.7.4 Hormonal factors: The hormonal interplay of testosterone and the gonadotropins (LH and FSH) play significant roles in the age at which puberty is attained in goat bucks. Prior to puberty, the gonadotropins are present but only at basal level. At puberty, the anterior pituitary is triggered by GnRH released from the hypothalamus to produce more FSH and LH. These hormones act on the testes to produce testosterone which is required for spermatogenesis and other secondary sexual characteristics the bucks display at the age of maturity (puberty). Hence, an optimal balance in the level of testosterone and these gonadotropins is required for the induction puberty and optimal functioning. This is regulated through the negative feedback mechanism between testosterone and the gonadotropins. Any imbalance in this, may affect the age at puberty in the goat buck (Hafez and Hafez, 2000; Daramola *et al*., 2006).   ).

2.8. Optimum breeding age in the Buck.

Optimum breeding age (OBA) is the age at which the animal attains full sexual maturity (Abebe, 2008). At this age, in the buck, all the reproductive organs are fully developed and functional particularly the testis and epididymis (Osinowo and Williams, 2008). Hence, represents the best time to start using the male animal for breeding. However, OBA is yet to be determined in WAD bucks. Whereas, its value has been highlighted in the bull; in a

herds studied, only about 35% of pubertal bulls i.e age 12 months, were reproductively matured with good quality semen. This increased to 60% in the 14 months old bulls and 95% was recorded in the 16 months old bulls in the herd. This implies that, although, puberty was at the age of 12 months but the OBA commenced at 16 months in these bulls (Barth, 2000; Barth and Waldner, 2002). Observing the OBA in rabbit breeding has been reported to be very valuable and profitable (Proverbs and Quintyne, 2008).

2.8.1 The benefits of Optimum Breeding Age

OBA represents the best time to start using the male animal for breeding. At OBA, energy is directed basically towards spermatogenesis (Henkel, 2015). Also, the rate of matured sperm cell replacement after depletion is faster at OBA compared to the pubertal age (Kinne, 2001). At OBA, there is increase in the rate of spermatogenesis, hence, increase in the number of does the buck can serve and fertilize successfully (Noble, 2004). Also, increase in litter sizes has been reported (Suckow *et al*., 2005).Females from the litters of a male animal introduced at OBA have been reported to reach puberty, cycle, become pregnant and litter successfully at younger ages (Raji *et al.*, 2008; Ugwu, 2009). In terms of health, at OBA, the male animal is less prone to health problems compared to the age of puberty (Henkel, 2015).

2.9 Fertility in the goat buck

Fertility in the WAD buck is its ability to procreate or successfully impregnate the doe. Infertility is the reversible inability of the goat buck to procreate while Sterility is the irreversible inability of the goat buck to procreate (Raczykowski, 2002). Breeding Soundness Examinations (BSE) is usually carried out to ascertain whether normal fertility can be expected from the goat buck, or for the diagnosis of infertility or sterility. This involves taking adequate history of the buck, a general examination, a detailed examination of the genital tract, observation of copulation, and collection and evaluation of semen (Memon *et al*., 2007). History-taking is an important part of BSE especially in a suspected infertile buck. Many of the infertility problems do not manifest until after a considerable period of time has elapsed from the original insult. Hence, the need to ask the ownerof the buck salient questions about the possible causes of the infertility conditions that may have

been considered trivial at the time of their occurrence. History-taking is also a useful way of assessing owners' expectations of the bucks, for many cases of so-called 'infertility' result from no more than an unrealistic expectation of a buck's capabilities.The history must establish whether or not the buck is likely to be the cause of the infertility, the duration of infertility and the circumstances of its onset. The number of does with which the sire's infertility has been manifested must be determined, as must the conditions under which mating has occurred. The system of production or mating practiced and the ratio of buck to doe must be enquired about.A common cause of apparent infertility in bucksderives from no more than using groups of too many does, especially if these have undergone synchronisation of oestrus or are being used in out-of-season breeding regimen. The time of year when the infertility wasnoticed may give helpful clues as to its cause, andmay help to determine whether does factors are likely to have been of importance. Similarly, information regarding the previous breeding records and success is of great importance in differentiating between congenital and acquired conditions, or between managemental and pathological causes (Memon *et al*., 2007). If records of the management and reproductive performance of the herd are available, they are invaluable in ascertaining the overall level of the fertility of the herd and bucks. Records may also provide useful comparative information for other contemporary bucks and may help to pinpoint the onset and duration of the period of low fertility. Observation of the normal environment of the buck is usually advisable. Seeing and asking questions on how the buck ishandled, how it is housed, fed and cleaned, the area in which it is required to serve, how it is moved there and how it is handled during service, all may assist with one's assessment of the infertility of the buck (Pezzanite *et al*., 2013).

2.9.1. Breeding Soundness Examination.

Breeding Soundness Examination (BSE) is the complete and systematic evaluation of the reproductive potential of a given male (Pezzanite *et al*., 2013); including physical examination and inspection of the genital organs, measurement of scrotal circumference (SC) and assessment of sperm production and quality (Bezzeria *et al*., 2009).

2.9.1.1 General physical examination: The general physical examination of the buck must take into consideration its age and likely sexual experience, body condition, the possibility

of intercurrent illness and the buck's temperament (Keith *et al.*, 2009; Okere *et al.*, 2011). Considerable importance can be attached to the body condition and general degree of maturity of young bucks; on one hand, puberty can be delayed in poorly grown bucks, while, on the other hand, animals that have achieved very high growth rates during rearing may have a body conformation that belies their sexual immaturity. Body Scoring (BS) is an assessment of the percentage of body fat the male has and is determined by feeling the ribs and spine of the buck as well as a visual assessment. Ideally, a buck should enter the breeding season with a BS of 3 to 3.5 on a scale of 1 to 5 (1 being very thin and 5 being overweight). If the buck is too thin, breeding stamina will be affected (Kinne, 2001). This can result in a longer kidding period because some does will cycle more than once before conception occurs. On the other hand, obese bucks may lack vigor to breed large numbers of does. Spermatogenesis tends to be limited when body condition is poor, and can also be limited by specific micronutrient deficiencies(Kinne, 2001). In general, chronic and continuing deficiencies of protein and energyare likely to be of greater overall importance thanmicronutrient deficiencies, although the effects upon fertility can be severe when these occur simultaneously (Crowder and Chheda, 1982). Bucks should be maintained in moderate conditions before, during and after the breeding season. It is also important to determine that the sizes of bucks and does they will be mating are compatible. Conditions of the locomotor system, conditions causing pain in the caudal abdomen and conditions that result in prolonged pyrexia should be thoroughly examined. In principle, it is important to note that the hindlimb or back pains are incompatible with normal mating in goat bucks. Furthermore, not only does locomotor pain limit mating directly, but also the stress of prolonged, unresolved pain may cause corticosteroid-mediated impairment of spermatogenesis. Systemic illness causing prolonged pyrexia can result in increased temperatures within the testis, thereby causing temperature-limitedimpairment of spermatogenesis ((Kinne, 2001; Browning and Leite-Browning, 2008).

BODY SCORE

Parts of the Loin

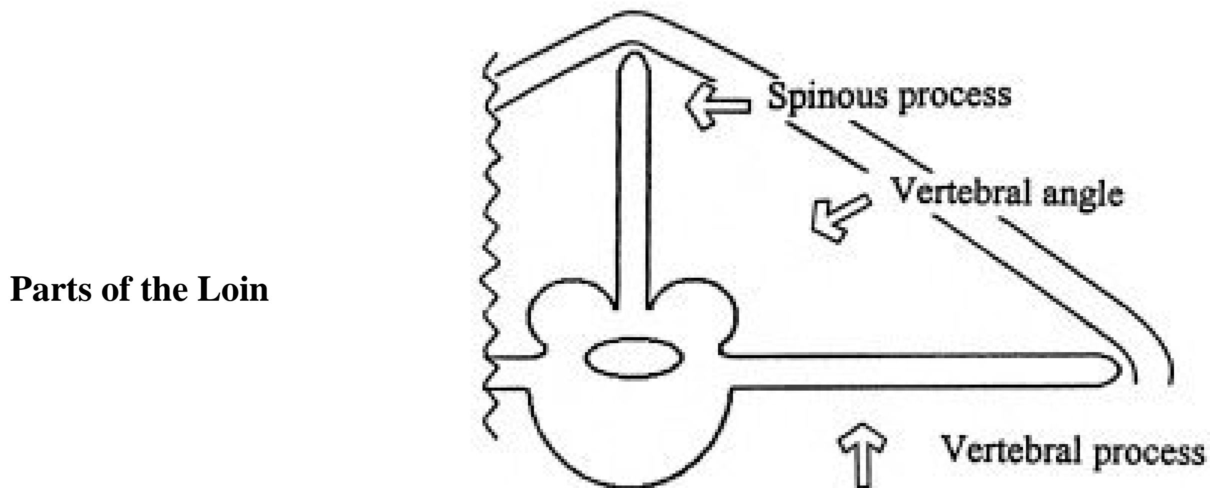


Figure2.4: Body condition scoring in goats

(Kinne, 2011).

Spinous processes: are the bones felt on top of the back.

Vertebral processes: are the long bones horizontal to the spine.

Vertebral angle: is the triangle between the top of the spinous process, the edge of the vertebral process andthe skin.

Longissimusor eye muscle isthe muscle inside the vertebral angle, a roast or part of a T-bone steak.

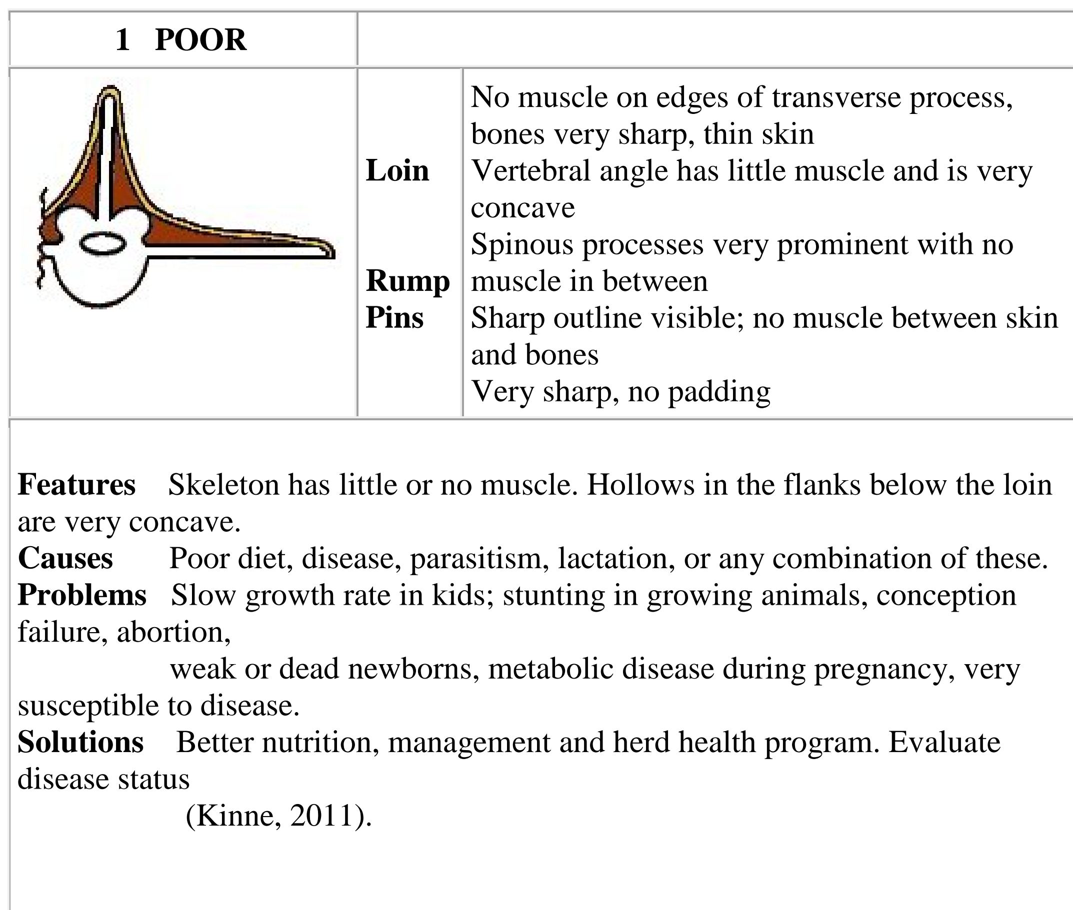
Body condition score: Scores 1-3 represent the longissimus muscle growth/expansion.

Muscle does not grow after score 3.

Scores 4 and 5 represent fat accumulation

(Kinne, 2011).

Body Score 1:

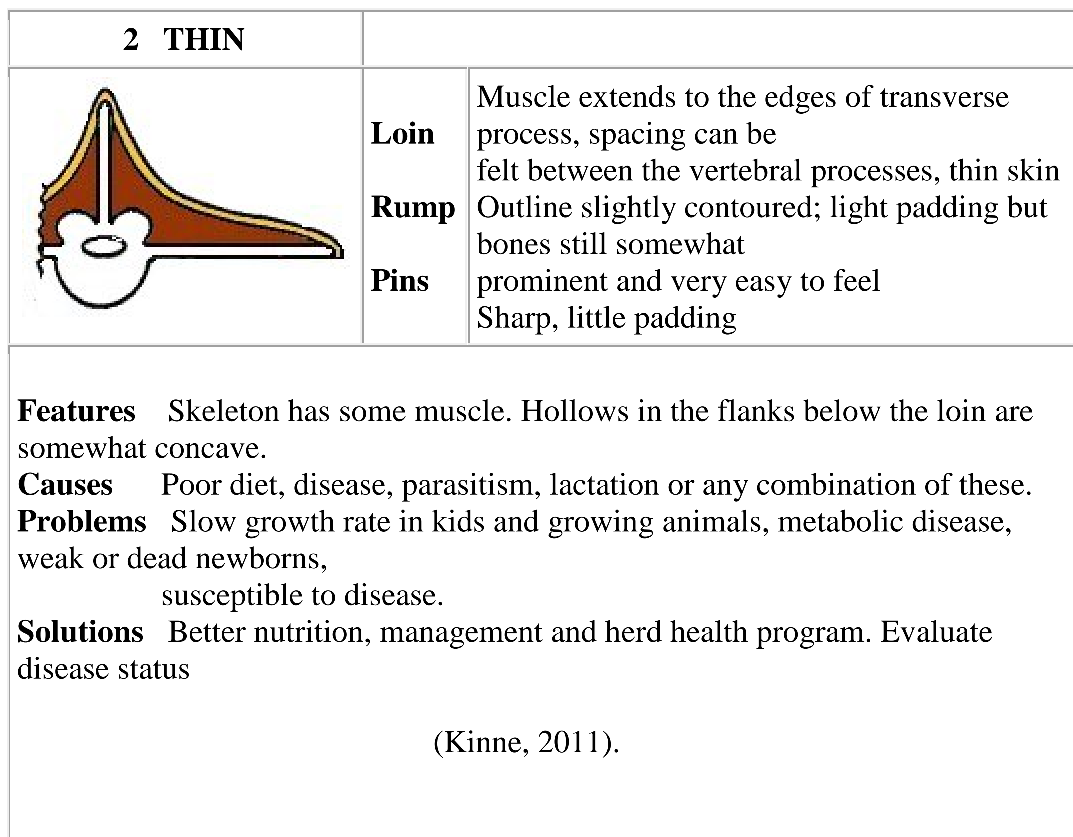


1 POOR		
	Loin **Rump** **Pins**	No muscle on edges of transverse process, bones very sharp, thin skin Vertebral angle has little muscle and is very concave Spinous processes very prominent with no muscle in between Sharp outline visible; no muscle between skin and bones Very sharp, no padding

Features Skeleton has little or no muscle. Hollows in the flanks below the loin are very concave.
Causes Poor diet, disease, parasitism, lactation, or any combination of these.
Problems Slow growth rate in kids; stunting in growing animals, conception failure, abortion,
weak or dead newborns, metabolic disease during pregnancy, very susceptible to disease.
Solutions Better nutrition, management and herd health program. Evaluate disease status
(Kinne, 2011).

Figure 2.5: Body score 1 in goats and associated features (Kinne, 2011).

Body Score 2:



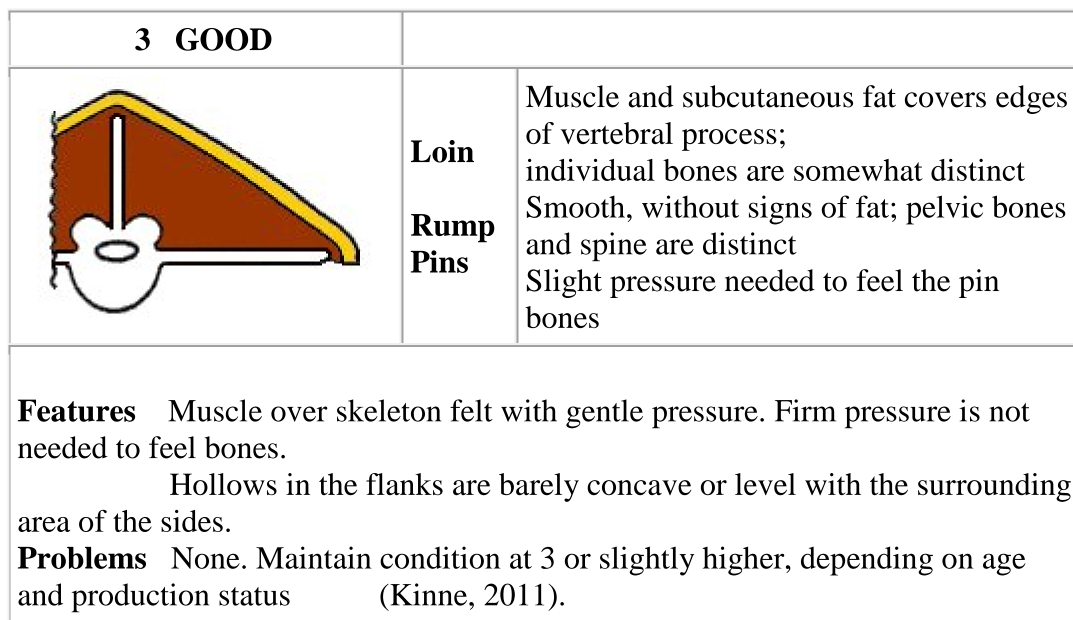
2 THIN		
	Loin **Rump** **Pins**	Muscle extends to the edges of transverse process, spacing can be felt between the vertebral processes, thin skin Outline slightly contoured; light padding but bones still somewhat prominent and very easy to feel Sharp, little padding

Features Skeleton has some muscle. Hollows in the flanks below the loin are somewhat concave.
Causes Poor diet, disease, parasitism, lactation or any combination of these.
Problems Slow growth rate in kids and growing animals, metabolic disease, weak or dead newborns,
susceptible to disease.
Solutions Better nutrition, management and herd health program. Evaluate disease status

(Kinne, 2011).

Figure 2.6: Body score 2 in goats and associated features (Kinne, 2011).

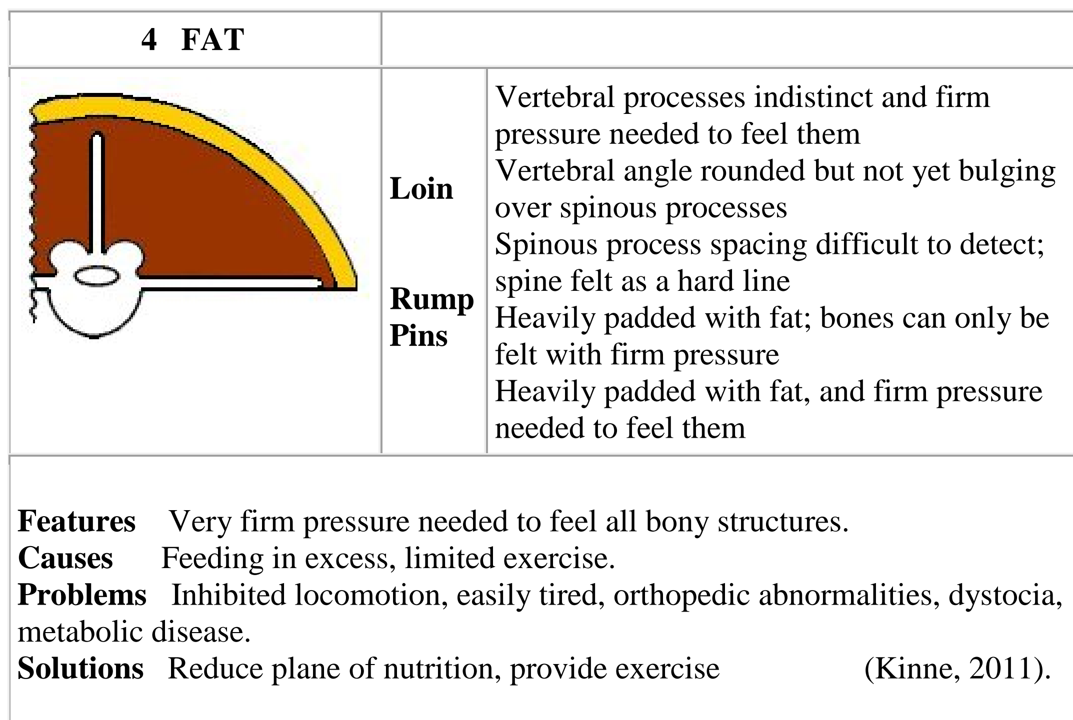
Body Score 3:



3 GOOD		
	Loin **Rump** **Pins**	Muscle and subcutaneous fat covers edges of vertebral process; individual bones are somewhat distinct Smooth, without signs of fat; pelvic bones and spine are distinct Slight pressure needed to feel the pin bones
Features Muscle over skeleton felt with gentle pressure. Firm pressure is not needed to feel bones. Hollows in the flanks are barely concave or level with the surrounding area of the sides. **Problems** None. Maintain condition at 3 or slightly higher, depending on age and production status (Kinne, 2011).		

Figure 2.7: Body score 3 in goats and associated features (Kinne, 2011).

Body Score 4:

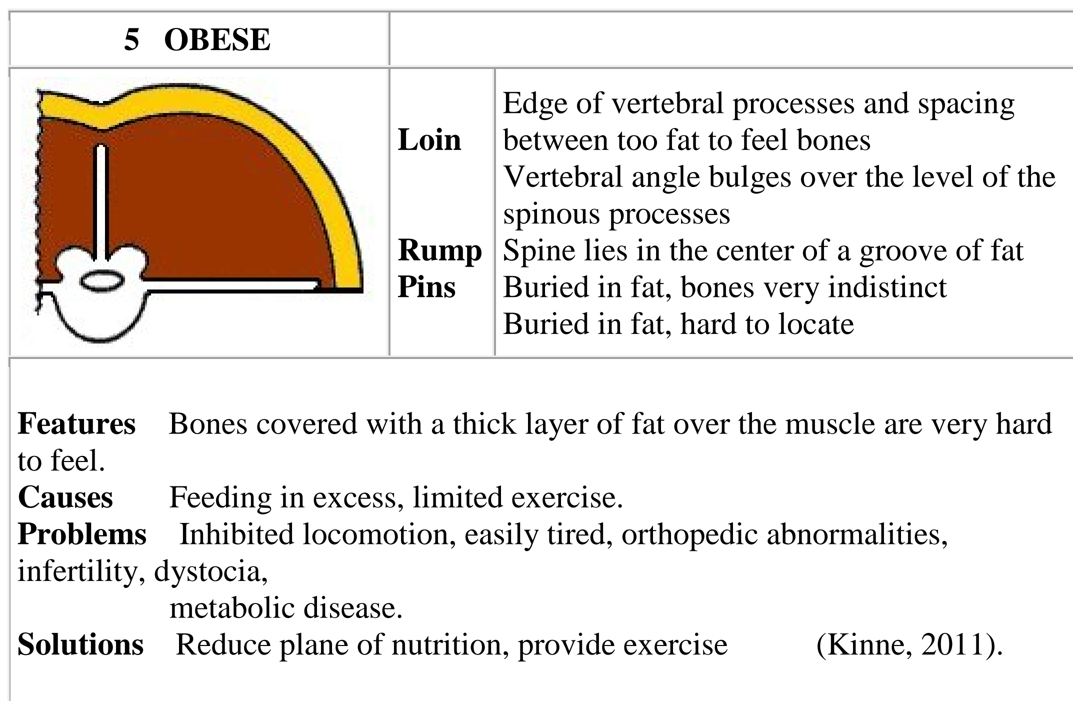


4 FAT		
	Loin	Vertebral processes indistinct and firm pressure needed to feel them Vertebral angle rounded but not yet bulging over spinous processes Spinous process spacing difficult to detect; spine felt as a hard line
	Rump	Heavily padded with fat; bones can only be felt with firm pressure
	Pins	Heavily padded with fat, and firm pressure needed to feel them

Features Very firm pressure needed to feel all bony structures.
Causes Feeding in excess, limited exercise.
Problems Inhibited locomotion, easily tired, orthopedic abnormalities, dystocia, metabolic disease.
Solutions Reduce plane of nutrition, provide exercise (Kinne, 2011).

Figure 2.8: Body score 4 in goats and associated features (Kinne, 2011).

Body Score 5:



5 OBESE		
	Loin **Rump** **Pins**	Edge of vertebral processes and spacing between too fat to feel bones Vertebral angle bulges over the level of the spinous processes Spine lies in the center of a groove of fat Buried in fat, bones very indistinct Buried in fat, hard to locate

Features Bones covered with a thick layer of fat over the muscle are very hard to feel.
Causes Feeding in excess, limited exercise.
Problems Inhibited locomotion, easily tired, orthopedic abnormalities, infertility, dystocia,
metabolic disease.
Solutions Reduce plane of nutrition, provide exercise (Kinne, 2011).

Figure 2.9: Body score 5 in goats and associated features (Kinne, 2011).

2.9.1.2 Specific examination of the buck's reproductive organs: During BSE, specific evaluation of the buck's reproductive tract is necessary in order to assess his ability to copulate and inseminate the doe and also to identify any potential reproductive tract abnormalities that may limit the buck's reproductive ability (Abba *et al*., 2014). This includes measurement of the scrotal circumference, observation and palpation of the penis, prepuce, sheath, testes and epididymides. The scrotum and testes should be examined and palpated for their tone and size. The testis should be firm (but not contain any areas of increased firmness or abscesses), movable within the scrotum, and of similar size. Pronounced differences in size may indicate fertility problems. Swelling of the testes or epididymis indicates injury or infection (Akpa*et al*., 2013). During testicular palpation, the epididymis should also be palpated and properly examined. This is because epididymitis is a common reason for the culling of breeding bucks. This may arise from a disease condition called Brucellosis. This disease causes swelling and hardening of the epididymis. It is transmitted during sexual activity, either from buck to buck, or through the doe or doe or buck to doe. Does can be carriers, and those that are bred by infected bucks may have abortions, stillbirths, or weak kids (Nasriddin *et al*., 2014). An important measurement taken during the BSE is the scrotal circumference of the buck. This measurement is strongly related to the semen production capacity of the buck. There is evidence that bucks with large scrotal circumferences will produce more semen of greater viability. Also, female progeny from bucks with larger scrotal circumferences reach puberty earlier than progeny from bucks with smaller scrotal circumferences. Scrotal circumference should be measured at the greatest circumference of the scrotum. Scrotal circumference can vary by season and with body condition (Raji *et al*., 2008; Bezzera *et al*., 2009).

2.9.2.1 Measurement of the scrotal circumference

Scrotal circumference of goat bucks is measured by taking the largest diameter of the testes. Before this is done, the consistency of the testes is usually evaluated by palpation. However, there are currently no age standards for SC in goat bucks generally. Since, SC is highly correlated with semen production; it will be highly desirable to establish this in the WAD goat buck (Ford *et al*., 2009; Keith *et al*., 2009).

2.9.3 Semen collection and analysis

This is a very important aspect of BSE of WAD goat bucks. The common methods of semen collection from the buck include by the use of Artificial Vagina (AV), the electroejaculation method and by vaginal collection. Semen can be collected without causing any injury to a buck once in two days (Bopape *et al*., 2015).

2.9.3.1 Artificial Vaginal (AV) method: The AV is one of the commonly used procedures for semen collection in the goat buck. It is painless, quicker and does not stress the buck at all. It resemblesa car radiator hose and is about six inches in length. It has an inner rubber liner (containing water ata temperature of 100°F) placed between the liner and the hose. The warmer water simulates thevagina of a doe. A latex rubber collection cone is placed in the AV and a graduated collection tubeis placed on the end of the cone.The buck is made to mount a doe inheat, another buck, or a wether. The usual procedure is to use a teaser doe that is in heat. This canbe a natural heat or one induced. A doe in heat usually stands better for abuck than a wether or another buck. She emits a smell when in heat that causes the buck to give abetter ejaculate. The doe is usually tied or held and the buck allowed to go through his courtingbehavior. The buck is allowed a few false mounts, and then the person with the AV collects the ejaculateby directing the penis into the AV. The test tube containing the ejaculate should be protected fromdirect sunlight and cold temperatures (Marjuki, 2011; Saxena, 2012).

2.9.3.2 Electroejaculation method (EE): This method is based upon electrical stimulation of erection and ejaculatory centers, thus erection and ejaculation can be obtained from bucks that are unwilling to serve. Electroejaculators come in a variety of shapes and sizes and are used when animals are nottrained for AV collection, are physically unable to mount, or a suitable mount is not available. The procedure is such that the prepuce is cleaned, hind limbs raised to about 45 degrees to the floor and thenthe bipolar electrode probe (that has been properly lubricated with a vaseline or lubricant) is inserted into the rectum of the buck. Stimulation is begun with the lowest possible voltage and then higher voltage stimuli are applied until erection and dripping of seminal plasma is observed. Stronger stimuli are given until the protrusion of penis is complete and more opaque fluid is discharged from the tip of the penis. At this point, a funnel put in a semen collection vial is

brought over the glans penis for collection of ejaculate (Sundararaman *et al*., 2007). Mostworkers have obtained a greater volume of semen but of lower concentration of spermatozoa with EE than with an AV. Some bucks do not respond well to the electrical stimulus, especially if asecond or third collection is desired. Furthermore, there is some danger of contamination of the semen sample with urine. The increased volume of seminal plasma obtained by EEappears to reduce the resistance of sperm to cold shock and decreases the post-thaw survival rate offrozen semen (Oyeyemi *et al*., 2001). Some studies showed that comparing these two methods, the conception rate at first service was higher when compared with semen that was collected with an AV (Malejane, 2013). However, recent advances in theanatomical and physiological knowledge of the male reproductive tract may lead to EE techniqueswhich permit acquisition of normal sperm to seminal plasma ratios in the ejaculate (Shawon, 2014).

2.9.3.3 Vaginal collection: Collection of semen from the vagina of goat is best done with a doe out of heat because the vagina is more apt to be dry and free of mucus. Any fluid in the vagina should be removed before service. The semen should be removed immediately after each service with a pipette. Contamination with urine and other foreign materials should be avoided (Wulster-Radcliffe *et al.,* 2001; Saxena, 2012).

2.9.4 Handling of WAD buck semen: Buck semen should be collected in insulated vials because exposure of semen to sudden temperature changes could result in death of sperm cells, tertiary abnormalities or agglutination of sperm cells. There should be no delay in semen evaluation after collection, especially motility. Contamination with chemicals and water should be avoided because this can alter the pH of the semen. Vigorous shaking of buck semen during transport and mechanical damage that can result during the making of smears should be avoided (Mann, 1964; Dorado *et al*., 2007).

2.9.5 Semen analysis

2.9.5.1 Colorand consistency: A good buck semen color ranges from a creamy white color to yellow. Off colors, however, is a suspect and whenever possibleshould be cultured. Red coloring of the sample indicates the presence of blood. This maybe due to adhesions or trauma that has occurred prior to collection. Yellow watery colorsmay indicate urine in the

ejaculate. A black or dark sample may indicate debris such as dirtor manure contaminating the sample due to an uncleaned prepuce or the presence of pus (Oyeyemi *et al*., 2001; Oyeyemi *et al*., 2008).

2.9.5.2 pH: The pH of the semen is sometimes measured at the time of semen collection. Thisdetermination is usually made by using litmus paper or a pH meter. The average pH of buck semen sample ranges between 6.5 and 7.4. The pH of the semen sample may also vary at different times of the year.Altered pH of semen samples can be corrected. For example, sodium bicarbonate added to acidic semen samples will bring the sample to thedesired pH. Anything that alters the pH of the semen above or below the normal range willcause sperm death. The presence of blood or infection can alter the pH enough to kill thesperm cells.If the pH of the ejaculate is low (acidic) that may not indicate poor quality sincehighly active sperm samples produce lactic acid as a metabolic waste product. However,the sperm will be killed if the acidity is not neutralized. All of the common semen extenders(dilutors that "extend" sperm cell life) are buffered. That is, they contain chemicals that tendto prevent pH changes and "force" the pH to remain in a safe range. Since it is a goodpractice to dilute buck semen as quickly after collection as possible, to prevent loss ofviability, the extenderwill usually correct any pH problem (Akpa *et al*., 2013).

2.9.5.3Volume: Volumeis an important criterion in semen evaluation. The quality of the semen may decrease asthe total volume of the ejaculate increases depending on the method of collection. But, generally larger volumesmean more sperm. Older bucks generally have a larger volume of ejaculate than youngerbucks. Therefore, age should be considered with respect to this parameter of semenevaluation. The volume is simply measured using the graduations on the collection tube while avoiding error due to parallax. Small volume in goat buck may not be harmful. The volume of the ejaculate WAD goat buck ranges between 0.3 – 2.0 ml(Oyeyemi *et al*., 2001; Ajala *et al*., 2009).

2.9.5.4 Mass activity: This is also referred to as swirl motion or gross motility. This reflects the combined effect of concentration and viability. It is carried out by using a pipette to put a drop of semen on a warm slide, cover with cover slip and observe under microscope using objective lens x10 and x40. Scoring may be done as + - fair, ++ - good, +++ - very good (Oyeyemi *et al*., 2011).

2.9.5.5 Motility: The motility of sample is defined as that percentage of the individual sperm cellin a sample that swims in a progressiveunidirectional or linear fashion. Circular or reversemotion often indicates cold shock or media that is not isotonic with semen. Theprogressive motility is determined by examining a drop of semen, diluted so that individualcells can be visualized.Physiological saline can be used as a diluter, but it is better to use a bufferedsolution containing an energy source such as glucose. Phosphate Buffered Saline (PBS)with 1% glucose works very well. Other standard buffered solutions (such as Ringerssolution, sodium citrate buffer, Tris buffer, and Tyrodes solution) can also be used.It is extremely important that the solutions, pipettes, and glassware (such as testtubes and slides) be at the same temperature as the semen. It is best to make the motilityestimate as soon after the semen is collected as possible. Astage warmer should be provided to keep the slides warm at 37°C while it is being examined to prevent the sperm cells from dying of cold shock. Motility scoring is subjective; hence slides should be easily readable with only a thin sample covered with cover slipwith only about 10 to 20 sperm cells per field under the microscope. Oyeyemi *et al*., (2009) recorded 90.75% motility of buck spermatozoa; while Daramola *et al*. (2010) recorded 62.5 + 6.46% motility of WAD buck sperm cells.

2.9.5.6 Live-dead ratio: This is scored in percentage similar to that of motility. The procedure for this is such that a drop or two of eosin-nigrosin stain is added to semen on a warm slide, mix gently using the edge of another slide, then make a smear with a slide at an angle of 45 degrees to the slide on which the semen was mixed with the stain. Observe under microscope. Live sperm cells do not pick up stain while dead sperm cells will pick up stain (Bitto *et al*. 2012).

2.9.5.7 Percentage normal Morphology: Morphologically, normal buck sperm cell consists of two main regions, the head and the tail. The tail is further divided into four regions namely, the neck, mid-piece, principal piece and the end piece. The procedure is the same as for live-dead ratio but wells and awa stain is the preferred stain. The percentage of normal against abnormal sperm cells are then scored (refer to motility scoring). Normal ejaculate expected range of spermatozoa abnormalities is between 8-10% with no effect on

fertility.If abnormalities exceed 25% of total ejaculate, reduced fertility can be anticipated.There are 3 major classifications of spermatozoa defects:

(A). Classification based on the site or portion of the spermatozoa having the defect e.g head, midpiece and tail abnormalities. Head abnormalities: pyriform head, small head, narrow head, twin heads etc. Midpiece abnormalities - bent midpiece, curved midpiece etc.Tail abnormalities - rudimentary tail, looped tail, coiled tail etc.

(B). Classification based on the region of reproductive tract where the defect originated (Bloom's method of classification). This is further classified into 3:

(i). Primary abnormality: Arises during spermatogenesis within the testes (seminiferous tubules) andare mainly defects of the head and the midpiece.

(ii). Secondary abnormality: Arises during epididymal transit and storage e.g proximal cytoplasmic droplet.

(iii). Tertiary abnormality: Arises after ejaculation, during semen processing or handling of the spermatozoa e.g exposure of the spermatozoa to cold, excessive heat, chemical etc. e.g looped tail, missing tail, may be as a result of vigorous shaking.

(C). Classification based on emperical effect on fertility or fertilizing ability of spermatozoa. Major abnormalities that can cause infertility include:

Acrosomal defect, knobbed acrosomal due to defective spermatozoa genes affecting 80-90% spermatozoa.

Diadem defect- spermatozoa having a necklace diadem consisting of 5-6 reddish pores placed tightly around the sperm head. This may arise from disturbances in spermatogenesis due to testicular degeneration. This affects ability of spermatozoa to penetrate the ova.

Cock-screw defect: This abnormality involves the tail and the midpiece, may arise from testicular degeneration thus affecting motility and fertilizing capabilities.

Pseudo-droplet: This is a midpiece defect often characterized or diagnonized as a dislocated cytoplasmic droplet. It consist of a rounded or elongated swellings or lumps of dense

centrally accumulated granules of unknown origin and the surface of mitochondrion surrounded by several layers of mitochondria normal layers (Oyeyemi *et al*., 2011; Menkveld *et al*., 2011).

2.9.5.8 Concentration: Currently the semen analyses are done by using the Computer Assisted Semen Analysis (CASA). It is the easiest and most accurate method but expensive. Buck semen concentration can also be estimated by its optical density (turgidity) using the nephelometer or photoelectric colorimeter. Semen samples with high concentration are usually slightly acidic in reaction while those with low concentration are slightly alkaline. An alkaline reaction of semen is often associated with poor quality and low fertility. The commonest way of estimating buck semen concencentration is by the use of haemocytometer which contains improved Neuber counting chamber with 25 squares and a red blood cell pipette. Suck semen to 0.5ml mark and then fill up to 1.01 mark on the pipette with formal saline and shake mixture gently. Discard formal saline at the top of pipette gently. Fix a cover slip on the counting chamber and then fill up with the diluted semen carefully. Count sperm cells in 5 squares diagonally or at the edges and center(as illustrated), add up,and multiply by 5, then by 10,000 and the dilution factor. The concentration of the ejaculate is a function of several parameters. They includethe degree of sexual preparation of the buck, the age of the buck, the time of year thecollection is made, the amount of sexual rest before collection, the health of the buck, hisnutritional state, inherent sperm storage, and the production capacity of the buck.Most of these factors can be controlled by employing good management practices. Theability of the buck to produce sperm (in the testes) and store sperm (in the epididymis) canbe assessed to some extent by palpation and measurement of the testes and epididymis (Oyeyemi *et al*., 2011; Bobape *et al*., 2015).

2.10 Infertility in the goat buck

Reproductive abnormalities causing absolute orrelative infertility in goat can be broadly categorized into two, namely,conditions causing reducedor complete inability to copulate(impotentia coeundi) and conditions causing inability or reduced ability to fertilize (impotentiagenerandi) (Jubb and Kennedy, 1970; Raczykowski, 2002; Parma, 2016).

2.10.1 Impotentia coeundi:

The conditions causing this can be further dividedinto three, firstly, conditions causing reduced or complete lack of libido (sexual drive) and ability to copulate; secondly, conditions that preventnormal copulation from occurring, despitenormal libido. Superimposed upon both groupsare considerations of whether the infertility represents a pathological condition of the genital (orother) system, or whether infertility is primarilymanagemental in origin and could simply be alleviated by modifying aspects of the husbandry ofthe animal. Much of the differentiation betweenthese major groups of conditions can be achievedby careful history-taking (Abba *et al*., 2014; Parma, 2016).

2.10.1.1 Lack of libido: This is one of the most difficult conditions for the clinician to diagnose. This is becauseapart from the fact thatthe condition can be due to genital pathologic conditions (which are most often visible or obvious), but it can also result from intercurrent diseases, management, age, maturity or seasonal effects. Also, delay in treatment or neglect of disease conditions, frequently result in sexually disinterested bucks. Many of the bucks that are presented for lack of libido, are either young or of advanced age. At times, this could just be due to the conditions under which a young buck has been reared, thereby affecting his mating behavior. For example, bucks that are reared in groups or in company of other bucks and/or does learn mounting and mating abilities through the social interactions amongst them, compared to a buck reared in isolation. Age at puberty could also be a major factor; an immature buck may show lack of libido.However, puberty

or libido can be induced by giving hormone therapy to goat bucks. In a study by Daramola *et al*. (2006), puberty was induced in WAD bucks using melatonin. Some farmers give large doses of human ChorionicGonadotrophin (hCG) orGnRH in order to elevate testosterone concentrations. The disadvantages of this practice include induction of unwanted aggression alongside libido; there may also be testicular oedema leading to impaired spermatogenesis. Also, hormonal imbalance may occur which sometimes cannot be corrected. On a worse scenario, the buck may not even respond to the hormone therapy, or if at all, response is only for a short time. Moreseriously, the correlation between the age of onsetof reproductive activity of bucks and their off-spring means that it is positively undesirable toattempt to breed from bucks that exhibit agross delay in the onset of sexual activity. Lack of libido can also result frompoor service management. Slippery floors, roofs that are too low, relatively too big does and insensitive handling of bucks by stockpersons, can all contribute to lack of libido in breeding bucks (Kerketta *et al*., 2014; Abba *et al*., 2014; Parma, 2016).

2.10.1.2 Locomotor dysfunction: Lesions or disease conditions affecting locomotion usually impair the abilityand willingness of bucks to copulate. The most important of these include lesions of the back and hindlimbs. Also, gross pathologyof the foot, such as penetrations of the sole, separations of the white line, foot rot, swelling in the foot,etc., can produce pains that may make the buck unwilling totake his weight on the foot during copulation. Forthis reason, valuable bucks' feet should be given considerable attention during BSE (Jubb and Kennedy, 1970).

2.10.1.3 Failure to copulate: The Conditions thatcause failure of copulation include failure of thepenis to become turgid or failure of erection, abnormalities of erection that prevent intromission, lesions of the penis and prepuce that prevent protrusion of the penis, and chronic back pains which may be as a result of spinal cord infections or traumatic injuries. Most of these conditions can be differentiated relatively easily, and a prognosis can usually be given at an early stage of investigation (Jubb and Kennedy, 1970; Abba *et al.*, 2014).

2.10.1.4 Failure of erection: This could arise from a number of factors or causes including injury of the ischiocavernosus muscleor if any aspect of thevascular system of the Corpus Cavernosum Penis (CCP) is perturbed or ruptured. It could also arise from the effect of

recurrent diseases such as urolithiasis (Jubb and Kennedy, 1970; Abba*et al*., 2014; Parma, 2016).

2.10.1.5 Persistence of the penile frenulum: This condition can either limit the amount of penis that can be protruded or cause the protruded penis to be deviated ventrally in the buck. Transection ofthe frenulum after ligating the frenular blood vessels, may give a good prognosis for the recovery of breeding ability (Jubb and Kennedy, 1970; Raczykowski, 2002).

2.10.1.6 Congenital abnormalities of the penis preventing protrusion: Considerable growth ofthe penis and changes in the relationships between the penis and the peripenile tissues occur duringthe prepubertal period. Failure of these developmental changes in these tissues can result in failure of normal erection. For example, failure of the penis to undergonormal growth causes a congenital shortness of the organ, such that normal intromission cannot be achieved. Alternatively, if such failure of growth is confined to the sigmoid flexure, it may be impossible to exteriorise the penis. Similarly, the retractor penis muscles can fail todevelop, causing inability to protrude the penis.Although treatable by myectomy, this condition is probably inherited,so correction should, perhaps, not be undertaken (Jubb and Kennedy, 1970; Abba *et al*., 2014; Parma, 2016).

2.10.1.7 Adhesion of the penis and prepuce: Adhesions between the peripenile tissues can arisefrom localised trauma, haemorrhage and/orabscessation in and around the prepuce. Infectionof the penis (balanitis) or prepuce (posthitis) notonly is painful, causing unwillingness to copulate,but also can result in development of adhesionsbetween the two organs, preventing protrusion ofthe penis (Jubb and Kennedy, 1970; Kerketta *et al*., 2014; Abba *et al*., 2014).

2.10.1.8 Balanoposthitis: Inflammation of the penis and prepuce often occur simultaneously presumably because of the close anatomical relationship between these two parts of the reproductive tract of the buck. This condition can be due to infectious and non-infectious causes. Infectious causes may include bacteria, moulds, protozoa and viruses often in the prepucial cavity e.g Streptococci, Staphylococci, caprine herpesvirus-1 etc; these are often associated or secondary to the non-infectious causes which could be due to

trauma, lacerations, abrasions etc of the glans penis and prepuce. These infectious agents may enter the deeper tissues of the prepuce and penis resulting in inflammation, discharge and pain. Occassionally, during courtship behavior, hair may be accidentally drawn into the prepucial cavity leading to swelling or inflammatory reactions. Severe balanoposthitis can cause pain, unwillingness to mate, preputial stenosis, adhesions between penis and prepuce, andperipenile adhesions. In mild cases of balanoposthitis, there may be mild seropurulent exudate from the prepuce which is rarelyindicative of clinical disease. Prognosis of balanoposthitis depends on the severity of the infection and the causative agents. In mild cases, prognosis is good as recovery could be spontaneous. But in severe and chronic cases, the prognosis is guarded. In mild cases, bucks respond well to treatments which may consist of douching the prepuce with antiseptics solution, hydrogen peroxide or antibiotics (irritant antibiotics should be avoided). This may aid in the prevention possible adhesions. Systemic antibiotics may be administered in severe cases. Tranquilizers, anesthetics, pudendal nerve block and/ or adequate restraints may be desirable in the application of treatment particularly in severe cases. Sexual rest during treatment and for sometime thereafter is required to avoid pain and promote recovery (Kerketta *et al*., 2014; Abba *et al*., 2014; Parma; 2016).

2.10.1.9 Phimosis (stenosis of prepucial orifice): Phimosis is the narrowing or stricture of the preputial orifice which prevents protrussion of the penis. This disease condition may be congenital or acquired (due to injuries, wounds and infections). The prognosis is guarded and depends upon the promptness in attendance to cases, extent of trauma and necrosis. Phimosis of congenital origin may be treated surgically by removing a wedge of preputial skin,fascia and mucosa, from just behind the ventral aspect of the preputial orifice. Thereafter, the mucosa and skin are then sutured together. Mild urine scalding may occur after surgery, as urine does not run away freely (Jubb and Kennedy, 1970; Abba *et al*., 2014; Parma, 2016).

2.10.1.10 Paraphimosis: This is the inability of the penis to retract back into the prepuce following protrusion. This may result from congenital oracquired strictures of the prepuce, paralysis ofthe penis (due to spinal diseases), swelling or oedema of the prepuce and occasionally, balanoposthitis and following copulation or spontaneous erection. Although

not strictly constituting paraphimosis, some coital injuries to the penis also prevent its return to the prepuce and thus, giving similar clinical signs. It may also occur when the preputial opening becomes constricted by a band of hair, thereby preventing return of the penis to the prepuce. In prolonged or neglected cases, the penis initially becomes oedematous, then swollen and inflamed, and suffers damage to its increasingly friable integument or may become strangulated within a relatively short space of time. The prognosis is guarded and depends upon the degree of trauma, necrosis and promptness of treatment. Treatment in mild cases consists of careful cleaning of the penis, removal of necrotic tissues or obstructive debris, application of cold packs to reduce small swellings, generous lubrication, liberal use of broad-spectrum antibiotic ointments and careful manipulation to return the penis back into the prepuce. In severe or chronic cases, the preputial orifice may need to be surgically enlarged before the penis can be replaced. The penis should be dressed or wrapped with guaze and replaced inside the prepucial sheath as soon as possible. Liberal use of only ointments or vaseline with daily withdrawal of penis should be done to prevent adhesions. Suspensory bandage can also be used to support prolapsed penis and sheath to minimize oedema. In long standing severe cases, amputation of the penis is recommended (Kerketta *et al*., 2014; Abba *et al*., 2014, Parma, 2016).

2.10.1.11 Strangulation and necrosis of the penis: This usually occur sequel toparaphimosis or as a result of constriction of thepenis by hair or maliciously placed objects. It ismost common in hairy buck goats. Similar to that of paraphimosis, the prognosis depends uponthe duration of the vascular constriction and thedegree of necrosis that has ensued. If gross necrosis of the penis has not occurred, healing is relatively good.However, prognosis is guarded, if strangulation is prolonged with pronounced ischaemia, there will be loss of function and impairment of ejaculation reflex. In severe or gross necrotic cases, amputation of the penis may be indicated (Jubb and Kennedy, 1970; Abba *et al*., 2014; Parma, 2016).

2.10.1.12 Tumour of the penis: Tumors of the penis and prepuce may be single or multiple and are firm and cauliflower like growths. Although not common, tumours can be found in intact and castrated bucks. Clinical effects vary according to the size andthe morphology of the lesions. Haemorrhage andulceration are the most common sequelae, which can result in

phimosis and paraphimosis. Prognosis is good especially if tumour is not multiple. Tumor can be removed surgically following tranquilizers and local anaesthesia. Topical and systemic antibiotics should be given after surgery. Treated bucks should be given sexual rest for quick recovery (Jubb and Kennedy, 1970; Abba *et al*., 2014).

2.10.2 Impotentia generandi

2.10.2.1 Conditions causing failure of ejaculation: Occasionally, ejaculation may not occur even after normal intromission and ejaculation. This may be as result of impaired ejaculation reflex and/or localized pain making the buck unwilling to ejaculate. The first condition generally occur when somedamage has occurred to the neural pathwaysbetween the glans penis and the spinal cord; also strangulation of the penis, with ensuing damageto the sensory dorsal nerve of the penis.Localised pain can also prevent ejaculation. This may arise from localised peritonitis in the caudal abdomenof the buck causing pain during the ejaculatorythrust and often making them willing tomount but less willing to ejaculate. Similar situation do occur in bucks withback pain, although they may beless willing to mount. Painful conditions of the penis particularly in cases of orf,may make a buck unwilling to achieve intromission and ejaculate, despite of an active libido (Jubb and Kennedy, 1970; Kerketta *et al*., 2014; Abba *et al*., 2014; Parma; 2016).

2.10.2.2 Conditions causing failure of fertilization: Some of the conditions causing failure of fertilization are often difficult to diagnose. The affected bucks may show normal libido and even copulate very well but may not be able to fertilize the does. The normal characteristics of such bucks' ejaculate may be reduced or absent. This may be associated with genetic (inherited sperm defects) or disease factors. These genetic factors may be difficult to trace or diagnose especially when source or histories of bucks are unknown. A number of disease conditions such as brucellosis, campylobacteriosis, trichomoniasis and trypanosomiasis have been reported to affect bucks' semen characteristics resulting in reduced or poor motility, morphology, concentration, color and volume. Occasionally, these disease conditions may not really affect the semen quality but may cause fertilization failure, early embryonic death, abortion and stillbirth. These disease conditions affect the reproductive organs resulting in testicular degeneration, orchitis, epididymitis, vesiculitis etc. These conditions can be diagnosed by careful examination of the external genitalia of

the bucks but at times may require semen analysis and confirmatory laboratory tests. However, it does occur that the fertilization failure may not even be related to the buck; this is because there might be ongoing identical infertility problems in the does which require a differential diagnosis. Hence does bred to suspect bucks should be thoroughly examined during BSE. Also, it is important to separate causes of fertilization failure that represent pathologies ofthe reproductive tract from causes that are non-pathological (Sundaraman *et al*., 2007; Parma, 2016).

2.10.2.3 Cryptorchidism: This is a common disease condition in WAD goat bucks. It occurs when there is incomplete or total lack of testicular descent. It could be unilateral or bilateral. A unilateral cryptorchid buck may still be able to fertilize with other descended testis. But in cases of bilateral cryptorchids, it is absolute sterility.The crytorchid buck may show some libido or sex drive as the level of testosterone is unaffected. Also, unskilled use of rubber rings for castrationcan result in one testis being forced back eitherinto the inguinal canal or, more commonly, into asubcutaneous position cranial to the scrotum. It is recommended that cryptorchid bucks should not be used for breeding even if they can perform (Igbokwe *et al*., 2009; Ozegbe, 2012).

2.10.2.4 Testicular degeneration: The testis (particularly the seminiferousepithelium) is highly susceptible to damage often resulting in reversible or irreversible testicular degeneration. Testicular damage may be due to raised intratesticular temperature, toxins, endocrine disturbances and infection. The effects of these causative agents are not often noticed immediately probably due to the protracted nature of spermatogenesis. There is therefore normally a lag interval, which may be of several weeks, between the time atwhich the testis is damaged and the time at which effects upon semen quality are first noted. Increased testicular temperature can be due to many factors one of which includes hot climatic condition. Excessive accumulation of fat deposits in the scrotum can also result in increased testicular temperature. Local infections, trauma, abscessation and inflammation to the scrotal skin or other structures in the scrotum can also raise the testicular temperature sufficiently to impair spermatogenesis. Any alterance or abnormalities of the testicular circulation, such as it occurs in varicocoele, perturbs the heat exchange mechanism responsible for maintaining the testis at a temperature below that of the body, again

resulting in temperature-dependent testicular degeneration. Toxic and heavy metal or radiation contamination can also cause testicular damage, but many other materials have been implicated at various times. Establishing causal relationships between such substances and infertility is particularly difficult, given the time interval between their ingestion and appearance of infertility. Stress-relateddegeneration occurs largely due to the inhibitionof LH secretion by the corticosteroids that are released during stress and management (Oyeyemi *et al*., 2011; Abba *et al*., 2014; Parma; 2016).

2.10.2.5 Orchitis and epididymitis.

Orchitis (inflammation of the testis) rangesfrom a mild infection of the testis, which is closely related to testicular degeneration, through togross suppurative or necrotic destruction of thetestis. It can arise from a primary infectionor by haematogenous spread of bacteria into thetestis superinfecting pre-existing traumatic viral orparasitic damage. It is often unilateral than bilateral and also involving the epididymis. In acute cases, the affected testis is inflamed or grossly enlarged (up to about two or three times its initial size), hot, hyperaemic and painful. The pain may be so serious to cause an altered gait. In chronic cases, the testis may become shrunken, fibrotic and adhered to the tunic and scrotum. If the orchitis is bilateral, theprognosis for future breeding is poor, and the affected bucks should be castrated. The causative microbial agents include *Brucella species, Mycoplasma species, Mycobacterium species* amongst many others. Epididymitis can also occur as a primary infection or by spread from an infected testis and vice-versa. The clinical signs of epididymitis are similar to those of orchitis. Depending on the severity, this condition can lead to total loss of function of the epididymis.Hence, unilateral epididymitis usually results in reduced fertility, whereas bilateral type may result in sterility. Similar to what occurs in cases of orchitis, unilateral epididymitis causes temperature-induced degeneration in the contralateral testis, so early removal of the affectedepididymis and its associated testis is highly recommended. In cases of bilateral epididymitis, treatment and culling of the bucks is better. This reducesthe incidence but does not eliminate the disease from the farm (Kerketta *et al*., 2014; Abba *et al*., 2014; Parma; 2016).

2.10.2.6 Testicular hypoplasia: Testicular hypoplasiais an incomplete development of the germinal epithelium of the seminiferous tubules, dueto inadequate numbers of germinal

cells withinthe testis. Lack of germinal cells may arise throughpartial or complete failure of the germinal cells todevelop in the yolk sac, failure to migrate to thegenital ridge, failure to multiply in the developinggonad, or widespread degeneration of embryonicgerminal cells within the primitive gonad. In mild cases, moderateoligospermia or poor sperm morphology may occur, but in severe cases the affected buck may be aspermic. The buck may still show signs of good libido because the leydig cells are usually not affected. This condition is mostly hereditary in nature. During, examination, it may be difficult to distinguish between testicular hypoplasia and testicular degeneration. For the diagnosis of this condition, measurement of scrotal circumference has been suggested, especially in breeds that have standards for scrotal circumference. Unfortunately, scrotal circumference standards are yet to be established in WAD goat bucks. Thorough palpation of both testes and semen analysis should be carried out. When these are not done, this condition may not be discovered on time until reduced pregnancy rate or even worse is observed. The use of hormones to correct testicular hypoplasia has been unsuccessful. Castration or slaughter policy for meat purposes can be recommended. It is better not to use affected bucks for breeding. Attempts attreatment with exogenous hormones are invariably unsuccessful, so castration and (for meatanimals) slaughter for recovery of the carcassvalue should be recommended(Jubb and Kennedy, 1970; Kerketta *et al*., 2014; Abba *et al*., 2014).

2.10.2.7 Testicular neoplasia (tumours): These conditions are not common in WAD goat bucks. But when they occur at all, it is usually during old age and they originate from interstitial cells, seroli cells and germinal epithelium. Large testicular neoplasiascause testicular degeneration either through their compressing effect or due to the excess steroid hormones produced by the interstitial cells/sertoli cell tumours (Jubb and Kennedy, 1970; Abba *et al*., 2014; Parma, 2016).

2.11 Ultrasonography

Ultrasonography (diagnostic ultrasound) is an imaging technique that uses high-frequency sound waves to produce images of body tissues or structures.It depends on the computerized analysis of reflected ultrasound waves, which non-invasively build up fine images of internal body structures. The resolutions attainable with these structures are higher with shorter wavelengths, with the wavelength being inversely proportional to the

frequency. However, the use of high frequencies is limited by their greater attenuation (loss of signal strength) in tissue and thus shorter depth of penetration.These imagesproduced, provide valuable information for diagnosing and treating a variety of disease conditions (Medan and Abd El-Aly, 2010).

Ultrasound is an image produced by ultrasonography. It is the term used to describe sound of frequencies above 20 000 Hertz (Hz), beyond the range of human hearing. Frequencies of 1–30 megahertz (MHz) are typical for diagnostic ultrasound; specifically, 3–5MHz for abdominal areas, 5–10 MHz for small and superficial parts and10–30 MHz for the skin or the eyes. This is done with the use of an ultrasound machine which comprises of the monitor (with a keyboard) and the transducer which comes in various designs or forms and frequencies (linear, convex, and rectal) depending on purpose and the type of body tissue or areas to be visualized. The transducers (probes) are made of thin discs of an artificial ceramic material (such as lead zirconate titanate) comprising of piezoelectric crystals. The thickness (usually 0.1–1 mm) determines the ultrasound frequency. The frequency of each transducer determines how deep it can penetrate structures. For instance, 3.5MHz, 5.0MHz and 7.5MHz tranducers can penetrate structures up to 17 – 20cm, 10 – 17cm and 5 – 7cm respectively. Invariably, the lower the frequency of the transducer, the deeper the penetration strength of the transducer.. The piezoelectric crystals are able to convert mechanical pressure (which causes alterations in their thickness) into electrical voltage on their surface (the piezoelectric effect). Conversely, voltage applied to the opposite sides of a piezoelectric material causes an alteration in its thickness (the indirect or reciprocal piezoelectric effect). If the applied electric voltage is alternating, it induces oscillations which aretransmitted as ultrasound waves into the surrounding medium. The piezoelectriccrystal, therefore, serves as a transducer, which converts electrical energy into mechanical energy and vice versa.Ultrasonography has been carried out in human and animals on the different parts and systems of the body (Medan and Abd El-Aty, 2010; Gilbert, 2014).

2.12 Testicular Ultrasound (Sonogram)

This is a test that uses reflected sound waves to show the picture of the testes, epididymis and scrotum. It is useful for checking for masses and swellings of the testes and

epididymides; testicular torsion and ischaemia; testicular cancer; undescended testis (unilateral or bilateral chrytorchidism); fluid in the scrotum (hydrocele); fluid in the epididymis (spermatocele); blood in the scrotum (hematocele); or pus in the scrotum (pyocele); guide a biopsy needle for testicular biopsy especially when checking for infertility; checking for testicular degeneration; traumatic injuries to the testis and other genitalia areas. Researches on testicular ultrasound in WAD bucks is scarce but it has been well documented in human and other various species of animals (Andrade *et al*., 2014; Carazo *et al*., 2014).

2.13 Recent studies on West African Dwarf bucks

Apart from the socio-economic importance of the WAD goat particularly the WAD buck, recent researches show that numerous works had been carried on WAD bucks. These also show their importance in livestock production as a whole as well as an experimental animal. Some of these research works on WAD bucks are reviewed.

2.13.1 Growth performance and Haematological studies on West African Dwarf goat bucks

A study was conducted by Amadi *et al*., (2015) in which haematological parameters of WAD bucks experimentally infected with *Trypanosoma vivax* and *Trypanosoma brucei* and response to treatment with Diminazene Aceturate were evaluated. Result showed that the WAD bucks are trypano tolerant. However, they concluded that although the effect of the parasite on the haematological features showed that anaemia was normocytic and normochronic for most periods. The intensity of the anaemia was related to the degree of parasitemia and in cases where the animals were infected adequate dietary measures and proper sanitation needed to be taken to ensure productivity was not hindered.

Opara *et al*., (2010) conducted an experiment in the South-eastern part of Nigeria in which haematological parameters and blood chemistry of WAD goats comprising of 30 adult bucks, 30 adult does, 56 buck-kids and 14 doe-kids were evaluated. They observed that male WAD goats had significantly higher lymphocytes, neutrophil and White Blood Cell (WBC) count than the females, while other parameters were similar. The values obtained for serum sodium, total protein and urea levels were 126.1 ± 2.2mmol / l, 5.2 ± 0.1g / dl and

37.9 ± 1.7mg / dl respectively, while the values obtained for serum enzymes such as Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline Phosphatase (ALP) were 6.7 ± 1.01 IU / l, 5.3 ± 0.7 IU / l and 63.2 ± 6.9 IU / l respectively. There was also a significantly higher percentage of PCV, Hb and RBC in female WAD goats than the males. The WBC, MCV and MCH were significantly higher in the male WAD goats. The percentage of lymphocytes was higher in male goats, while that for neutrophil was higher in the female goats. Age did not significantly influence the haematological parameters of the WAD, except for the significantly higher Neutrophils in the does than other groups. Eosinophil values were significantly higher in the WAD doe kids than other age groups examined. Serum sodium was higher in younger WAD goats, than the older ones.

A study was conducted by Ogunleke *et al*., (2014) on the performance and blood profile of WAD goat fed concentrate supplement containing varying levels of corncobs. They observed that bucks fed on 30% corncob inclusion had the highest dry matter intake, feed conversion ratio and weight gain. There werealso no significant differences in the haematological parameters of WAD goats across all the dietary treatments. It was concluded that inclusion of corncobs in the diets of WAD goats had no deleterious effects on the haematological and serum biochemical parameters and could therefore be included in ruminant diets up to 30%.

A comparative evaluation of haematological profile of WAD and red sokoto goats reared in humid Southeastern Nigeria by Obua *et al*., (2012) involving thirty goats, made up of fifteen WAD and fifteen Red Sokoto goats with each breed comprising of four adult male, four adult female, four young female and three young male goats. The age of the goats ranged from 6 months to 4 years.They observed that the haematological values of WAD and Red Sokoto goats did not differ significantly. Similarly, haematological values showed non-significant ($p>0.05$) age and sex differences and were therefore, similar in both adult and young and male and female goats, respectively. They concluded that the goats were averagely healthy and may have adapted to the study area.

2.13.2 Reproduction related studies on West African Dwarf bucks

A study was conducted by Oyeyemi *et al*., (2011) to verify the anabolic and androgenic effect of Mesterolone (Poviron®) using twelve WAD bucks that the testes were pre-insulated with cellophane bags and cotton wool for 30 days. Testicular degeration was observed post insulation which was evidenced by a significant reduction in motility. This was reversed and with improved motility following treatment with 100mg of Poviron®.

Bitto and Egbunike (2012) conducted a study to compare the semen characteristics of pubertal and adult WAD bucks. Pre-pubertal WAD bucks of 148 to 156 days were evaluated for puberty using the preputial smear technique, however age of puberty was not stated in this study. Semen was later collected from pubertal and adult bucks using the electroejaculation method. It was observed that adult bucks were significantly superior ($p < 0.05$) to pubertal bucks in semen volume, massactivity, sperm progressive motility, sperm concentration, live sperm, total sperm/ejaculate and normal spermmorphology. They concluded that pubertalbuck semen, though inferior in quantity and quality to that of the adult, may be sparingly used for AI, and thatgood sires may be selected at puberty on the basis of the physical characteristics of their semen.

Sperm morphological studies of four healthy WAD bucks of the age of twenty four to thirty months treated with pumpkin plant (*Cucurbita pepo*) was carried out by Oyeyemi *et al*., (2008). They observed that there were significant differences between the control and experimental values for both primary and secondary morphological spermatozoa abnormalities with the treated group having superior values. They concluded that the pumpkin plant should be used to treat and prevent infertility problems in male animals. However, they recommended that further works on the plant could result in the extraction its essential elements that could be of great resource in the pharmaceutical industry.

A study on the effect of successive ejaculations on the spermiogram was carried out byOyeyemi *et al.*, (2001) on twenty WAD bucks divided into four groups comprising of five bucks per group. Semen was collected once and twice a week for a period of eight weeks for group A and B respectively; once a day for 21 days in group C, and twice daily at an interval of five hours for 21 days in group D. Live body masses, height at withers, scrotal length and scrotal circumference were not affected by successive ejaculations across the groups. Also, the ejaculate colour , mass activity, motility and percentage live

spermatozoa were not significantly different. However, the ejaculate volume decreased as the frequency of ejaculations increased, although the decrease was not significant. There was a significant increase in abnormal spermatozoa as the frequency of ejaculation increased.

Semen characteristics and sperm morphological studies of the West African Dwarf Buck treated with Aloe vera gel extract was also carried out by Oyeyemi *et al*., (2011). It was observed that the continuous administration of *Aloe vera* extract significantly reduced sperm motility, concentration and percentage livability and increased sperm abnormalities in WAD bucks. They concluded that *Aloe vera* adversely affected the spermiogram of bucks and that the plant can reduce fertility in male animals and is therefore not recommended for medicinal purpose in male animals especially those used for breeding.

In a review Article by Etim (2015) on testicular and epididymal morphometric characteristics as viable indicators of reproductive ability of farm animals, it was stated that testicular and epididymal development is influenced by several factors among which are species, breed, body weight, age, nutrition; and that a positive relationship exists between semen quality and testicular dimension, giving an indication that improvement in one would lead to improvement in the other. However, in a bid to increase and improve animal production, the testicular and epididymal morphometry is essential for a maximum and rational utilization of the breeding stock.

A study was carried out to evaluate the effect of guava leaf as feed on the scrotal and testicular characteristics of WAD bucks by Abu *et al*., (2016). It was observed that testicular and epididymal parameters of the bucks fed guava leaf meal at 10% inclusion were significantly higher than the control. It was concluded that this non-conventional feedstuff might be useful in improving spermatogenesis and enhancing sperm production.

2.13.3 Need for further research on West African Dwarf bucks

Based on literature review, there is need for more research works on WAD bucks particularly in the area of reproduction and more specifically in the area of standardizing the BSE programme of these invaluable sources of protein animals. In doing this, the gaps such as that of no current standard for SC in WAD bucks which would be useful in the

assessment of puberty and onset of OBA which are essential components of a successful reproduction management in any species of animal. Also studies in the areas of morphology and morphometry of the testes and epididymides which are the very important organs of reproduction. As well as studies in the area of TU as a non-invasive diagnostic and biometric tool which can be introduced into the BSE programmes of WAD bucks (Chukwuka *et al*., 2010).

CHAPTER THREE

3.0 SCROTAL CIRCUMFERENCE AS A DETERMINANT OF OPTIMUM BREEDING AGE INTHE WEST AFRICAN DWARF BUCKS.

3.1 INTRODUCTION

The buck plays significant roles in West African Dwarf (WAD) goat reproduction. It can sire many does on the farm (Nolte, 2012). Also, it can detect estrus in WAD does (Abebe, 2008). However, the quality of these capabilities is relative to and largely depends on the age at puberty and the Optimum Breeding Age (OBA).

Puberty is the first appearance of matured spermatozoa in the ejaculate (Osinowo and Williams, 2008).It is the age at which the buck becomes capable of reproduction. However, during early puberty, much energy is directed towards enhancing and increasing the functionality of the testis; thereby affecting other body development; and also making the bucks more susceptible to health problems (Henkel, 2015).

The OBA is the age at which the buck attains full sexual maturity (Abebe, 2008); and most importantly, the testes and epididymis are fully developed and functional (Osinowo and Williams, 2008). The OBA represents the best time to start using the buck for breeding because at OBA, energy is directed basically towards spermatogenesis (Henkel, 2015).Also the rate of matured spermatozoa replacement after depletion is faster at OBA (Kinne, 2001). There is increase in the number of does the buck can serve and fertilize successfully especially via Artificial Insemination (AI)(Noble, 2004; Arrebola *et al*., 2012); and thus increase in litter sizes (Suckow *et al*., 2005). Also, the bucks are less prone to health problems (Henkel, 2015).The OBA is determined by an assessment of reproductive indices which are indicatives of the functions of the reproductive organs which are evaluated during Breeding Soundness Examination (BSE).

The BSE of WAD bucks is valuable and represents the most practical and economic tool with which to select potentially excellent sires for breeding (Petherick, 2005). It involves general physical examination, palpation of external genitalia, measurement of the Scrotal Circumference (SC), semen collection and analysis.

An important but relatively easy parameter that does not require serious expertise to be taken during BSE of bucks especially on the farm is the SC (Pezzanite *et al.*, 2013). Research has shown that SC is a significant correlate of fertility and is highly related to improved semen quality and reproductive soundness in bucks (Bongso*et al.*, 1982; Bezerra*et al.*, 2009). Bucks with larger SC, that is, bigger testicles have been reported to sire does with relatively better results (Raji*et al.*, 2008). However, there are closely related factors such as age and body weight that significantly affect SC in bucks (Rege*et al.*, 2000; Karakus*et al.*, 2010). Shoyombo*et al.*, (2012) reported increases in SC with age and that SC can be influenced by BW in Savanah brown bucks. Presently in Nigeria, there are no guidelines for evaluating SC of WAD bucks for BSE purposes. Also, its relation to puberty and particularly the OBA is yet to be determined in the WAD bucks. Therefore, this study was carried out to evaluate the value of SC (in correlation with age, BW, BS and semen production) as a determinant of the OBA in WAD bucks.

3.2.0 MATERIALS AND METHODS

3.2.1 Study design

3.2.2 Phase 1:

A field study involving 450 semi-intensively raised WAD bucks by small scale farm holders was conducted from October 2012 to October 2013 during the dry and raining seasons. The bucks studied (one to fifteen months old) were grouped into 15 comprising of 30 bucks per group, for the determination of BS, BW and SC. Fifteen bucks were studied per group for the dry and rainy seasons.

3.2.3 Study Areas

This study was carried out in five different Local Government (LG) areas in Abia State, South-eastern Zone of Nigeria, namely: Ikwuano LG, Umuahia North LG, Umuahia South

LG, Umu Nneochi and Osisioma LG. Abia State has a latitude-longitude coordinates of 5.4309ºN, 7.5247 ºE. It has usually, a rainy season between the months of March and October; and a dry season between the months of November and February (Akpanta *et al*., 2015).

3.2.4 Parameters studied

The parameters studied included:

3.2.4.1 Age: The bucks' ages were determined by records and history from the owners and by dentition using the method specified by Wosu (2002).

3.2.4.2 Bodyweights: These were measured by using bathroom scale (Camry®). The procedure was such that an individual carried the buck, stood on the bathroom weighing scale, then the total weight was recorded. Thereafter, the individual's weight was then deducted from this weight after putting the buck down. This gave the actual weight of the buck.

(Individual BW + Buck BW) – Individual BW = Buck BW.

3.2.4.3 BodyScore (BS): This was evaluated subjectively as described by Ford *et al*., (2009) and Okere *et al*.,(2011). The scores ranged from 1 = emaciated to 5 = obese.

3.2.4.4 ScrotalCircumference: The largest diameter of the scrotum using a flexible tape was measured while ensuring the testes lie side by side to each other. Before measuring SC, scrotal shape, scrotal anatomy, scrotal content and testicular consistency were examined. Bucks with ovoid and long ovoid scrota which are undivided and split were considered normal. Scrotal content were palpated to observe freely moving testes. Testicular consistency were scored as 1 - very soft, 2 - soft, 3 - normal, 4 - hard and 5 - very hard (Keith *et al.,* 2009; Philip and Okere 2011).

3.2.5 Phase II

3.2.6 Semen collection

Semen samples were collected twice from 6 randomly selected bucks per group for dry and rainy seasons by the electro-ejaculation method. The prepucial hairs of the bucks were clipped using a sharp sterile scissors and then cleaned gently with tissue paper. Vaseline (lubricant) was applied on the probe of the battery operated electro-ejaculator. The bucks were then restrained and the hind limbs were elevated from the ground at an angle of 45° and the lubricated probe was inserted into the rectum touching the mucous membrane. Stimulation was done by alternately increasing and decreasing the voltage until full erection and ejaculation was achieved. The ejaculate was then collected into a clean sterilized warm graduated semen collection vial through a funnel with a handle brought over the glans penis around the prepucial area (Bitto *et al*., 2000; Bitto and Egbunike, 2012).

3.2.7 Semen analysis

The semen characteristics analyzed were as follows:

3.2.7.1 Color: The appearance or color of the semen collected were observed and recorded (Ajala *et al*., 2009).

3.2.7.2 Volume: The volume of the semen was observed avoiding error due to parallax and recorded(Zemjanis, 1970; Bitto *et al*., 2000).

3.2.7.3 Mass activity: This was done by putting a drop of semen on a warm slide (at 37^oC) using a pipette, covered with cover slip and then observed under microscope. Scoring was as follows: 0 – immotile sperm cells and no wave motion (very poor semen); 1 – weak oscillatory sperm cells movements with no wave motion (poor semen); 2 – less than 50% progressively motile or oscillatory sperm cells movements with little or no wave motion (fair semen); 3 – more than 50% progressively motile sperm cells with apparent wave motions and eddies (good semen); 4 – 80% and above progressively motile sperm cells with distinct dark wave motion and eddies (very good semen); 5 – About 100% very vigorous progressively motile sperm cells with distinctly dark and extremely rapid wave motion and eddies (Zemjanis, 1970; Bitto *et al*., 2000).

3.2.7.4 Motility: To study this, a drop of warm normal saline was added to semen on a warm slide, covered with cover slip and observed under microscope. This was scored in percentage(Zemjanis, 1970; Bitto *et al*., 2000).

3.2.7.5 Live-dead ratio/Livability: One to two drops of warm eosin-nigrosin stain was added to semen on warm slide, mixed gently using the edge of a slide, then a smear was made with the slide at an angle of 45 degrees on another slide, air dried and observed under microscope. Scoring was in percentage(Zemjanis, 1970; Bitto *et al*., 2000).

3.2.7.6 Percentage normal Morphology: The same process as described for live-dead ratio but Wells and Awa stain was used in this case. This was also scored in percentage(Zemjanis, 1970; Bitto *et al*., 2000).

3.2.7.7 Concentration: This was determined by the use of haemocytometer which contained improved Neuber counting chamber with 25 squares and a red blood cell pipette. Semen was sucked up to 0.5ml mark and then filled up to 1.01 mark with formal saline on the pipette. This mixture was shaken gently and the formal saline at the top of pipette was discarded. A cover slip was fixed on the counting chamber and then filled up with the diluted semen carefully. Sperm cells in 5 squares were counted diagonally (ensuring sperm heads were within the square), added up, multiplied by 5, then by 10,000,000 and the dilution factor (Zemjanis, 1970; Bitto *et al*., 2000).

3.2.8 Statistical analysis

Selected characteristic measures in this study were analyzed using Analysis of variance and Pearson Product Moment Correlation (PPMC) at 5% level of significance ($\alpha_{0.05}$) using SPSS version 20.

3.3.0 RESULTS

There were positive correlations amongst the parameters studied (Table 3.1). The relationship between SC and age was strong (r = 0.8), older bucks had bigger SC compared with younger bucks; while that of SC and BW was not as strong (r = 0.6) compared with that of SC and age. However, the positive correlation between SC and BS was very weak and not significant (r = 0.05). Age had a very strong relationship with BW (r = 0.9), heavier

weights were observed in older bucks. BW had a strong relationship with BS; heavier bucks had higher BS (r = 0.8).Results also revealed that there were no significant differences amongst the parameters studied during the rainy and dry seasons at $\alpha_{0.05}$ (Figure 3.1). However, SC [$F_{1, 29}$ = 7678.8] significantly increased from one month (7.9±0.1 cm) to eight months (17.6±0.2 cm) without further significant increase up to 15 months.The SC of the dry season bucks were higher than those of the rainy season bucks but the differences were not significant ($\alpha_{0.05}$). It was also observed that BW[$F_{1, 29}$ = 11372.8] significantly increased from one month (3.4±0.1 kg) to fifteenmonths (13.6±0.1 kg); while BS was relatively constant in all the age groups with an average value of 3.2 ± 0.5 (Table 3.1 and Figure 3.1). However, the BW of the rainy season bucks were higher than those of the dry season bucks but the differences were not significant ($\alpha_{0.05}$). The results of the semen analysis of selected bucks in the groups for the dry and rainy seasons are as presented on Tables3.2, 3.3 and 3.4 respectively. There were no evidences of spermatozoa in the ejaculate of WAD bucks of one to five months. The first evidence of matured spermatozoa in the ejaculate was observed in the 6 months old WAD bucks. The spermiogram showed that volume, motility, morphology, livability and concentration increased significantly ($F_{1,5}$= 42561.3, 21774.2, 35182.1, 25146.2 and 14139.4, respectively) from six months through to eight months. The semen characteristics of the eight months WAD bucks were not significantly different from those of the nine to fifteen months old WAD bucks ($\alpha_{0.05}$).

Table 3.1: Correlation of scrotal circumference, age, body weight and body condition score of bucks for each age group.

	Parameters	N	Mean±SEM	1	2	3	4
1	Scrotal circumference (cm)	450	14.8± 2.9	-	0.81*	0.63*	0.05
2	Age	450	8.0 ± 5.5		-	0.89*	0.21*
3	Body weight (Kg)	450	9.1± 3.7			-	0.78*
4	Body Score	450	3.2± 0.5				-

** Significant at 0.05 (2-tailed)*

N – number of bucks; SEM - standard error of mean

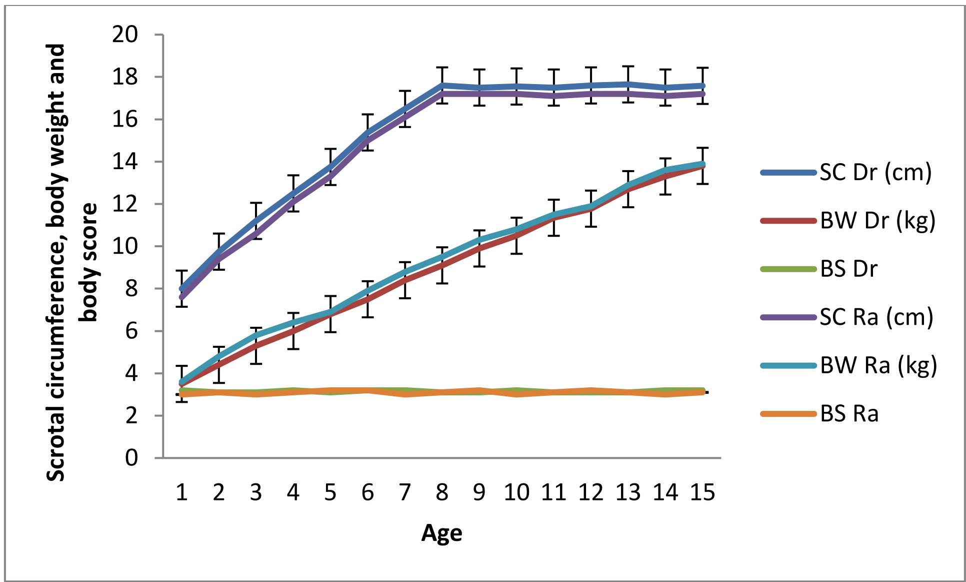


Figure 3.1: The trend for SC, BW and BCS for WAD bucks for each age group during the rainy and dry seasons.

Table 3.2: Semen characteristics of West African Dwarf bucks for each age group during the dry Season expressed in Mean ± Standard Deviation.

Age	N	Color	Volume (cm^3)	Mass activiy (%)	Motility	%Normal Morphology(%)	%Live spermatozoa (%)	Concentration (Cells/mL)
1	6	-	-	-	-	-	-	-
2	6	-	-	-	-	-	-	-
3	6	-	-	-	-	-	-	-
4	6	-	-	-	-	-	-	-
5	6	-	-	-	-	-	-	-
6	6	Milky	$0.16{\pm}0.07^{*fh}$	4	$86{\pm}4.16^{*fh}$	$87{\pm}2.83^{*fh}$	$88{\pm}3.61^{*fh}$	$1.74{\pm}0.04^{*fh}$
7	6	Milky	0.26±0.04	4	90±5.12	89±3.16	90±4.03	1.90±0.06
8	6	Creamy	$0.34{\pm}0.06^{*hf}$	5	$93{\pm}5.08^{*hf}$	$92{\pm}3.48^{*hf}$	$93{\pm}4.60^{*hf}$	$2.07{\pm}0.15^{*hf}$
9	6	Creamy	0.29±0.03	5	92±4.71	93±2.45	95±3.72	2.10±0.09
10	6	Creamy	0.32±0.05	5	94±5.16	91±3.09	92±4.27	2.05±0.13
11	6	Creamy	0.36±0.02	5	93±4.28	94±2.56	94±2.95	1.97±0.10
12	6	Creamy	0.29±0.08	5	92±3.94	92±4.01	93±3.52	2.02±0.07
13	6	Creamy	0.32±0.04	5	91±5.26	92±3.81	91±4.38	1.94±0.17
14	6	Creamy	0.37±0.02	5	94±3.72	93±2.60	93±4.12	2.01±0.11
15	6	Creamy	0.33±0.05	5	91±5.02	91±3.54	92±3.26	1.98±0.15

* *Significant at 0.05*; f, h are rows; N – number of bucks

Table 3.3: Semen characteristics of West African Dwarf bucks for each age group during the rainy season expressed in Mean ± Standard Deviation

Age	N	Color	Volume (cm^3)	Mass activity (%)	Motility (%)	%Normal Morphology (%)	%Live spermatozoa (%)	Concentration (Cells/mL)
1	6	-	-	-	-	-	-	-
2	6	-	-	-	-	-	-	-
3	6	-	-	-	-	-	-	-
4	6	-	-	-	-	-	-	-
5	6	-	-	-	-	-	-	-
6	6	Milky	$0.15 \pm 0.08^{*fh}$	4	$83 \pm 3.69^{*fh}$	$86 \pm 2.71^{*fh}$	$87 \pm 3.72^{*fh}$	$1.72 \pm 0.03^{*fh}$
7	6	Milky	0.24±0.03	4	89±4.93	88±4.06	90±3.92	1.87±0.04
8	6	Creamy	$0.32 \pm 0.08^{*hf}$	5	$93 \pm 4.65^{*hf}$	$91 \pm 3.27^{*hf}$	$92 \pm 4.51^{*hf}$	$2.06 \pm 0.12^{*hf}$
9	6	Creamy	0.28±0.07	5	92±3.98	93±2.26	94±3.54	2.07±0.08
10	6	Creamy	0.31±0.06	5	93±4.59	91±2.71	92±3.05	2.03±0.10
11	6	Creamy	0.30±0.05	5	92±3.81	92±2.36	93±2.82	1.98±0.09
12	6	Creamy	0.29±0.06	5	91±4.03	92±3.92	92±3.41	2.03±0.05
13	6	Creamy	0.31±0.02	5	91±3.74	91±3.68	91±3.92	1.95±0.06
14	6	Creamy	0.33±0.07	5	92±3.47	92±2.71	92±3.88	2.00±0.07
15	6	Creamy	0.31±0.08	5	91±4.63	91±3.40	91±3.23	1.99±0.06

* *Significant at 0.05*; f, h - are rows; N – number of bucks.

Table 3.4: Average Semen characteristics of WAD bucks for each age group for the dry andrainy seasons expressed in Mean ± Standard Deviation.

Age	N	Color	Volume (cm^3)	Mass activiy (%)	Motility (%)	%Normal Morphology (%)	%Live spermatoza (%)	Concentration (Cells/mL)
1	6	-	-	-	-	-	-	-
2	6	-	-	-	-	-	-	-
3	6	-	-	-	-	-	-	-
4	6	-	-	-	-	-	-	-
5	6	-	-	-	-	-	-	-
6	6	Milky	$0.16 \pm 0.08^{*fh}$	4	$85 \pm 4.03^{*fh}$	$87 \pm 2.72^{*fh}$	$88 \pm 3.67^{*fh}$	$1.73 \pm 0.03^{*fh}$
7	6	Milky	0.26±0.04	4	90±5.12	89±3.16	90±4.03	1.90±0.06
8	6	Creamy	$0.33 \pm 0.07^{*hf}$	5	$93 \pm 5.01^{*hf}$	$92 \pm 3.38^{*hf}$	$93 \pm 4.56^{*hf}$	$2.07 \pm 0.14^{*hf}$
9	6	Creamy	0.29±0.03	5	92±4.71	93±2.45	95±3.72	2.10±0.09
10	6	Creamy	0.32±0.05	5	94±5.16	91±3.09	92±4.27	2.05±0.13
11	6	Creamy	0.36±0.02	5	93±4.28	94±2.56	94±2.95	1.97±0.10
12	6	Creamy	0.29±0.08	5	92±3.94	92±4.01	93±3.52	2.02±0.07
13	6	Creamy	0.32±0.04	5	91±5.26	92±3.81	91±4.38	1.94±0.17
14	6	Creamy	0.37±0.02	5	94±3.72	93±2.60	93±4.12	2.01±0.11
15	6	Creamy	0.33±0.05	5	91±5.02	91±3.54	92±3.26	1.98±0.15

* *Significant at 0.05 (2-tailed)*; f, h are rows; N – number of buck

3.4.0 DISCUSSION

In this study, the scrotal circumferences (SC) of the WAD bucks were significantly correlated with age and BW. SC increased with age, especially between one and eight months. However, there was no further significant increase between eight to fifteen months when SC value was between 17 – 18cm, average of 17.6 ± 0.2 cm. There was no significance difference between SC of bucks taken during dry and rainy seasons accross the groups. Although, the SC of the bucks taken during dry season were slightly higher than those taken during the rainy season especially amongst the older groups of bucks. This could be attributed to the fact that the bucks of the dry season were given birth to in the rainy season, hence they had more access to food for their general body growth and testicular development. Secondly, during the dry season, the scrotum expands in order to maintain the testicular temperature which is required to be between 2 to 5°C below the body temperature for normal functioning.Also, BW increased with age from one to fifteen months. At eight months, bucks of at least 9.1 ± 3.7 kgBW had SC of 17.6 ± 0.2 cm. This is similar to the report of Shoyombo *et al.,* (2012) in Savanah brown bucks that SC can be influenced by age and BW. BS was highly correlated to BW. Although, the BW of the rainy season bucks were slightly higher than those of the dry season, however the differences were not significant. This could be attributed to access and availability of food during the rainy season as stated earlier. The bucks' BS were consistent with their BW showing that they were in good health conditions (Akpa *et al*., 2013). It was also observed that there were no spermatozoa in the ejaculate of the youngest groups of bucks' i.e age one to five months. The colorsof the ejaculates were also clear. The semen characteristics of the bucks in these groups were similar to those reported by Daramola *et al*., (2006) for immature WAD bucks. The first matured spermatozoa were observed in the six months old bucks; the ejaculates were milky in color. Since Puberty is defined as the first appearance of matured spermatozoa in the ejaculate (Osinowo and Williams, 2008); this suggests that the age at Puberty in the WAD bucks is 6 months. However, the SC was still increasing indicating that full reproductive maturity was yet to be attained. This is in corroboration with the fact that the best semen characteristics were observed starting from the 8 months group of bucks i.e the age at which the SC peaked. The semen characteristics of this group of bucks were similar to those earlier reported in sexually matured WAD bucks with full

fertilizing capacity (Oyeyemi *et al*., 2011; Jerimaiah and Osuagwuh, 2014). The color of the semen of these bucks was cream. The mass activity of the sperm cells also showed gross progressive motility and high concentration with distinct dark rapid wave motions and eddies. These findings suggest that 8 months is the OBA in WAD bucks. OBA is the age at which the bucks attain full sexual maturity and the reproductive organs (most importantly the testes and epididymides) are fully developed and functional (Osinowo and Williams, 2008). The OBA is the best age to introduce the bucks for breeding. At this age, much energy is directed towards spermatogenesis and the rate of matured spermatozoa replacement after depletion is faster; There is increase in the number of does the buck can serve and fertilize successfully especially via artificial insemination and leading to increase litter sizes (Kinne, 2001; Noble, 2004; Suckow *et al*., 2005).

The findings in this study corroborate with earlier reports that SC is an important correlate of semen production (Bezerra *et al*., 2009). However, this study further highlighted the value of SC as a parameter that could be used for determining OBA particularly in correlation with the quality of semen production. This is in relation with earlier reports that the size of testes (i.e SC), determines the amount of sperm-producing tissue (Ugwu, 2009). Bucks with bigger testicles can sire does at younger age (Raji *et al*., 2008). Increase in testicular size was also reported as an indicator of onset of spermatogenesis and that SC is highly related to semen quality and reproductive soundness (Bongso *et al*., 1982). This study has been able to relate SC to puberty (i.e onset of spermatogenesis) and the OBA (i.e age at which maximum output of spermatogenesis commences) in WAD bucks. The findings of this study suggest that puberty occurred at about six months while the OBA is eight months in WAD bucks. Hence, suggesting 8 months to be the best age to introduce WAD bucks for breeding(with maximum output expectations) on the farm.

In conclusion, SC (in correlation with BW, BCS and most importantly, the quality of semen production) was valuable in determining the optimum breeding age which is 8 months in WAD bucks. Hence, it is recommended that WAD bucks of 8 months with at least SC of 17.6 ± 0.2 cm) and BW of9.1 ± 3.7 kg and a corresponding BS of 3.2 ± 0.5can be used to breed does successfully under the semi-intensive system of production. In addition, improved selection index based on fertility rates should be carried out to confirm data

obtained in this study. This will fill the missing gap in BSE examination of WAD bucks which is an integral part of WAD goat production. Adoption and use of these findings will lead to improved goat production and greater socio- economic gains not only to farmers but also to other stakeholders in goat production in Nigeria and elsewhere in the world.

CHAPTER FOUR

4.0 TESTICULAR ULTRASOUND AS A TOOL FOR BREEDING SOUNDNESS EXAMINATION AND DETERMINANT OF OPTIMUM BREEDING AGE IN THE WEST AFRICAN DWARF BUCK.

4.1 INTRODUCTION

Breeding Soundness Examination (BSE) is a simple technique that is used to predict the potential fertility of West African Dwarf (WAD) goats. It is particularly more important in the buck because at least five does can be bred by one buck successfully under natural mating system (Nolte, 2012). The ratio is even many folds higher in Artifitial Insemination (AI) programmes (Arrebola *et al*., 2012; Ajala *et al*., 2013).

The BSE includes the examination for physical soundness, testicular consistency and size, semen quality and mating ability (Pezzante *et al*., 2013). During this examination in WAD bucks, particular attention is given to the testisas it is the site of production of sperm cells and testosterone. The sperm cells are important for the fertilization of the ova from the doe while testosterone is responsible for the production of sperm cells and other major WAD buck characteristics required for efficient reproduction (Udeh and Oghenesode, 2011; Daramola *et al*., 2006).

However, in the routine BSE, the internal structures and condition of the testes cannot be visualized except when opened up. But this can be resolved with use of testicular ultrasonography (TU). The TU or sonogram uses reflected sound waves to produce images of the testes, epididymis and scrotum. It is a safe, painless and non- invasive procedure. It does not use x-rays or other radiations and no side effects have been reported so far. The TU technique involves the use of a probe, called a transducer, to send sound waves into the testicular tissues. Reflective structures are referred to as being echogenic while the non-

reflective ones are referred to as anechoic. Highly reflective structures are termed hyper-echoic while structures with low reflections are referred to as hypo-echoic (Gilbert, 2014). With this, the characteristic features of the testes and other associated structures like the epididymis can be viewed on the monitor and abnormalities can be easily detected and correction can be duly made.

Studieson TU had been carried out in cattle (Yimer *et al*., 2011), camel (Pasha *et al*., 2011), sheep (Andrade *et al*., 2014), foreign breeds of goats (Ali *et al*., 2011) and dogs (Camara *et al*., 2014), however, TUon WAD goat bucks is yet to be fully established. Also, works on the use of TU to take biometric parameters like testicular width, height, length and volume is yet to be fully explored on WAD bucks. Some of these parameters are significant and have been reported to be important correlates of fertility.

Testicular volume (TV) is an index of spermatogenesis. This is because ninety eight percent of testicular volume is made up of seminiferous tubules which are responsible for spermatogenesis (Kollin *et al*., 2006). Slight or significant increase or decrease in TV above or below the normal level, could be an indicator of disease conditions. This study was therefore carried out to evaluate the value of TU as a BSE tool and in taking some important biometric parameters of the testes with particular emphasis on TV especially in relation to the OBA; and its possible introduction into the BSE programme of WAD buck goats.

4.2.0 MATERIALS AND METHODS

4.2.1 Study area

This study was carried out at the Department of Surgery and Theriogenology goat unit, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, during the dry and rainy seasons.

4.2.2 Animals

One hundred and eighty healthy WAD bucks of one to fifteen months (as in study 1) comprising of six bucks per group for the dry and rainy seasons were used for this study. The bucks were acquired from small scale farmers periodically in groups according to age

groups i.e one to fifteen months age group as described in study one. The bucks were disposed i.e sold after the completion of all studies, periodically according to age groups.

4.2.3Testicular Ultrasound Protocol and Biometrics.

In carrying out the TU, each buck was restrained by an assistant holding the animal firmly with the two hind limbs separated such that the testes were freely hanging caudal to but at a distance of about 10 cm away from the Ultrasound Machine (UM). The testes were thoroughly cleaned with tissue paper before Ultrasound Gel (UG) was applied generously covering the entire testicular surface. The testes of the WAD buck is only covered by a thin layer of hair, hence there was no need for shaving. The UM was connected to a stabilizer which was fixed to a source of light. The convex shaped transducer was then connected carefully to the monitor and switched on to a single B – mode, real time ultrasound scanner at a frequency of 7.5 MHz. The UG was also applied on the probe covering the entire surface and then pressed gently on the surface of the testes and the images produced on the monitor were viewed and recorded. The TU protocol for the bucks was by viewing on the Transverse Planes (TP) for the right testis and the left testis; and on Longitudinal Planes (LP) for the right testis and the left testis. The testicular parameters measured were: length, height and width of each testis. The Length of the testis (L) was taken on the LP and was measured from the most cranial point on the testis (right or left depending of the testis to be measured) to the most caudal point of the testis using the electronic caliper (Figure 4.1). The Height of the testis (H) was measured by placing the electronic caliper on the widest ventro-dorsal points on the testis on the LP (Figure 4.1); while the Width of the testis (W) was measured by placing the electronic caliper at the widest diameter on the TP (Figure 4.2) (Gilbert, 2014). The volume of each testis for the different age groups was then calculated using three different formulae namely the Prolate Ellipsoid Formula (PEF) - $L \times H \times W \times 0.52\ cm^3$, Prolate Spheroid Formula (PSF) - $L \times W^2 \times 0.52\ cm^3$and the Lambert Formula (LF) - $L \times H \times W \times 0.71\ cm^3$. The true TV using the water displacement method (WDM) as described by Mbaeri *et al*., (2012) was also determined for each age group and then contrasted with the TV obtained using the PEF, PSF and LF. The parameters taken were recorded and analyzed (Sotos and Tokar, 2012). The UM used for this study was

Biocare Ultrasonic Diagnostic Equipment (Model: BU - 907). Images were displayed on grey scale.

4.2.4 Statistical analysis

The data obtained in this study were analyzed using Analysis of variance (ANOVA) at 5% level of significance using SPSS version 20.

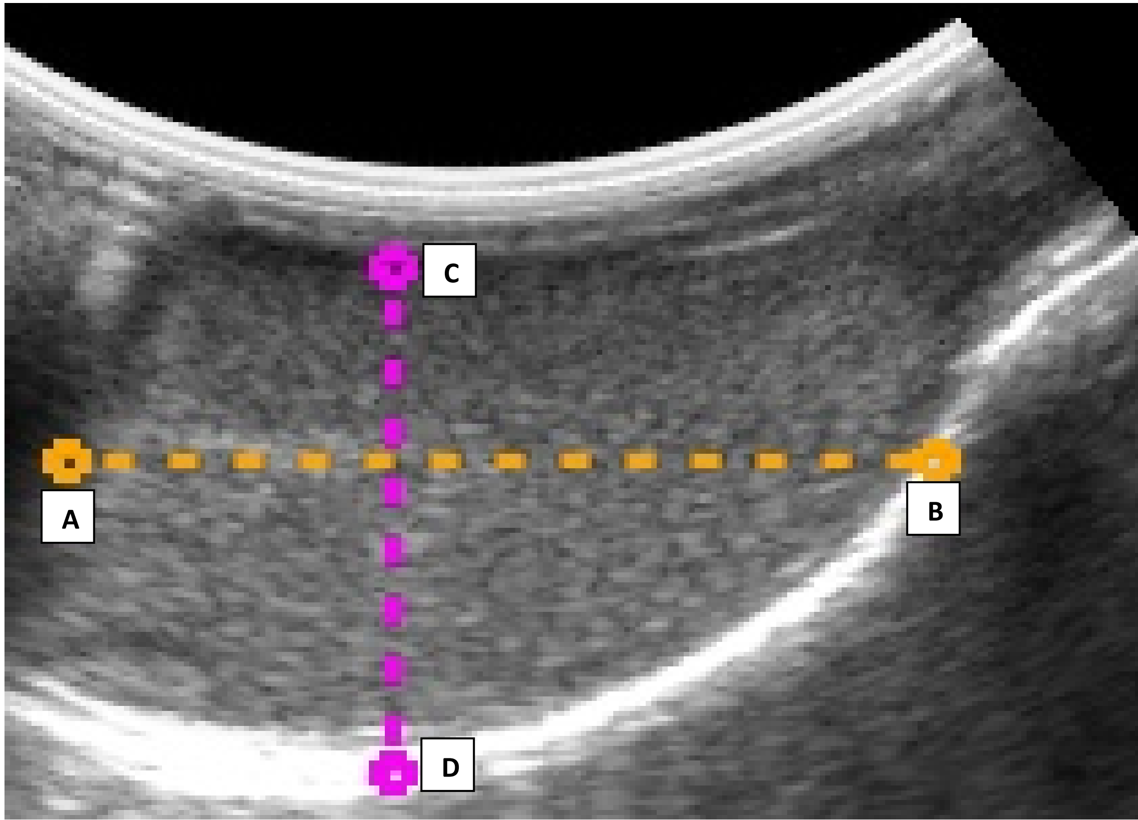


Figure 4.1: Sonogram showing the length (A-B) and height (C-D) of the right testes on longitudinal plane.

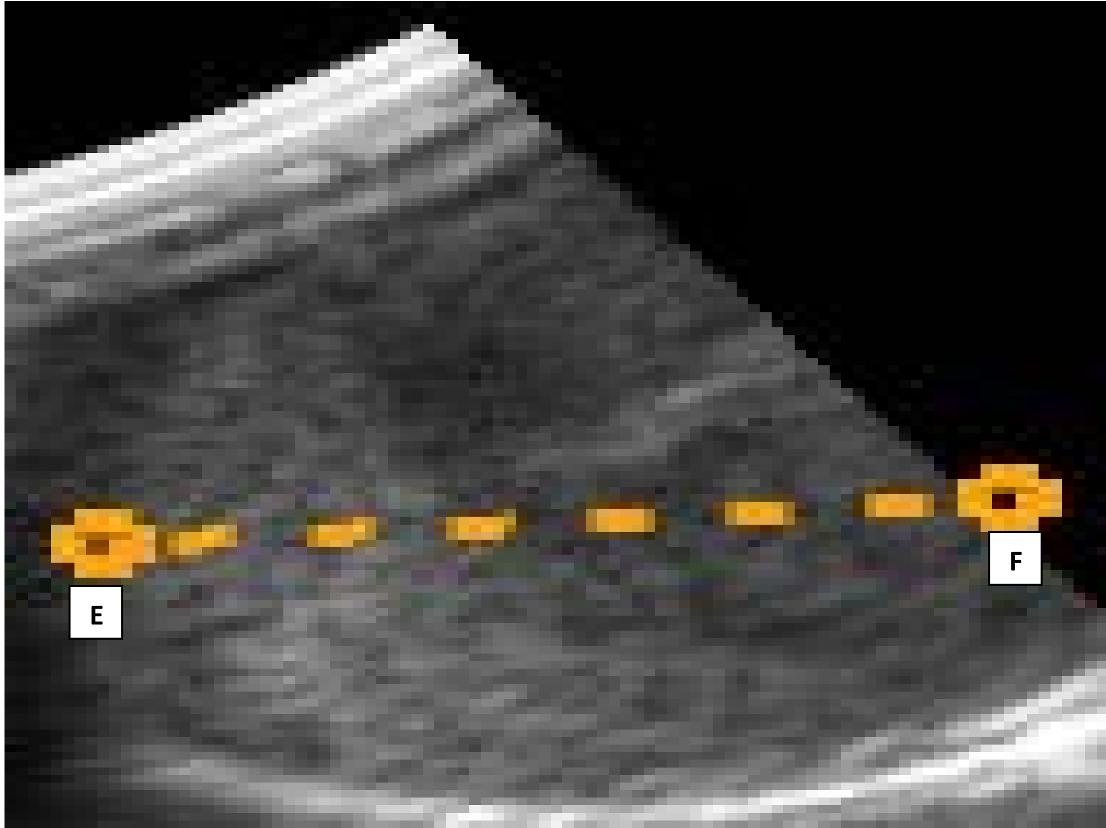


Figure 4.2: Sonogram showing the width of testis (E-F) on transverse view.

4.3.0Results

Across all the age groups, TU revealed that on the TP and LP for the right and left testes, testicular parenchyma appeared homogenously greyish while the mediastinum testes appeared as a white hyper-echoic thin line in the mid-section of the testes. The scrotal wall appeared as a thick hyper-echoic semi-circular layer forming a border line at the caudo-dorsal portion of each testis on the TP (Figure 4.3). The head of the epididymis of the right testis appeared as a small roundish dark hypo-echoic structure on the caudo-ventral part of the testis on the LP (Figure 4.4); while the head of the epididymis for the left testis appeared similar to that of the right testis but on the cranio-ventral portion of the testis also on the LP (Figure 4.5). The results for the TV obtained using the PEF, PSF, LF and WDM for the different age groups are as presented on Tables 4.1, 4.2 and 4.3. The TV obtained ultrasonographically using PEF, PSF and LF significantly increased [$F_{1,5}$=113183.9, 4430.1; 17656.2 respectively] from one month (16.1±0.1, 23.6±1.7; 22.0±0.1 cm^3 respectively) to eight months (28.3±0.2, 40.0±0.3; 38.6±0.3 cm^3 respectively) without further significant increase up to 15 months. The TV obtained by WDM significantly increased [$F_{1,5}$=13234.2] from one month (16.2±0.1cm^3) to eight months (28.5±0.3 cm^3) without further significant increase up to 15 months. Comparison of TV [$F_{3,21}$=48.7] procedures showed that TV by PEF did not significantly differ[Scheffe =0.2] from those measured by WDM while TV by PSF and LF were significantly higher [Scheffe=10.8; 8.9 respectively] than those measured by WDM. TheTV increased with age using the three formulae from one to eight months but thereafter remained constant within a range of 28.26 ± 1.14 - 29.40 ± **1**.35; 40.25 ± 2.98**-**41.82 ± 2.47; and 37.76 ± 1.66 - 39.62 ± 2.14 for PEF, PSF and LF respectively (for the rainy and dry seasons). There were no significant differences in the means of TV of the right and left testes across the age groups using these formulae for each season; also the when these values for each season were compared ($\alpha_{0.05}$).

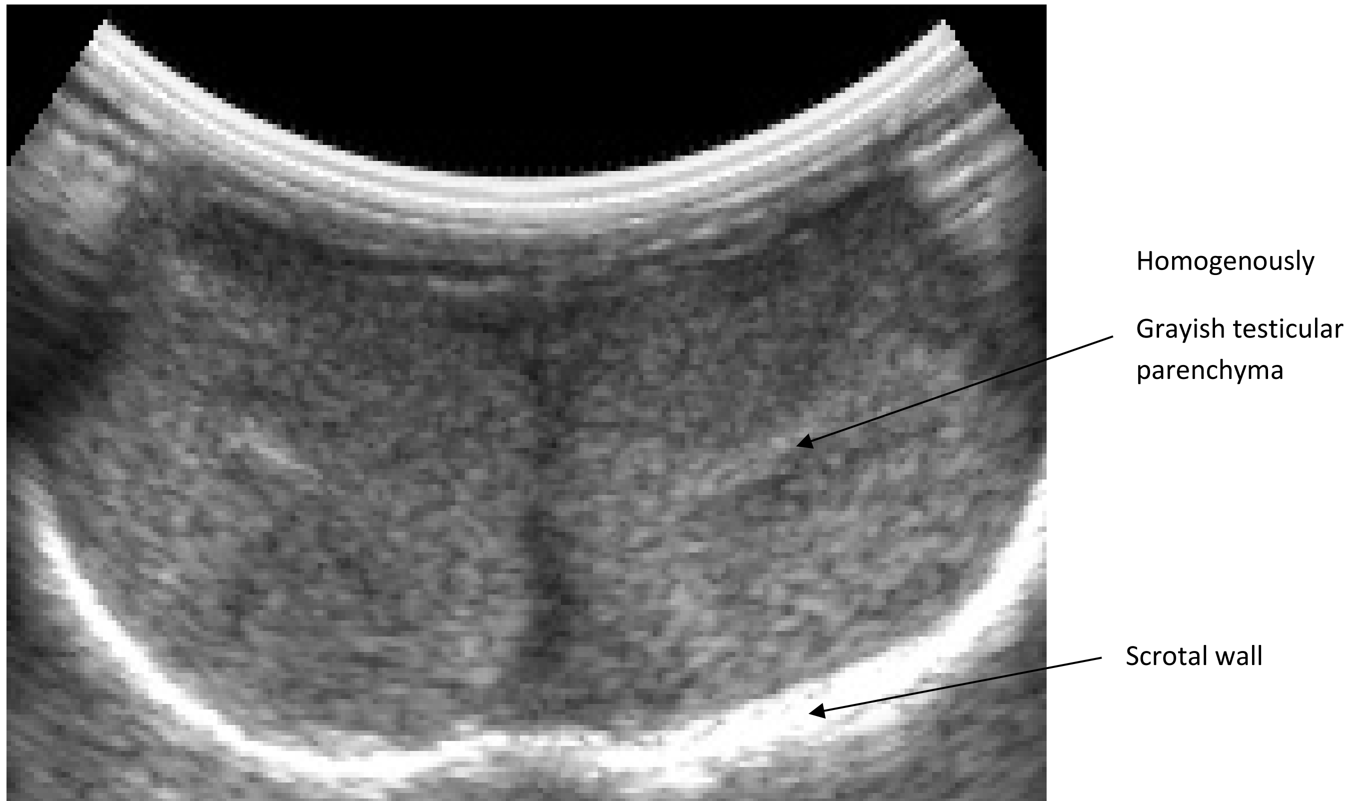


Figure 4.3: Sonogram showing the homogenously grayish testicular parenchyma, and scrotal walls on the transverse view of the right and left testes

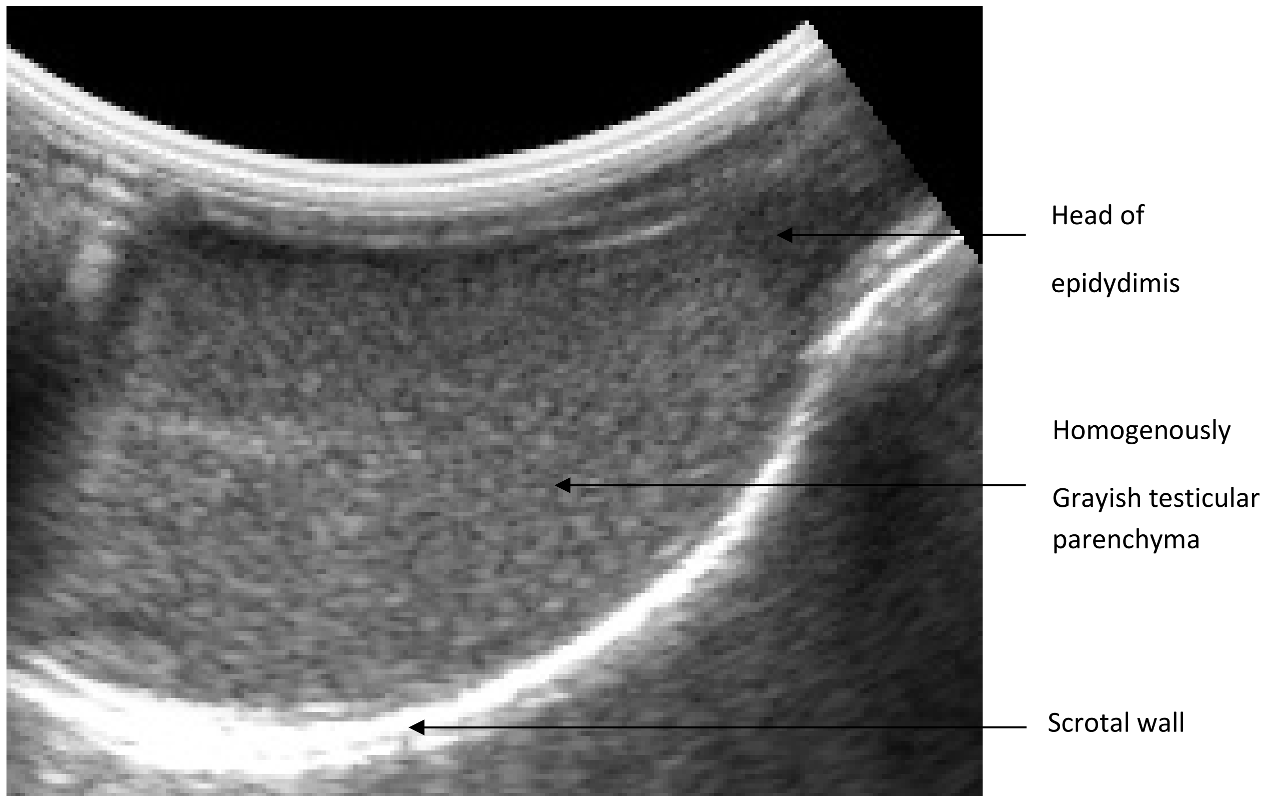


Figure 4.4: Sonogram showing the head of the epidydimis and scrotal wall of the right testis.

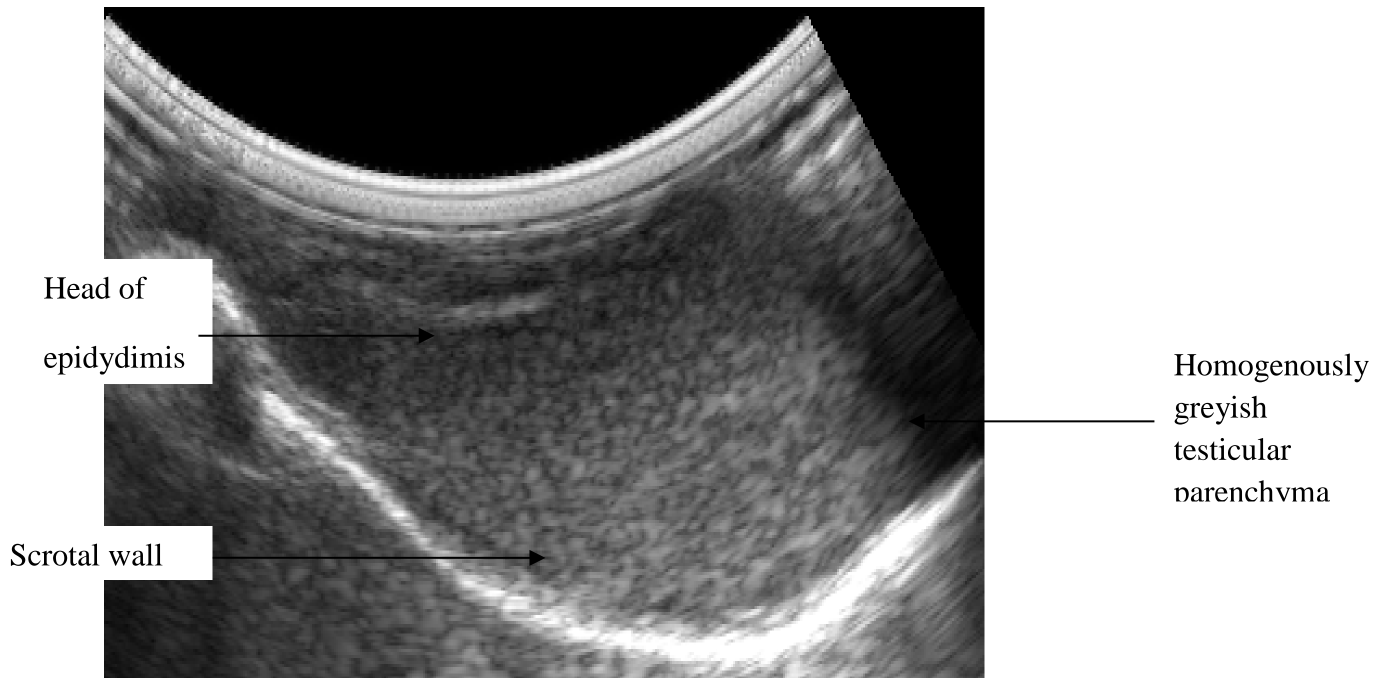


Figure 4.5: Sonogram showing the head of the epidydimis, testicular parenchyma and scrotalwall of the left testis on the Longitudinal view.

Table 4.1: The testicular volume of West African Dwarf bucks taken using the prolate ellipsoidformula, prolate spheroid formula and Lambert formula (mean ± standard deviation)for the dry season.

Age	N	Right testis				Left testis			
		TVWDM (cm^3)	TVPEF (cm^3)	TVPSF (cm^3)	TVLF (cm^3)	TVWDM (cm^3)	TVPEF (cm^3)	TVPSF (cm^3)	TVLF (cm^3)
1	6	16.4±0.1	16.3±0.1	23.8±1.1	22.4±0.1	16.1±0.1	16.2±0.0	23.6±0.4	22.1±0.0
2	6	18.5±0.1	18.7±0.5	26.6±0.2	25.8±0.1	18.7±0.1	18.4±0.6	26.5±0.1	25.6±0.5
3	6	20.9±0.2	20.7±0.4	28.7±0.3	28.6±0.3	20.7±0.1	20.8±0.2	28.4±0.1	28.8±0.2
4	6	21.8±0.2	22.1±0.3	31.9±0.2	30.2±0.2	21.9±0.2	21.9±0.2	31.6±0.3	30.4±0.3
5	6	24.5±0.2	24.1±0.9	34.3±0.2	32.4±0.1	24.4±0.2	24.2±0.6	34.5±0.2	32.5±0.3
6	6	25.6±0.1	25.7±0.3	36.6±0.4	34.5±0.2	25.8±0.2	25.6±0.2	36.8±0.2	34.6±0.1
7	6	26.7±0.1	26.9±0.4	38.7±0.1	35.9±0.4	26.5±0.2	26.7±0.2	38.4±0.2	35.6±0.2
8	**6**	**28.8±0.2**	**28.6±0.1**	**40.2±0.3**	**38.9±0.1**	**28.5±0.1**	**28.7±0.1**	**40.1±0.2**	**38.7±0.2**
9	6	28.9±0.2	28.8±0.3	40.2±0.1	37.9±0.2	28.7±0.1	28.9±0.2	40.1±0.1	40.0±0.2
10	6	28.7±0.1	28.9±0.2	41.3±0.2	39.8±0.1	28.6±0.2	28.8±0.3	41.5±0.1	39.7±0.3
11	6	28.8±0.2	28.6±0.1	41.7±0.1	38.9±0.3	28.7±0.2	28.7±0.2	41.5±0.2	38.6±0.2
12	6	28.7±0.1	28.7±0.3	41.5±0.2	38.9±0.1	28.8±0.2	28.6±0.1	41.6±0.2	38.7±0.3
13	6	28.9±0.1	28.6±0.2	40.4±0.3	38.6±0.3	28.7±0.2	28.5±0.2	40.5±0.1	38.4±0.2
14	6	28.8±0.2	28.6±0.1	40.5±0.1	38.6±0.2	28.6±0.1	28.5±0.2	40.3±0.2	38.5±0.2
15	6	28.5±0.1	28.7±0.2	41.2±0.2	39.5±0.2	28.7±0.2	28.8±0.1	41.3±0.1	39.6±0.1

*Significant at $\alpha_{0.05}$, N – Number of bucks, TV –Testicular Volume,

PEF– prolate ellipsoid formula, PSF – prolate spheroid formula, LF – Lambert formula.

Table 4.2: The testicular volume of West African Dwarf bucks taken using the prolate ellipsoidformula, prolate spheroid formula and Lambert formula (mean ± standard deviation)for the rainy season.

Age	N	Right testis				Left testis			
		TVWDM (cm^3)	TVPEF (cm^3)	TVPSF (cm^3)	TVLF (cm^3)	TVWDM (cm^3)	TVPEF (cm^3)	TVPSF (cm^3)	TVLF (cm^3)
1	6	16.1±0.1	16.0±0.1	23.6±1.1	21.7±0.1	16.2±0.1	16.1±0.2	23.5±0.4	21.9±0.1
2	6	18.1±0.2	18.0±0.1	26.1±0.1	25.2±0.2	18.3±0.1	18.3±0.2	26.0±0.2	25.3±0.2
3	6	20.2±0.1	20.3±0.2	28.0±0.3	28.4±0.2	20.4±0.2	20.2±0.3	28.2±0.1	28.2±0.1
4	6	21.6±0.1	21.7±0.1	31.4±0.4	29.9±0.1	21.5±0.2	21.6±0.3	31.3±0.2	30.0±0.2
5	6	24.2±0.2	24.0±0.3	34.1±0.1	32.1±0.2	24.1±0.1	23.8±0.1	34.0±0.3	32.2±0.2
6	6	25.3±0.2	25.2±0.1	36.2±0.1	34.2±0.1	25.4±0.2	25.4±0.2	36.4±0.1	34.1±0.2
7	6	26.2±0.2	26.4±0.2	38.1±0.2	35.3±0.2	26.3±0.2	26.3±0.3	38.2±0.1	35.2±0.1
8	**6**	**28.1±0.2**	**28.1±0.2**	**40.0±0.1**	**38.3±0.1**	**28.3±0.1**	**28.2±0.1**	**39.8±0.2**	**38.5±0.2**
9	6	28.3±0.1	28.4±0.3	39.8±0.3	37.6±0.2	28.4±0.2	28.3±0.1	40.0±0.2	37.7±0.1
10	6	28.4±0.2	28.6±0.2	41.1±0.2	39.5±0.1	28.3±0.2	28.5±0.2	41.0±0.1	39.4±0.2
11	6	28.4±0.2	28.4±0.2	41.1±0.1	38.3±0.2	28.5±0.2	28.3±0.2	41.2±0.2	38.4±0.1
12	6	28.5±0.1	28.3±0.1	41.2±0.2	38.4±0.1	28.4±0.1	28.4±0.1	41.1±0.3	38.5±0.2
13	6	28.4±0.1	28.3±0.2	40.1±0.2	38.1±0.3	28.5±0.2	28.2±0.1	40.0±0.1	38.2±0.1
14	6	28.2±0.2	28.2±0.1	40.0±0.3	38.1±0.1	28.3±0.1	28.3±0.2	40.1±0.1	38.2±0.2
15	6	28.1±0.1	28.4±0.2	40.9±0.1	39.2±0.1	28.2±0.2	28.5±0.1	41.0±0.2	39.3±0.1

*Significant at $\alpha_{0.05}$, N – Number of bucks, TV –Testicular Volume,

PEF– prolate ellipsoid formula, PSF – prolate spheroid formula, LF – Lambert formula.

Table 4.3: The average testicular volume obtained by testicular ultrasound using the Prollate Ellipsoid, Prolate Spheroid, Lambert formulae and Water Displacement Method in West African Dwarf bucks.

Age (months)	TVWDM (cm^3)	TVPEF (cm^3)	TVPSF (cm^3)	TVLF (cm^3)
1	16.2±0.1 ^{*acd}	16.1±0.1 ^{*bcd}	23.6±1.7 ^{*cab}	22.0±0.1 ^{*dab}
2	18.4±0.2 ^{*acd}	18.4±0.1 ^{*bcd}	26.2±0.1 ^{*cab}	25.5±0.1 ^{*dab}
3	20.6±0.2 ^{*acd}	20.5±0.1 ^{*bcd}	28.3±0.1 ^{*cab}	28.5±0.2 ^{*dab}
4	21.7±0.2 ^{*acd}	21.8±0.2 ^{*bcd}	31.5±0.2 ^{*cab}	30.1±0.2 ^{*dab}
5	24.3±0.2 ^{*acd}	23.9±0.2 ^{*bcd}	34.2±0.1 ^{*cab}	32.3±0.3 ^{*dab}
6	25.5±0.2 ^{*acd}	25.4±0.2 ^{*bcd}	36.5±0.2 ^{*cab}	34.3±0.2 ^{*dab}
7	26.4±0.2 ^{*acd}	26.5±0.2 ^{*bcd}	38.3±0.3 ^{*cab}	35.5±0.2 ^{*dab}
8	**28.4±0.2 ^{*acd}**	**28.3±0.2 ^{*bcd}**	**40.0±0.3 ^{*cab}**	**38.6±0.3 ^{*dab}**
9	28.6±0.2 ^{*acd}	28.5±0.2 ^{*bcd}	40.0±0.2 ^{*cab}	37.8±0.3 ^{*dab}
10	28.5±0.2 ^{*acd}	28.7±0.2 ^{*bcd}	41.0±0.2 ^{*cab}	39.6±0.2 ^{*dab}
11	28.6±0.2 ^{*acd}	28.5±0.2 ^{*bcd}	41.4±0.2 ^{*cab}	38.5±0.2 ^{*dab}
12	28.6±0.2 ^{*acd}	28.5±0.2 ^{*bcd}	41.3±0.2 ^{*cab}	38.6±0.3 ^{*dab}
13	28.6±0.2 ^{*acd}	28.4±0.3 ^{*bcd}	40.2±0.3 ^{*cab}	38.3±0.3 ^{*dab}
14	28.5±0.2 ^{*acd}	28.4±0.2 ^{*bcd}	40.2±0.3 ^{*cab}	38.3±0.3 ^{*dab}
15	28.4±0.2 ^{*acd}	28.6±0.2 ^{*bcd}	41.1±0.3 ^{*cab}	39.4±0.2 ^{*dab}

*Significant at $\alpha_{0.05}$; *abc – when a, b and c are compared.

TV –Testicular Volume, PEF– prolate ellipsoid formula,

PSF – prolate spheroid formula, LF – Lambert formula.

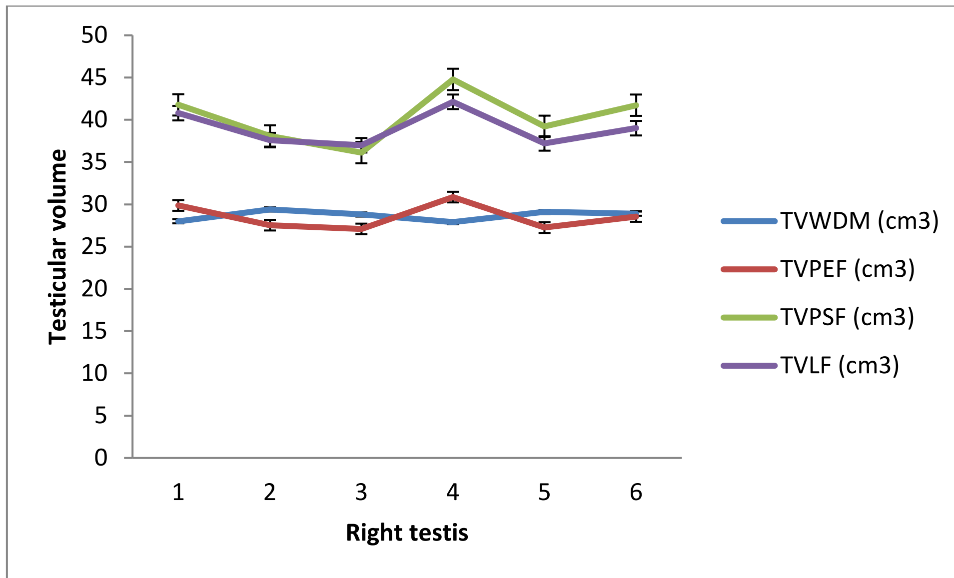


Figure 4.6: The comparison of the true testicular volume by water displacement method with the testicular volume by the Prolate ellipsoid, Prolate spheroid and Lambert formulae forthe right testes during the dry season.

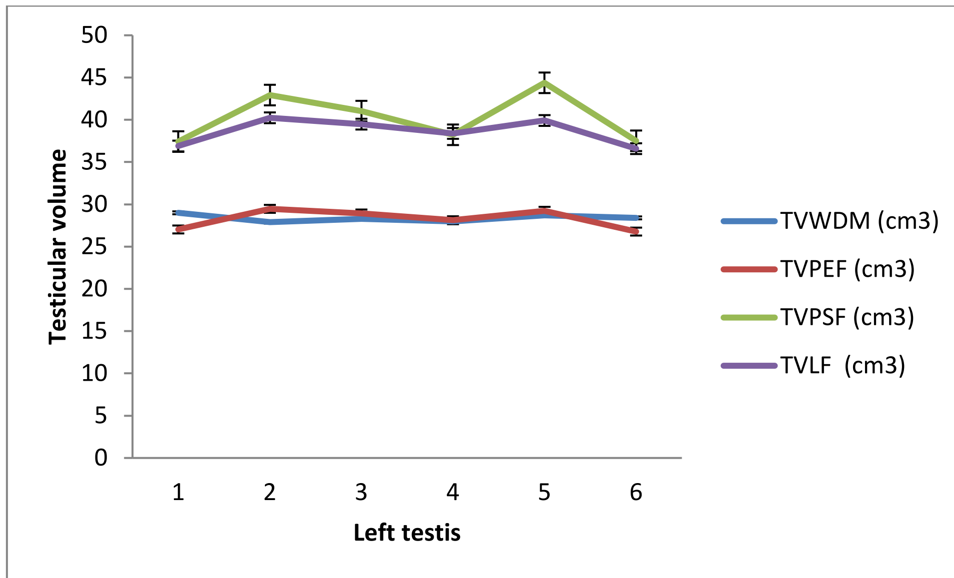


Figure 4.7: The comparison of the true testicular volume by water displacement method with thetesticular volume by the Prolate ellipsoid, Prolate spheroid and Lambert formulae forthe left testes during the dry season.

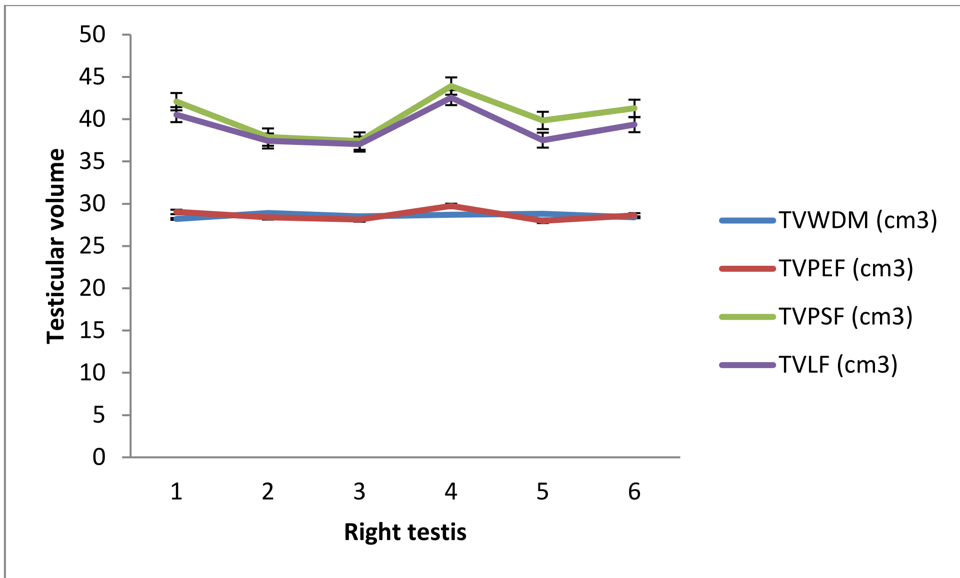


Figure 4.8: The comparison of the true testicular volume by water displacement method with the testicular volume by the Prolate ellipsoid, Prolate spheroid and Lambert formulae forthe right testes during the rainy season.

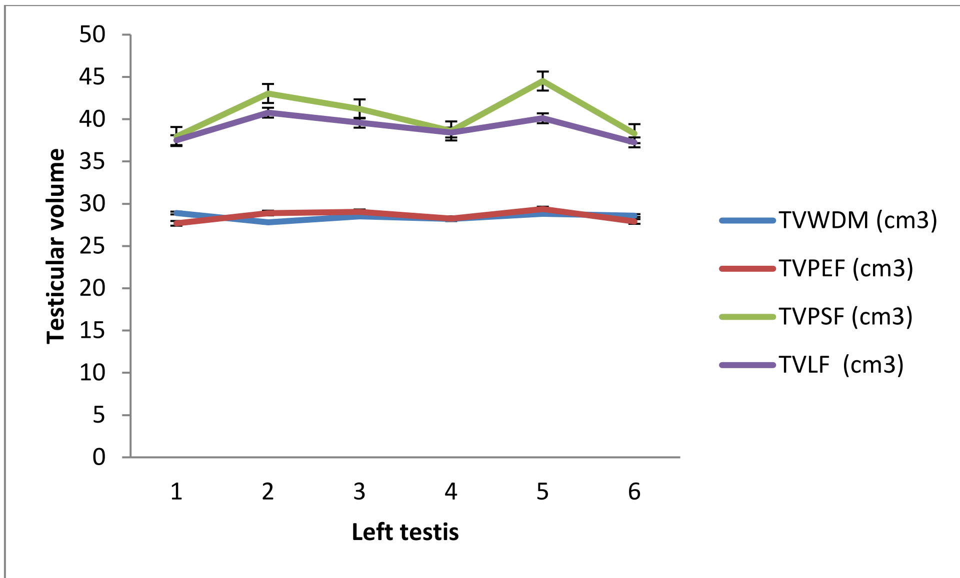


Figure 4.9: The comparison of the true testicular volume by water displacement method with the testicular volume by the Prolate ellipsoid, Prolate spheroid and Lambert formulae for the left testes during the rainy season.

4.4.0 DISCUSSION

This study showed that TU is valuable in estimating TV particularly by using the PEF which was useful as a parameter or determinant of the OBA in WAD goat bucks. The electronic caliper on the ultrasound was used in taking testicular length, height and width from which TV was calculated using the PEF, PSF and LF. It was observed that TV increased with age from one to eight months but thereafter without any further significant increase up to the fifteenth month using the three formulae across the different age groups of WAD bucks studied. Since TV is an index of spermatogenesis (Kollin *et al*., 2006), this suggests that full sperm production i.e (OBA) commences at eight months in WAD bucks. The TV obtained using the three different formulae were compared with the true TV. This is usually estimated using the WDM (Behre *et al*., 1989; Kiridi *et al*., 2012). It was observed that the PEF estimated TV was closest to the true TV obtained by WDM without any significant difference. However, TV by PSF and LF were significantly higher than the true TV by WDM in these WAD bucks. This suggests that the PEF is the best of the three formulae, in estimating the TV of WAD bucks. Further studies should be carried out to fully establish this in WAD bucks; also in other breeds of goats and species of livestock as well. Souza *et al.*, (2012) used the PEF to estimate testicular volumes in canine species. But they did not compare this with the other formulae and method used in this study. However, Paltiel *et al*., (2002), compared these three formula also in canine and recommended that the LF as the preferred formula for clinical practice in canine. Also in human, the LF was reported to be the preferred formula for estimating TV (Kiridi *et al*., 2007; Sotos and Tokar, 2012). This variation may be due to species differences. However, the present study has demonstrated that the PEF is the preferred formula for estimating TV in the WAD goat bucks. These results may offer a new, more accurate, none invasive method of evaluating TV which can be adopted to replace the invasive methods which require the removal of testes before testicular volume can be calculated (Franca *et al*., 2000).

The observations of TU also revealed the normal testicular echo-texture which was homogenously greyish with hyper-echoic scrotal wall border line at the caudo-dorsal aspect of each testis. The head of the epididymis of the right and left testes appeared as small oval hypoechoic structure at the caudo-ventral most and cranio-ventral most portions of the each

testis respectively. These findings were similar to those reported in fertile bulls (Ali *et al*., 2011), rams (Ulkera*et al*., 2005), camels (Pasha *et al*., 2011) and Alapine goats (Carazo *et al*., 2014).

In conclusion, this study has shown that testicular ultrasoundwas valuable in determining the optimum breeding age by the estimation of testicular volume (an important parameter or correlate of fertility) in the West African Dwarf bucks; of which the prolate ellipsoid formula was the most valuable in estimating the testicular volume. Testicular Ultrasound was also useful in taking the normal testicular echo-texture in these bucks. Therefore, its use and introduction into the BSE programmes of these bucks should be encouraged. Adoption and use of the findings in this study will go a long way in improving WAD goat buck production through the rapid use of improved bucks for increasing genetic progress.

CHAPTER FIVE

5.0 DETERMINATION OF THE OPTIMUM BREEDING AGE USING MORPHOLOGICAL AND MORPHOMETRIC CHARACTERISTICS OF THE TESTIS AND EPIDIDYMIS IN WEST AFRICAN DWARF GOAT BUCKS

5.1 INTRODUCTION

The testis is the main organ of reproduction of the West African Dwarf (WAD) goat buck (which plays major roles in WAD goat production (through animal protein supply as stated in the previous chapters). It is the sole producer of sperm cells and major producer of testosterone. The sperm cells are required for fertilization of the ova from the doe without which there will be no conception and subsequent production of kids. Testosterone is important for spermatogenesis, development of the testes, prostate and secondary sexual characteristics in WAD bucks (Daramola *et al*., 2006; Ugwu, 2009). The normal testes of WAD bucks are paired except in disease conditions like chryptorchidism where only one testis descends into a pouch of skin housing the testes known as scrotum. They are usually ovoid or long ovoid that could be undivided or split and symmetrical or asymmetrical but the asymmetry should not be too much otherwise would be representative of diseased testes (Ozegbe, 2012).

Located behind the testis, is a highly convoluted duct called epidydimis which is meant for sperm storage and passage prior to ejaculation (Oyeyemi *et al*., 2002). However, there is only a dearth of information on the testes and epidydimis of WAD bucks especially ex-situ.

Also, it is important to know the normal histology and histomorphometry of these tissues especially in relation to semen production and storage and variations due to conditions. An understanding of the histology of the normal testicular and epididymal structures and functions are necessary for disease diagnoses. Furthermore, causes of the diseases, possible

treatments as well as the efficacy of treatments can also be identified or known. Histomorphometry on the other hand, provides information on the amount of tissues, cellular activities as well as normal measurements of the various testicular and epididymal structures (Gofur, 2015). This study was therefore conducted to investigate the morphological and morphometrical characteristics of the testes and epididymides of WAD bucks between the ages of one to fifteen months; with a view to identifying the growth and developmental changes of these organs in relation to puberty and especially the OBA.

5.2.0 MATERIALS AND METHODS

5.2.1 Study area

The study was carried out at the Department of Surgery and Theriogenology goat unit, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

5.2.2 Study design

Folloing completion of testicular ultrasound of the WAD bucks used in study two (i.e chapter three), the bucks testes and epididymides were collected for study three (i.e the present study).

5.2.3 Collection of testes and epididymides

The testes and epididymides were harvested using a size 4 scapel blade to make an incision from the dorso-medial aspect of the testis proximal to the external inguinal orifice following disinfection of the incision site. The skin was reflected laterally and the subcutaneous tissue and scrotal fascia were incised to expose the vagina tunic. The *tunica albuginea* was carefully removed from the testis. The epididymis was carefully separated from the testis using the scapel blade and thumb forceps.

5.2.4 Gross anatomicand volumetric parameters taken

The gross anatomic and volumetric parameters of the testis and epididymis measured for the bucks included:

5.2.4.1Weights of the right and left testis: This was taken by using a digital weighing balance.

5.2.4.2Length, width and height of the right and left testis: The testis was placed on an X-Y axis while the length, width and height taken using a measuring ruler.

5.2.4.4Volume of testis: This was estimated using the PEF, PSF and LF as described earlier (in study two i.e chapter four). These were then compared with the WDM. Briefly, WDM was carried out by adding normal saline to a calibrated measuring cylinder (1000 ml) filling it up to a known volume and then the testes is gently dropped into the cylinder and the volume of water displaced which is calculated by deducting the increased volume of the normal saline in the cylinder from the initial known volume (e.g 320ml – 300ml = 20ml) while avoiding error due to parallax.

5.2.5 Histological procedure

Slices of various regions of the right and left testes, head (caput), body (corpus) and tail (caudal) part of the right and left epidydimis were fixed in Bouin's fluid and transfered to 70% alcohol after 24 hours. The specimens were processed by placing them in ascending grades of alcohol in the following order, first 95% alcohol for 1 hour and second 95% alcohol for 1¼ hours, first absolute alcohol for 1½ hours and second absolute alcohol for 2 hours to ensure proper dehydration of the tissues. It was then transferred to a mixture of equal volumes of alcohol and xylene where it was left overnight. It was later cleared in two changes of xylene for 1 hour each. It was then infiltrated twice for 1 hour each with molten paraffin wax in the oven at 60ºC. The tissues were embedded in paraffin wax, trimmed and mounted on wooden chuck, and then taken to the microtome for sectioning at 5µm thickness. The sections were floated in floating-out bath from where they were picked with clean albuminized slides. The slides were placed in a staining dish and excess wax was removed by two changes of xylene, hydrated by descending grades of alcohol in the following order- absolute alcohol, 95% alcohol and 70% alcohol for 2 minutes each. The slides were put in water and then stained by infiltrated Ehrlich hematoxylin for 15 minutes, and then washed in water for 5 minutes, differentiated in 10% acid alcohol and blued in running tap water for 10 minutes. It was then counter stained with filtered eosine for 2

minutes. Excess eosine was removed in ascending grades of alcohol in the following order- 75% alcohol, 95% alcohol and absolute alcohol for 2 minutes each. It was then cleared in two changes of xylene and cover slipped with depex mountant. The slides were viewed under a light microscope and selected images were captured using Moticam 2.0 digital camera attached to a Laptop computer (hp Model).

5.2.6 Histomorphometry

The images of the histology of testes and epidydimes were taken using Moticam 2.0 Digital camera attached to Laptop computer (hp model). Thereafter, measurements were taken and analyzed using the Motic Images Plus (MIPlus). The measurements taken included Seminiferous Tubular Diameter (STD), Seminiferous Luminal Diameter (SLD) and Germinal Epithelial Height (GEH). Ten measurements were taken per section for each parameter.

5.2.7 Statistical analysis

The data obtained in this study were analyzed using Analysis of Variance (ANOVA) at 5% level of significance ($\alpha_{0.05}$) using SPSS version 20.

5.3.0RESULTS

5.3.1 Gross morphological and morphometric characteristics

Each of the testes was ellipsoidal in shape and covered by a capsule of dense irregular connective tissue called *tunica albuginea* and a network of finely arranged blood vessels of arteries and veins (Figure 5.1). Also, on the medial aspect lied the epididymis extending from the cranio-dorsal to the caudo-dorsal aspect of the testis. The epididymis was made up of three regions namely the head, body and tail regions. The head of the epididymis was flat and irregularly oval to polygonal in shape at the caudo-dorsal aspect of the testis. The body of the epididymis was a slender long cylindrical tissue lying on the medial aspect of the testis. The tail of the epididymis was a raised short stocky firm tissue extending a little beyond the caudo-dorsal aspect of the testis (Figure 5.2). On sagittal incision, the mediastinum testis appeared as a densely white longitudinal structure at the centre of the testis. The morphometric data of the testes for the WAD bucks are as presented on Tables

5.1 and 5.2 respectively. There was a high correlation between the weight and volume of the right and left testes ($r = 0.87$, $\alpha = 0.05$; and $r = 0.83$, $\alpha = 0.05$ respectively).

5.3.2 Histology

Histological studies revealed that the WAD buck testis is divided into regularly arranged lobules or compartments (by the septae of the *tunica albuginea*). Each lobule contains mostly rounded and some irregularly shaped Seminiferous Tubules (ST). Each ST is lined by a basement membrane and a layer of germinal epithelium. The lumen of the ST was filled with sperm cells surrounded by an adluminal space (Figure 5.11). This was first observed in the six months old WAD bucks. The germinal epithelia layer revealed two major categories of cells namely the proliferating population of spermatogonic cells and a non-proliferating sustentacular or sertoli cells. The sertoli cells appeared elongated with pale staining nuclei and were fewer in number (compared to the spermatogonic cells) but on a collective view, occupied most of the volume of the ST forming a blood-testis barrier extending from the basal membrane into the lumen of the testis. The spermatogonic cells were arranged in successive concentric layers according to maturity from the basal layer to the lumen of ST. The earliest cells called the spermatogonia lied next to the basal layer and were small cells with dark oval nuclei. These were closely followed by the large cells with large round nuclei called spermatocytes which together with the sertoli cells form the tight junctional complex referred to as the blood testis barrier. Above the spermatocytes were the spermatids which appeared as two generations of cells close to the lumen of the ST. The first generation of these cells appeared roundish with centrally located nucleus while the second generation was identified by their dark-blue heads and eosinophilic thin like flagella extending into the lumen (Figure 5.11). In between adjacent ST were cells that appeared polyhedral and uninucleated called Leydig cells, occupying a considerable portion of the interstitial tissue (Figures 5.10 and 5.11). There were three segments of the epididymis namely the head, body and tail. The head was characterized by tall columnar epithelium with basal nuclei and scanty sperm cells. The free ends of the surface of these cells possessed small branching microvilli called stereocilia (Figure 5.30). The body was characterized by low columnar epithelium with prominent stereocilia. Small groups of sperm cells were observed within the lumen of most the duct in this part (Figures 5.31 and

5.32). The tail was characterized by cuboidal epithelia but without stereocilia. Large amount of spermatozoa were observed within the lumen of the duct (Figures 5.33 and 5.34).

5.3.3 Histomorphometry

The results of the histomorphometric studies are as presented on Table 5.3. The STD, SLD and GEH [$F_{1,5}$=10451.3; 1715624.8 and 175260.1 respectively] significantly increased from one month to eight month without further significant increase up to 15 months.

Table 5.1: The average testicular weight and testicular volume obtained by morphometry usingthe Prolate Ellipsoid, Prolate Spheroid, Lambert formulae and by Water Displacement Method in West African Dwarf bucks.

Age (months)	TW (grams)	TVWDM (cm^3)	TVPEF (cm^3)	TVPSF (cm^3)	TVLF (cm^3)
1	16.3±0.2	16.2±0.1^{*bde}	16.3±0.1^{*cde}	23.4±0.6^{*dbc}	22.1±0.2^{*ebc}
2	18.6±0.1	18.5±0.2^{*bde}	18.2±0.2^{*cde}	26.0±0.1^{*dbc}	25.3±0.1^{*ebc}
3	20.5±0.1	20.7±0.2^{*bde}	20.4±0.1^{*cde}	28.5±0.3^{*dbc}	28.6±0.1^{*ebc}
4	21.4±0.2	21.3±0.1^{*bde}	21.6±0.2^{*cde}	31.4±0.2^{*dbc}	30.3±0.2^{*ebc}
5	24.6±0.1	24.8±0.1^{*bde}	23.9±0.2^{*cde}	34.6±0.1^{*dbc}	32.4±0.2^{*ebc}
6	25.8±0.2	25.6±0.2^{*bde}	25.7±0.1^{*cde}	36.8±0.2^{*dbc}	34.6±0.3^{*ebc}
7	26.2±0.1	26.5±0.2^{*bde}	26.8±0.2^{*cde}	38.5±0.2^{*dbc}	35.2±0.1^{*ebc}
8	**28.5±0.2**	**28.7±0.1^{*bde}**	**28.4±0.1^{*cde}**	**40.2±0.3^{*dbc}**	**38.4±0.3^{*ebc}**
9	28.3±0.1	28.2±0.1^{*bde}	28.6±0.2^{*cde}	40.0±0.1^{*dbc}	37.5±0.2^{*ebc}
10	28.4±0.1	28.6±0.2^{*bde}	28.3±0.2^{*cde}	41.3±0.2^{*dbc}	39.7±0.1^{*ebc}
11	28.5±0.2	28.4±0.1^{*bde}	28.7±0.2^{*cde}	41.5±0.3^{*dbc}	38.2±0.3^{*ebc}
12	28.8±0.2	28.5±0.2^{*bde}	28.6±0.2^{*cde}	41.1±0.1^{*dbc}	38.4±0.2^{*ebc}
13	28.4±0.1	28.7±0.2^{*bde}	28.8±0.1^{*cde}	40.4±0.2^{*dbc}	38.6±0.2^{*ebc}
14	28.7±0.2	28.4±0.1^{*bde}	28.3±0.2^{*cde}	40.3±0.1^{*dbc}	38.4±0.2^{*ebc}
15	28.3±0.2	28.6±0.1^{*bde}	28.2±0.2^{*cde}	41.2±0.2^{*dbc}	39.7±0.1^{*ebc}

*Significant at $\alpha_{0.05}$; *abc – when columns a, b and c are compared etc.

TW – Testicular Weight, TV –Testicular Volume, PEF– prolate ellipsoid formula,

PSF – prolate spheroid formula, LF – Lambert formula.

Table 5.2: The average testicular volume by morphometry of West African Dwarf bucks taken using the prolate ellipsoidformula, prolate spheroid formula and Lambert formula (mean ± standard deviation) for the dry and raing seasons.

Age	N	Right testis				Left testis			
		TVWDM (cm^3)	TVPEF (cm^3)	TVPSF (cm^3)	TVLF (cm^3)	TVWDM (cm^3)	TVPEF (cm^3)	TVPSF (cm^3)	TVLF (cm^3)
1	6	16.3±0.1	16.4±0.1	23.5±0.2	22.2±0.1	16.1±0.1	16.2±0.3	23.3±0.4	22.0±0.2
2	6	18.7±0.1	18.3±0.1	26.0±0.2	25.2±0.1	18.5±0.2	18.1±0.2	26.2±0.1	25.4±0.1
3	6	20.6±0.2	20.2±0.4	28.7±0.3	28.7±0.3	20.8±0.1	20.6±0.2	28.3±0.1	28.5±0.2
4	6	21.5±0.2	21.8±0.3	31.6±0.2	30.1±0.2	21.1±0.2	21.4±0.2	31.2±0.3	30.5±0.3
5	6	24.7±0.2	24.1±0.3	34.5±0.2	32.2±0.1	24.9±0.2	23.7±0.3	34.7±0.2	32.6±0.3
6	6	25.7±0.1	25.6±0.3	36.6±0.2	34.5±0.2	25.5±0.2	25.8±0.2	37.0±0.2	34.7±0.1
7	6	26.7±0.1	26.9±0.2	38.7±0.1	35.1±0.3	26.3±0.2	26.7±0.2	38.3±0.2	35.3±0.2
8	**6**	**28.8±0.2**	**28.6±0.1**	**40.1±0.3**	**38.6±0.1**	**28.6±0.1**	**28.2±0.1**	**40.3±0.2**	**38.2±0.2**
9	6	28.1±0.2	28.8±0.3	39.9±0.1	37.4±0.2	28.3±0.1	28.4±0.2	40.1±0.1	37.6±0.2
10	6	28.7±0.1	28.2±0.2	41.5±0.2	39.8±0.1	28.5±0.2	28.4±0.3	41.1±0.1	39.6±0.3
11	6	28.8±0.2	28.6±0.1	41.7±0.1	38.9±0.3	28.7±0.2	28.7±0.2	41.5±0.2	38.6±0.2
12	6	28.7±0.1	28.7±0.3	41.5±0.2	38.9±0.1	28.8±0.2	28.6±0.1	41.6±0.2	38.7±0.3
13	6	28.9±0.1	28.6±0.2	40.4±0.3	38.6±0.3	28.7±0.2	28.5±0.2	40.5±0.1	38.4±0.2
14	6	28.8±0.2	28.6±0.1	40.5±0.1	38.6±0.2	28.6±0.1	28.5±0.2	40.3±0.2	38.5±0.2
15	6	28.5±0.1	28.7±0.2	41.2±0.2	39.5±0.2	28.7±0.2	28.8±0.1	41.3±0.1	39.6±0.1

*Significant at $\alpha_{0.05}$, N – Number of bucks, TV –Testicular Volume,

PEF– prolate ellipsoid formula, PSF – prolate spheroid formula, LF – Lambert formula.

Table 5.3: Average Histomorphometry of the testes of West African Dwarf Bucks of one to fifteen months

Age (months)	**STD (μm)**	**SLD (μm)**	**GEH (μm)**
1	83.1±0.2	47.5±0.2	28.4±0.2
2	102.4±0.4	71.4±1.1	42.0±0.2
3	198.4±0.2	120.6±0.2	56.2±0.2
4	273.3±0.2	154.5±0.2	96.3±0.1
5	361.6±0.2	185.4±0.2	124.4±0.2
6	497.1±0.3	203.5±0.2	146.4±0.2
7	524.6±0.3	228.2±0.4	171.2±0.3
8	**574.1±0.3**	**261.3±0.3**	**197.6±0.2**
9	572.5±0.3	257.4±0.2	194.5±0.2
10	575.7±0.3	257.5±0.2	196.5±0.3
11	570.8±0.4	259.5±0.3	193.8±0.4
12	573.9±0.4	261.3±0.4	197.4±0.4
13	575.0±0.4	258.2±0.4	197.5±0.2
14	571.9±0.5	257.9±0.2	197.8±0.3
15	573.1±0.5	259.4±0.3	197.0±0.4

*Significant at $\alpha_{0.05}$; N – Number of bucks.

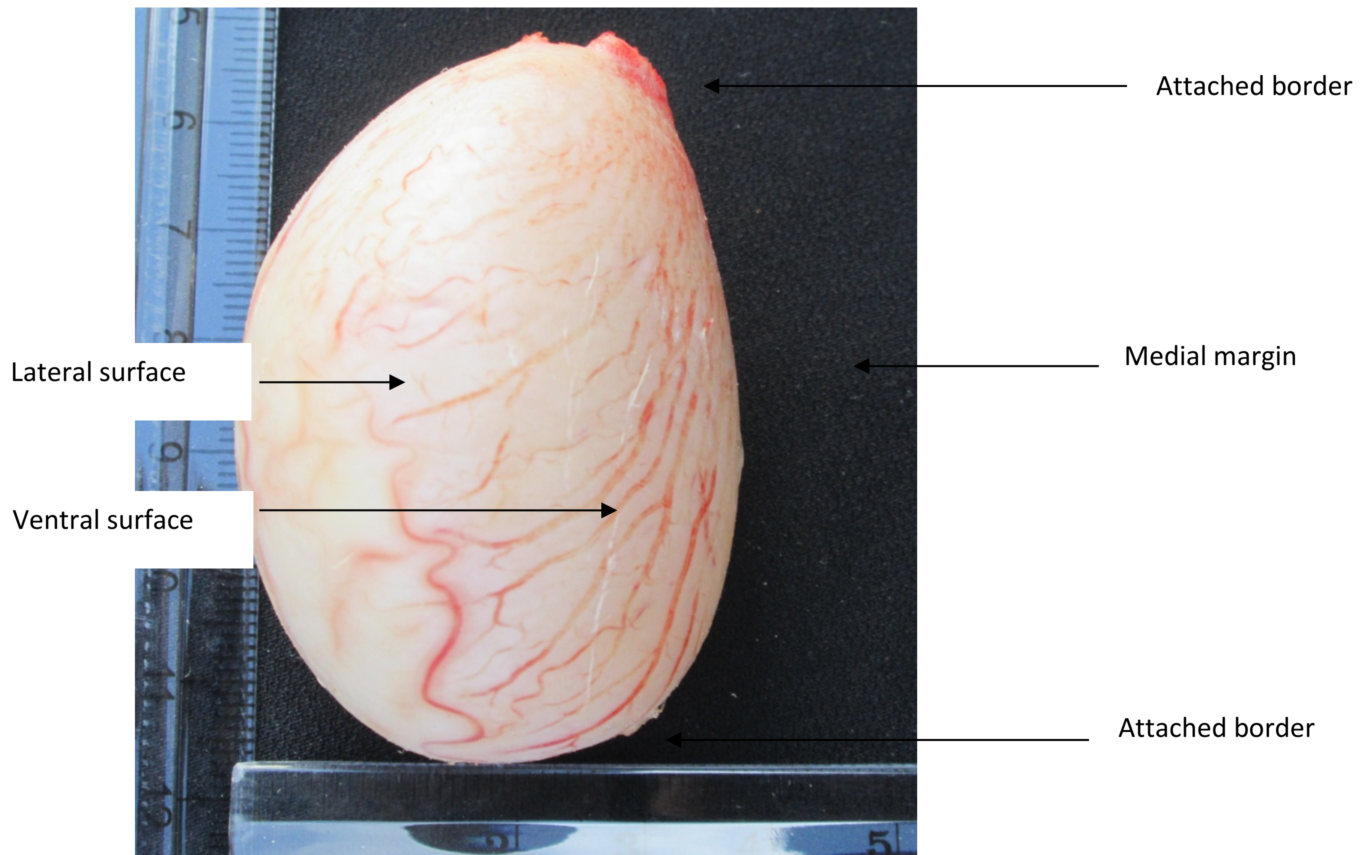


Figure 5.1: West African Dwarf buck testis covered by a fine network of blood vessels.

2160 × 3840 pixels.

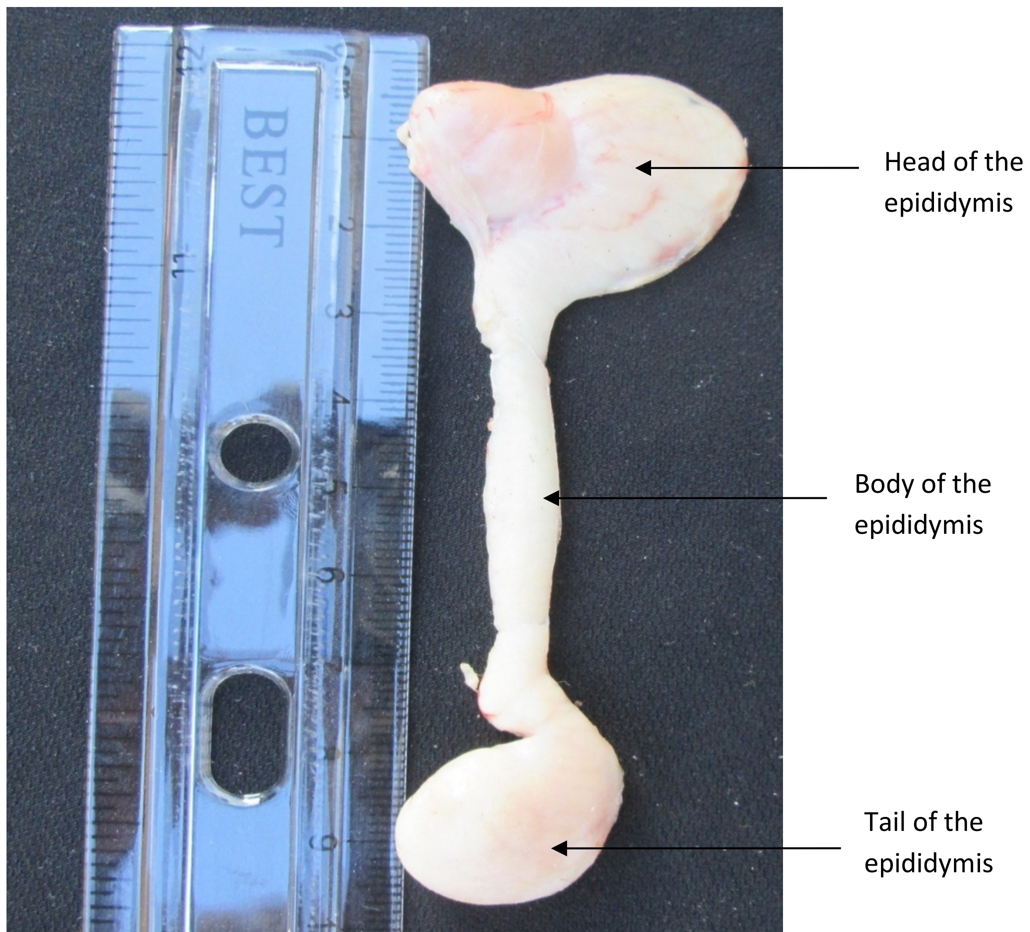


Figure 5.2: Epididymis of West African Dwarf buck, 2160 × 3840 pixels.

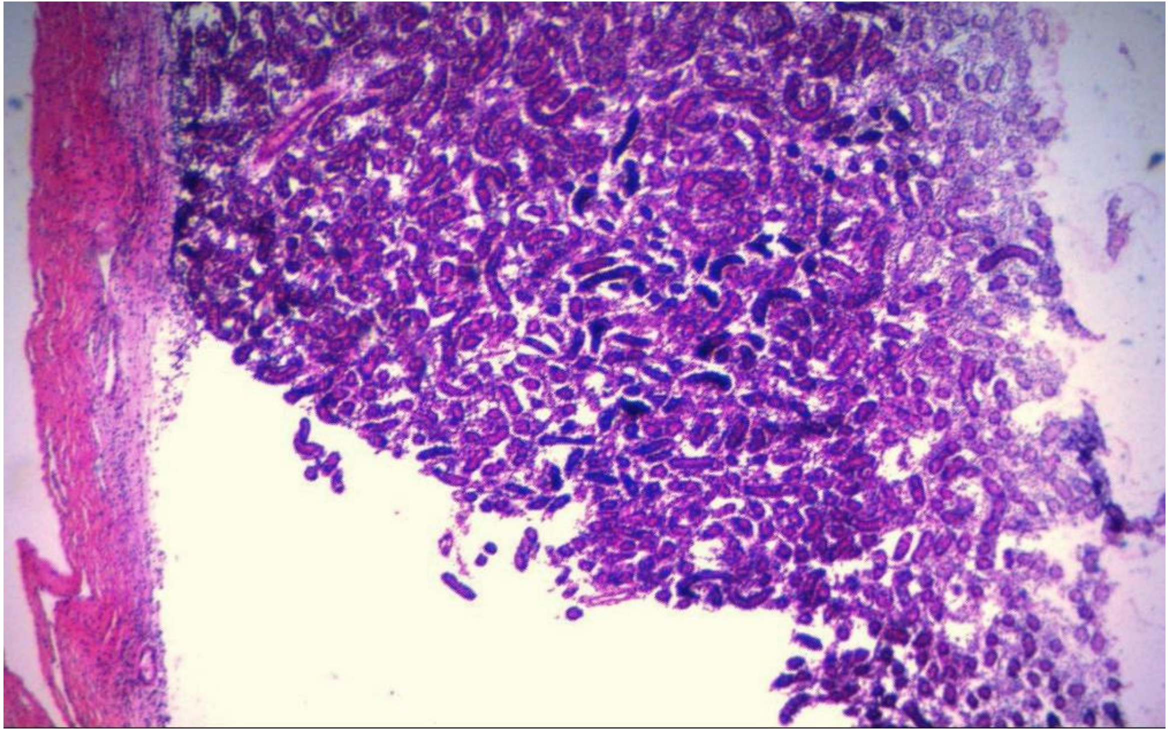


Figure 5.3: Seminiferous tubules of the testis of one month old West African Dwarf buckH & E × 40.

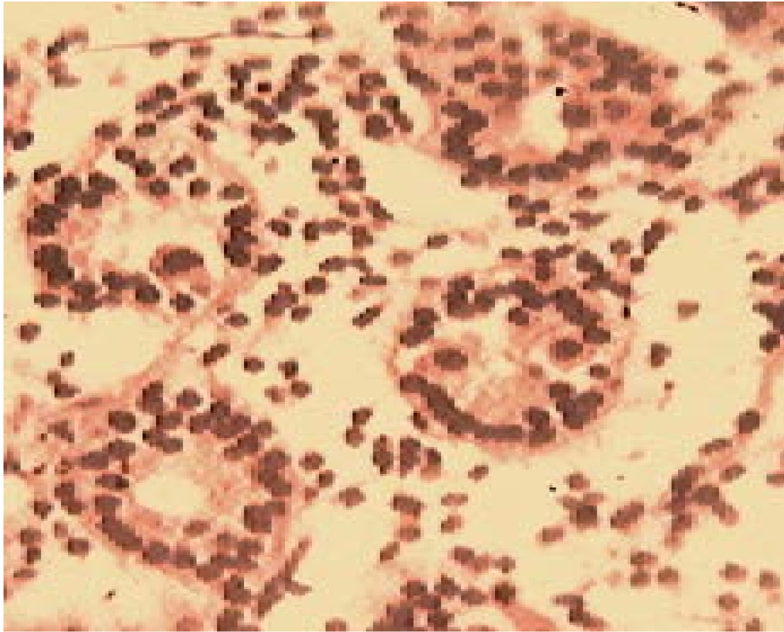


Figure 5.4: Seminiferous tubules of the testis of two months old West African Dwarf buckH & E $\times$ 400.

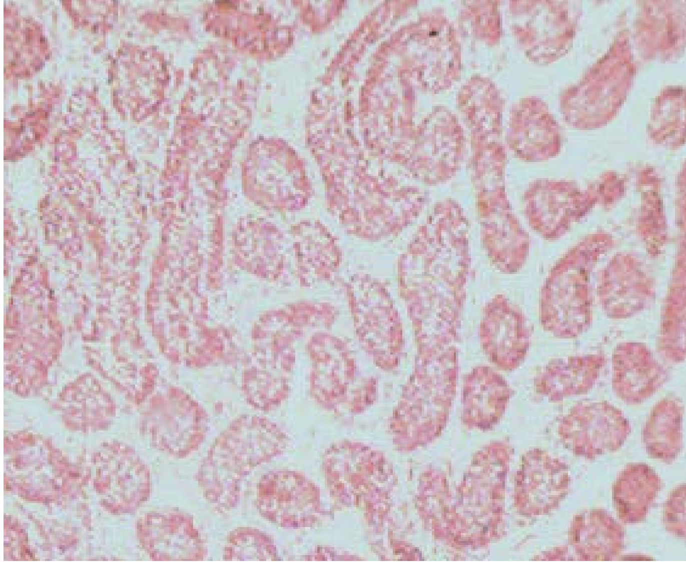


Figure 5.5: Seminiferous tubules of the testis of three months old West African Dwarf buck H& E $\times$ 40.

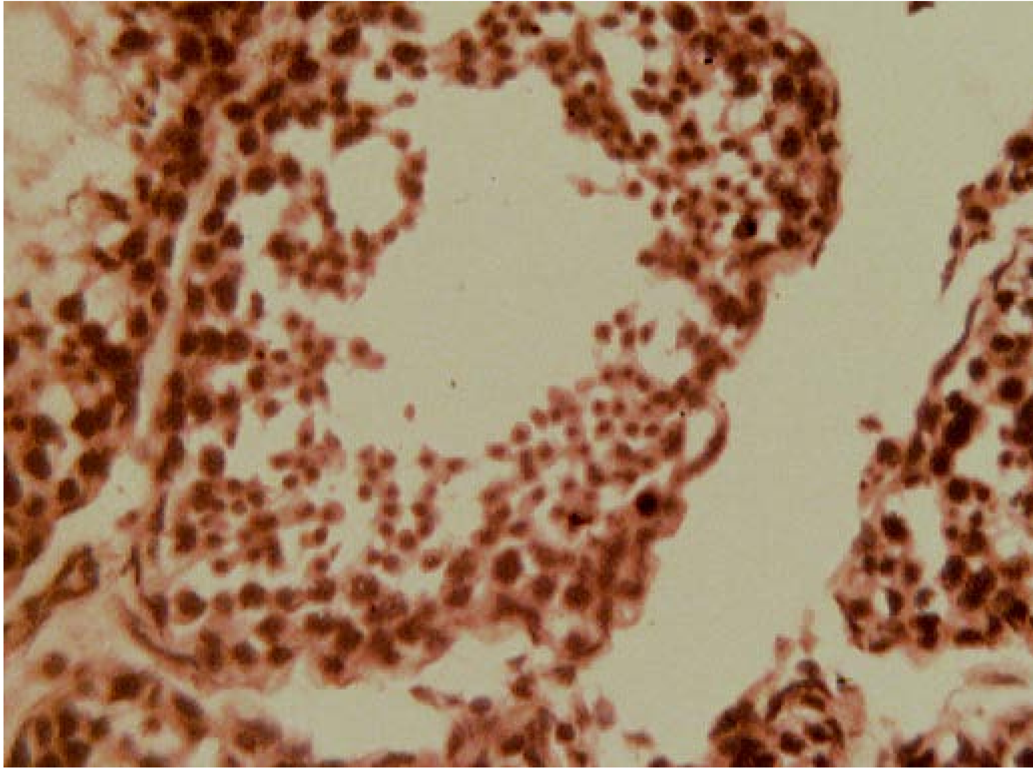


Figure 5.6: Seminiferous tubules of the testis of four months old West African Dwarf buckH & E × 400.

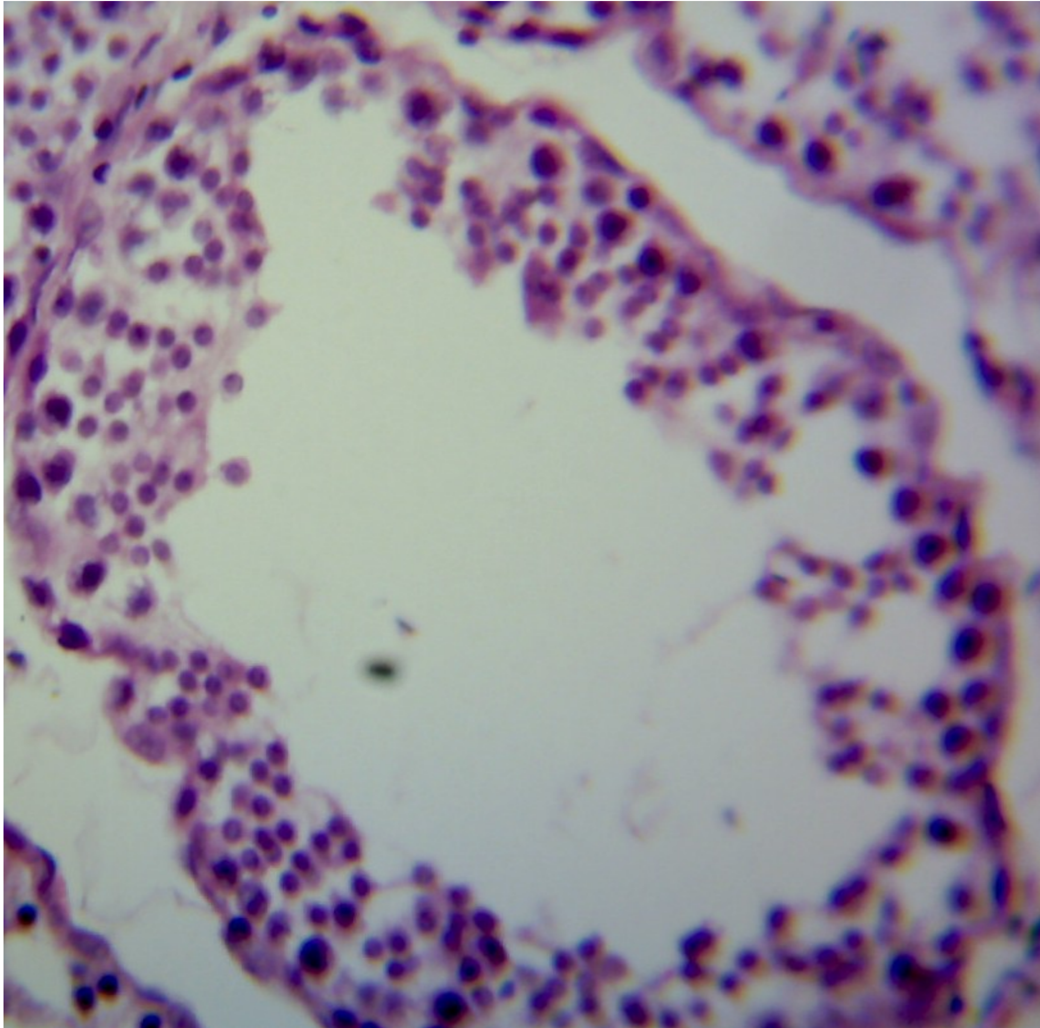


Figure 5.7: Seminiferous tubules of the testis of five months old West African Dwarf buckH & E × 400.

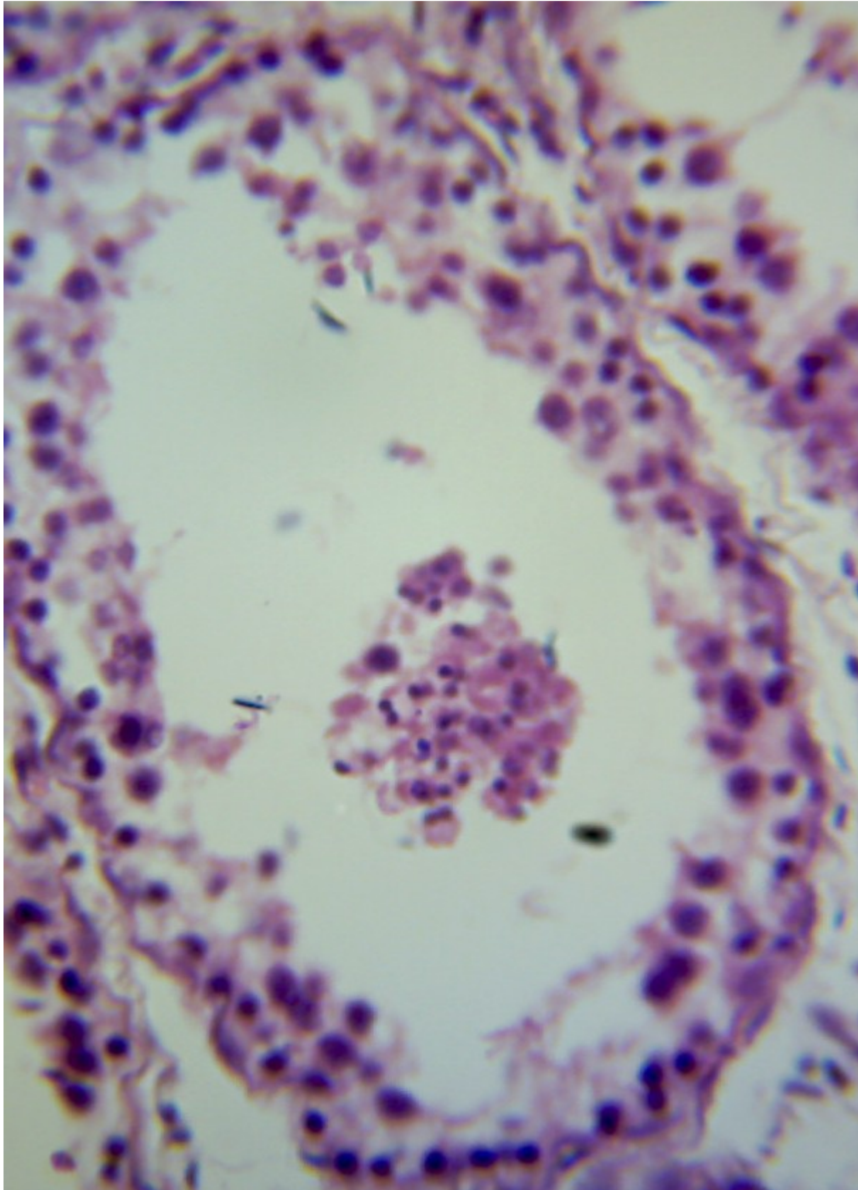


Figure 5.8: Seminiferous tubules of the testis of six months old West African Dwarf buck H & E $\times$ 400.

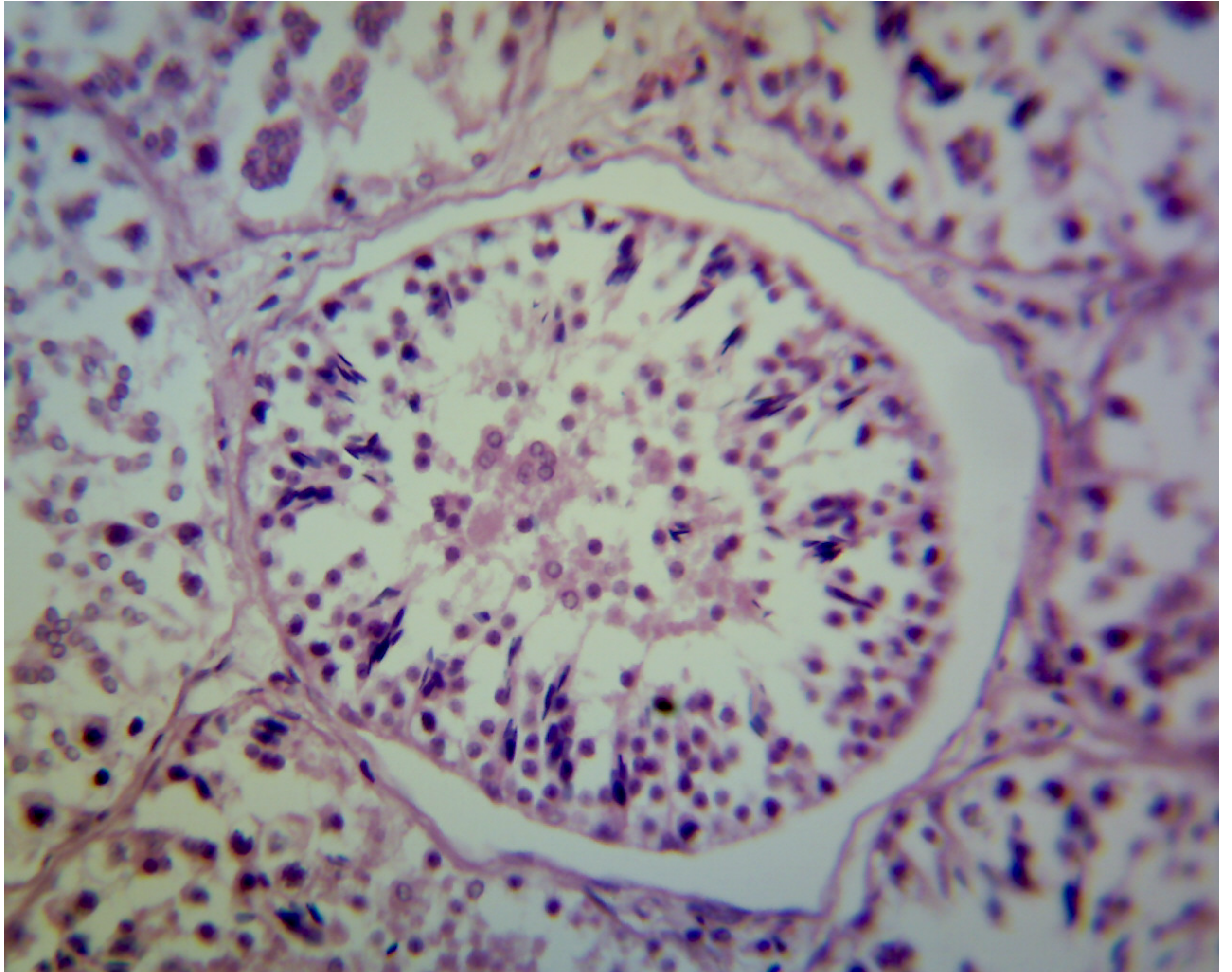


Figure 5.9: Seminiferous tubules of the testis of seven months old West African Dwarf buckH & E × 400.

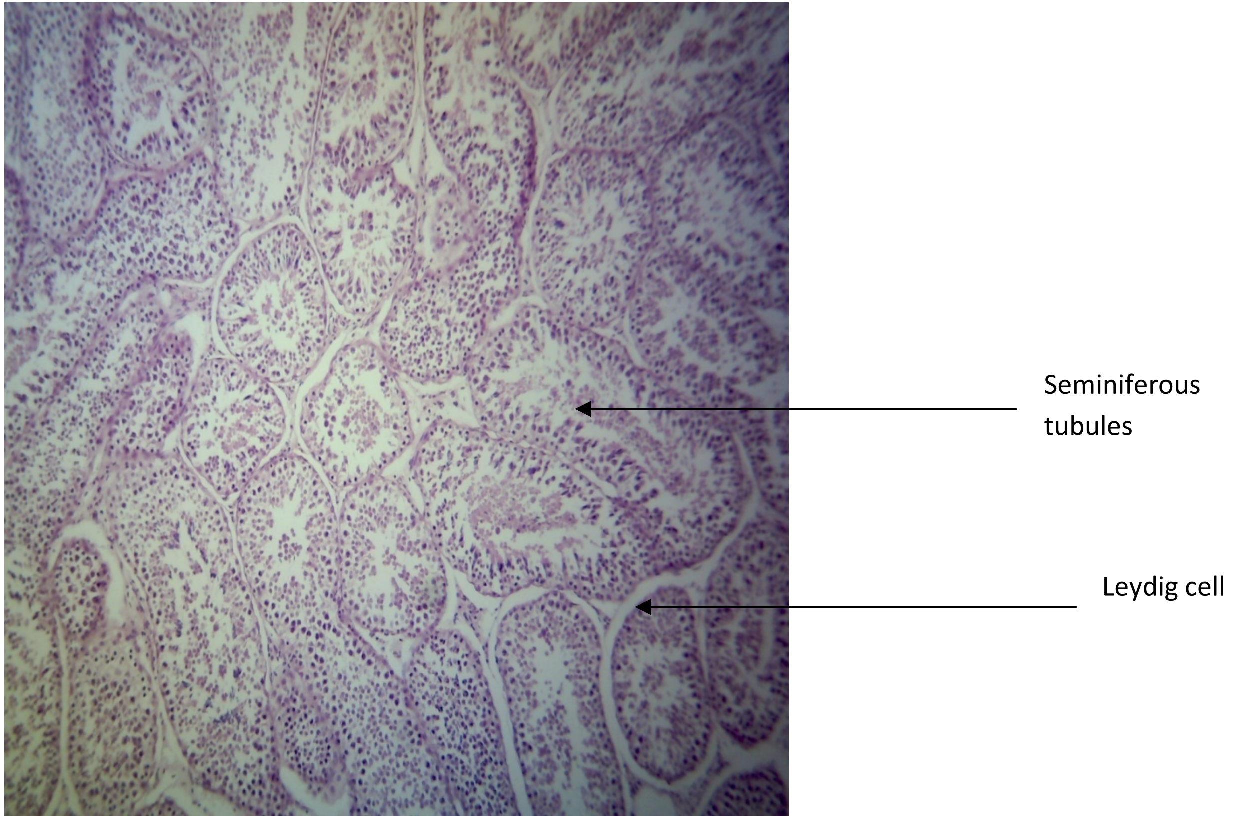


Figure 5.10: Seminiferous tubules of the testis of eight months old West African Dwarf buckH & E × 40.

Leydig cell

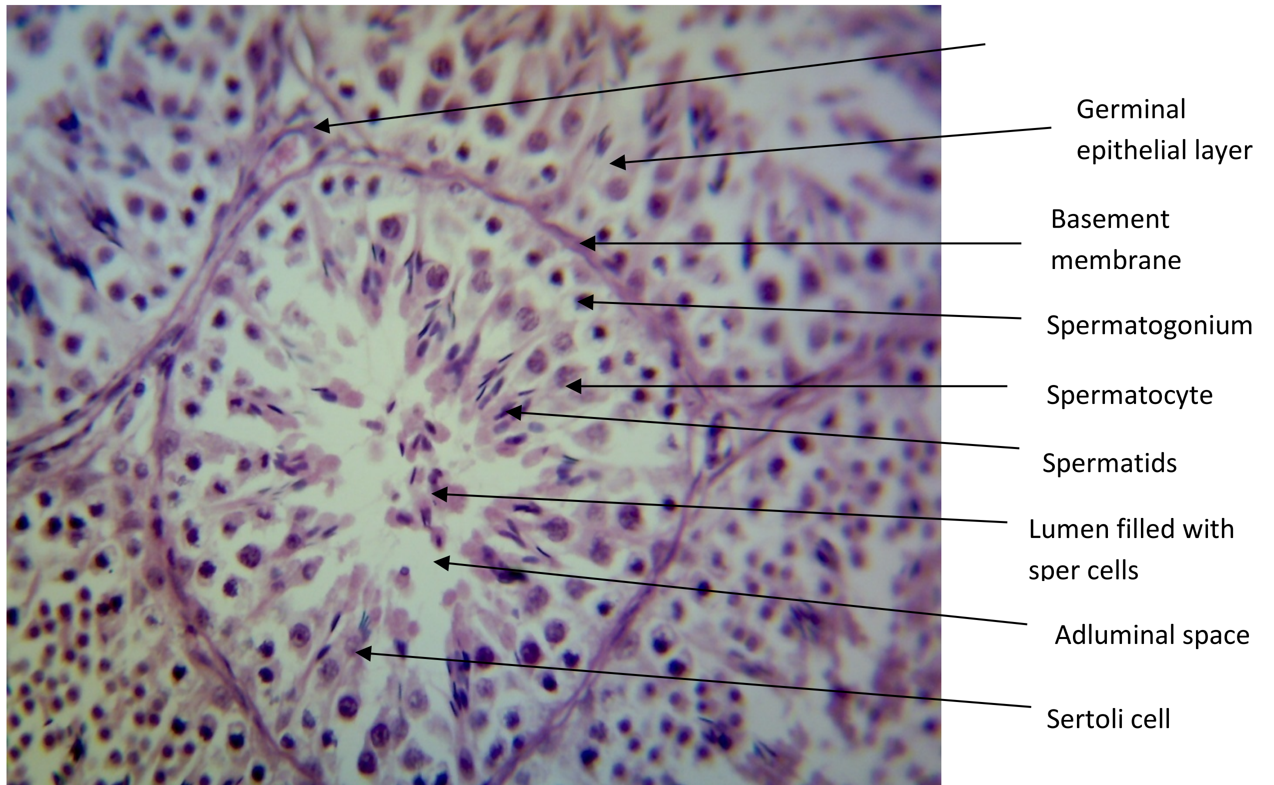


Figure 5.11: Seminiferous tubules of the testis of eight months old West African Dwarf bucks H & E × 400

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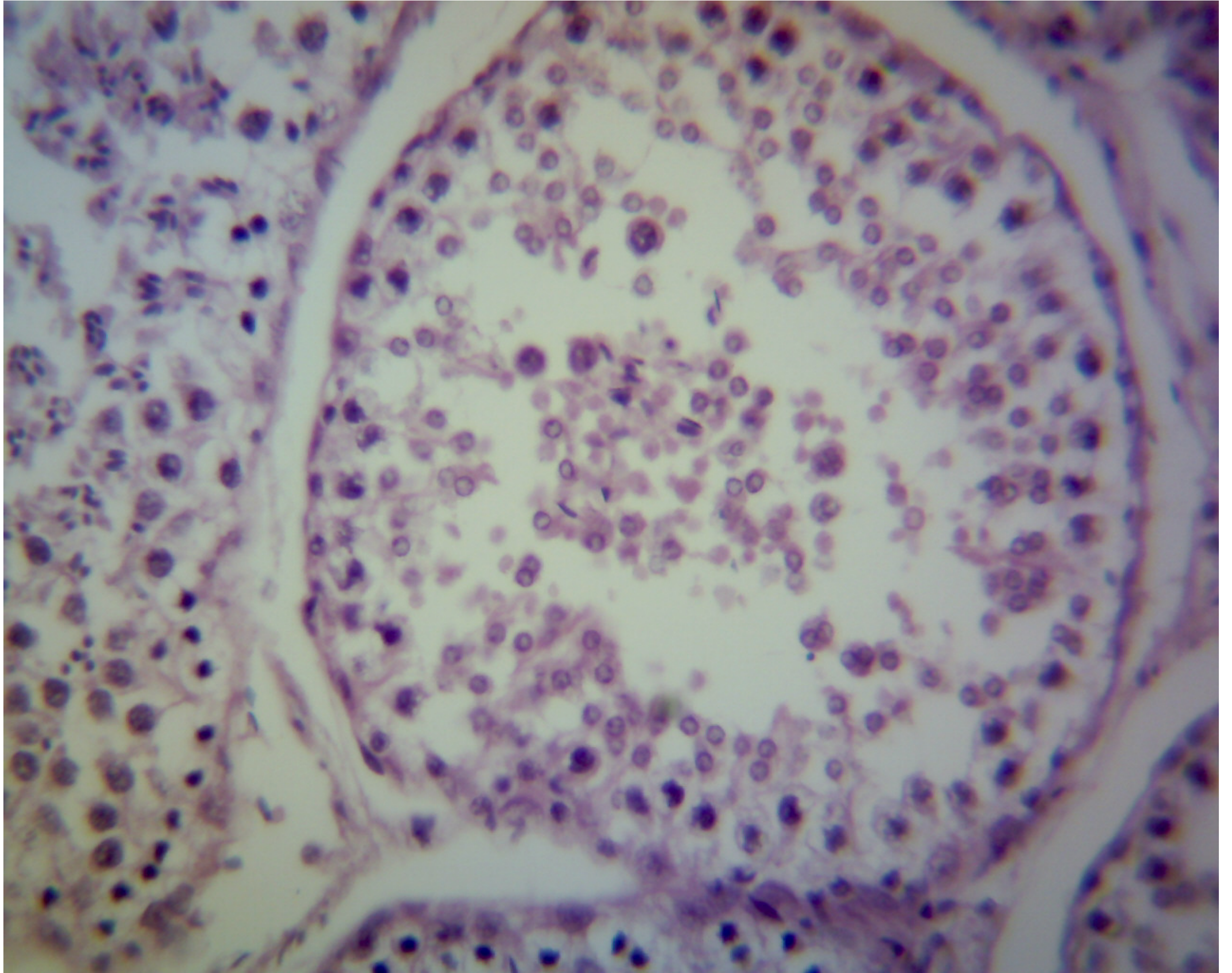

Figure 5.12: Seminiferous tubules of the testis of nine months old West AfricanDwarf bucksH & E × 400

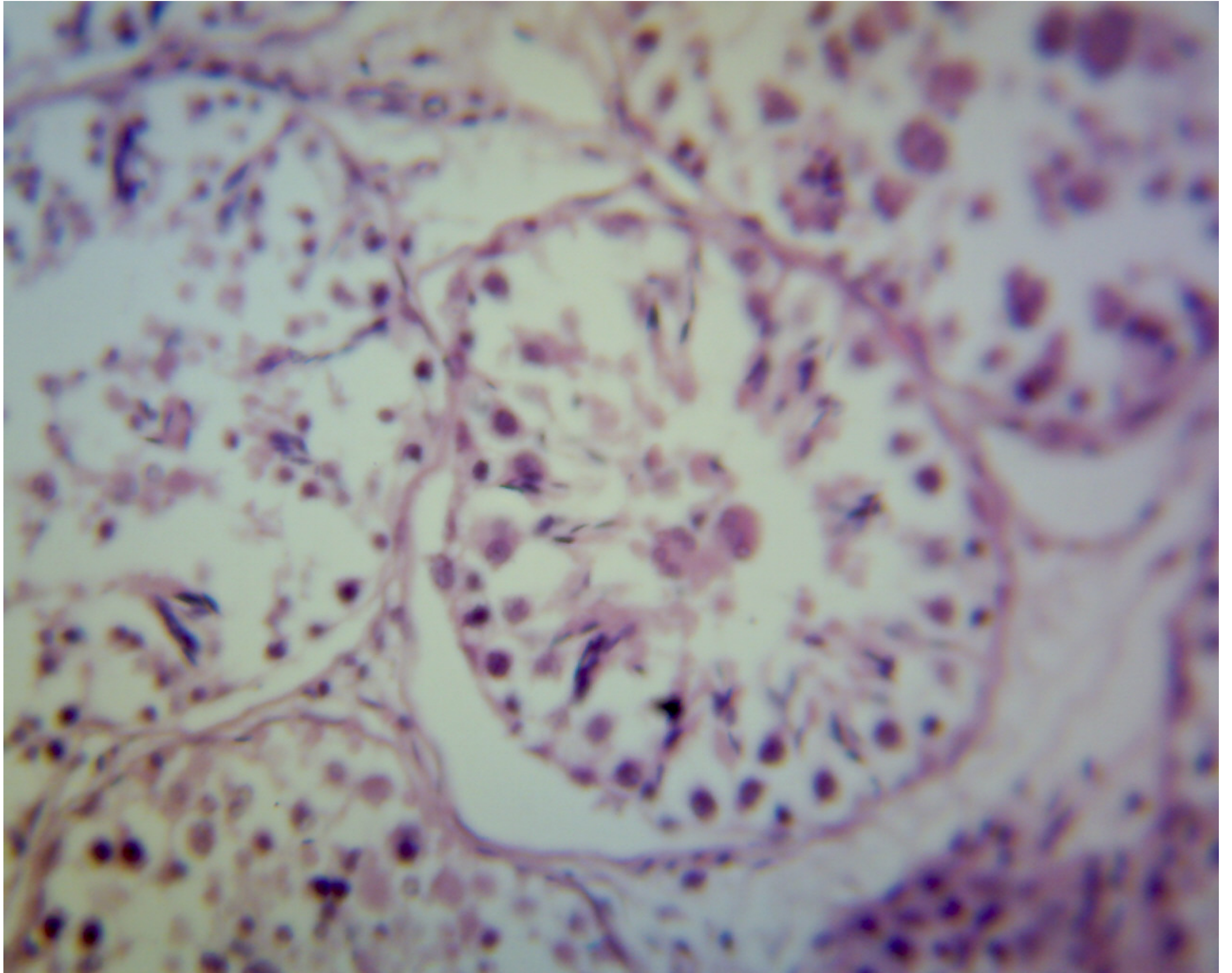

Figure 5.13: Seminiferous tubules of the testis of ten months old West AfricanDwarf bucksH & E $\times$ 400

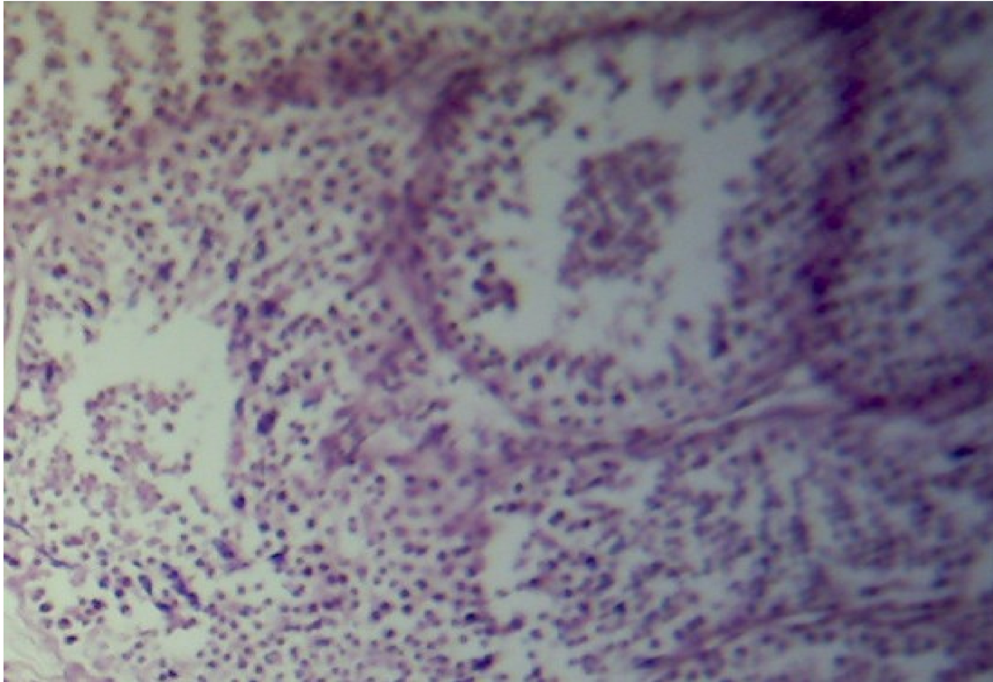

Figure 5.14: Seminiferous tubules of the testis of eleven months old West AfricanDwarf bucksH & E $\times$ 400

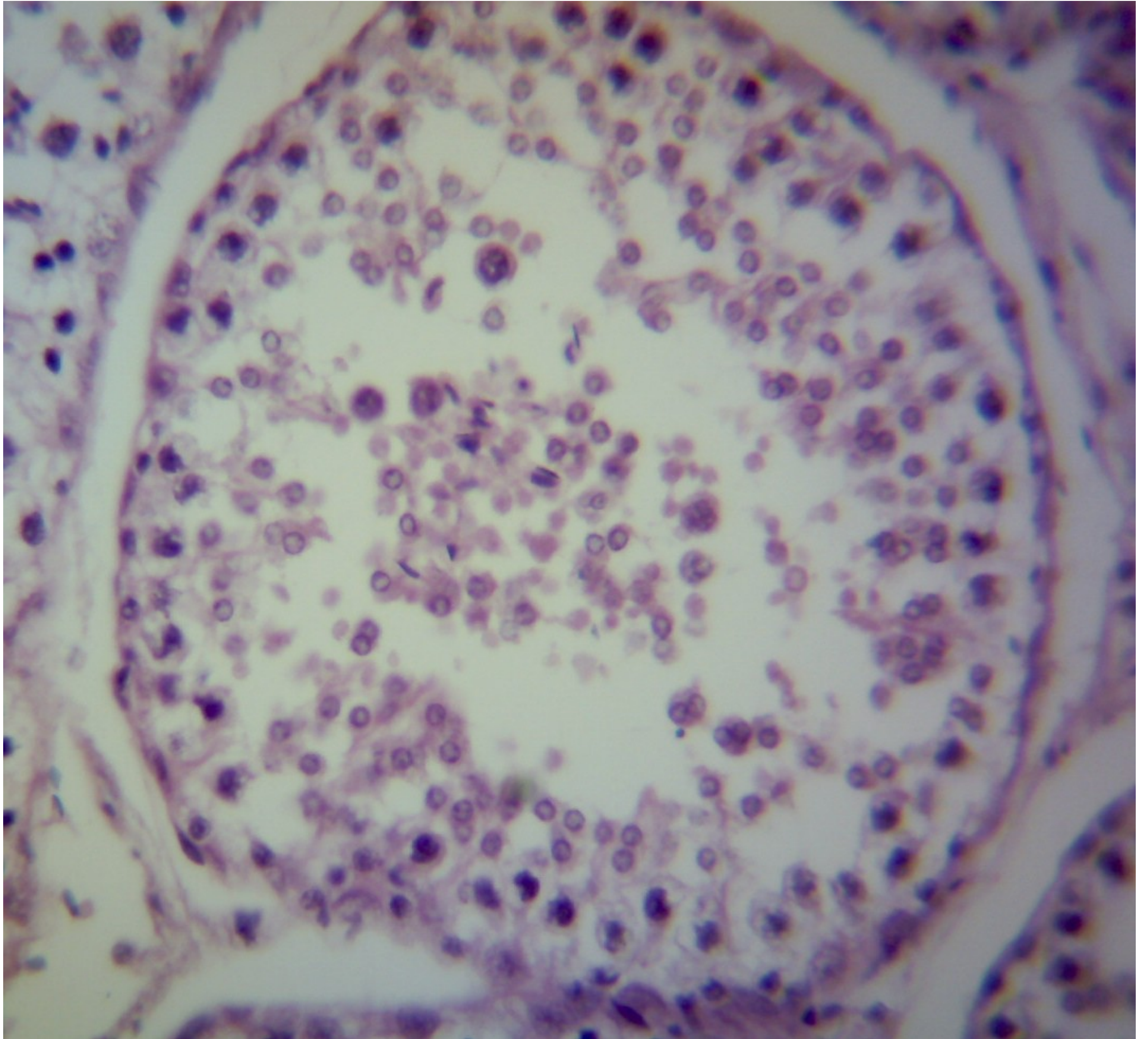

Figure 5.15: Seminiferous tubules of the testis of twelve months old West AfricanDwarf bucksH & E × 400

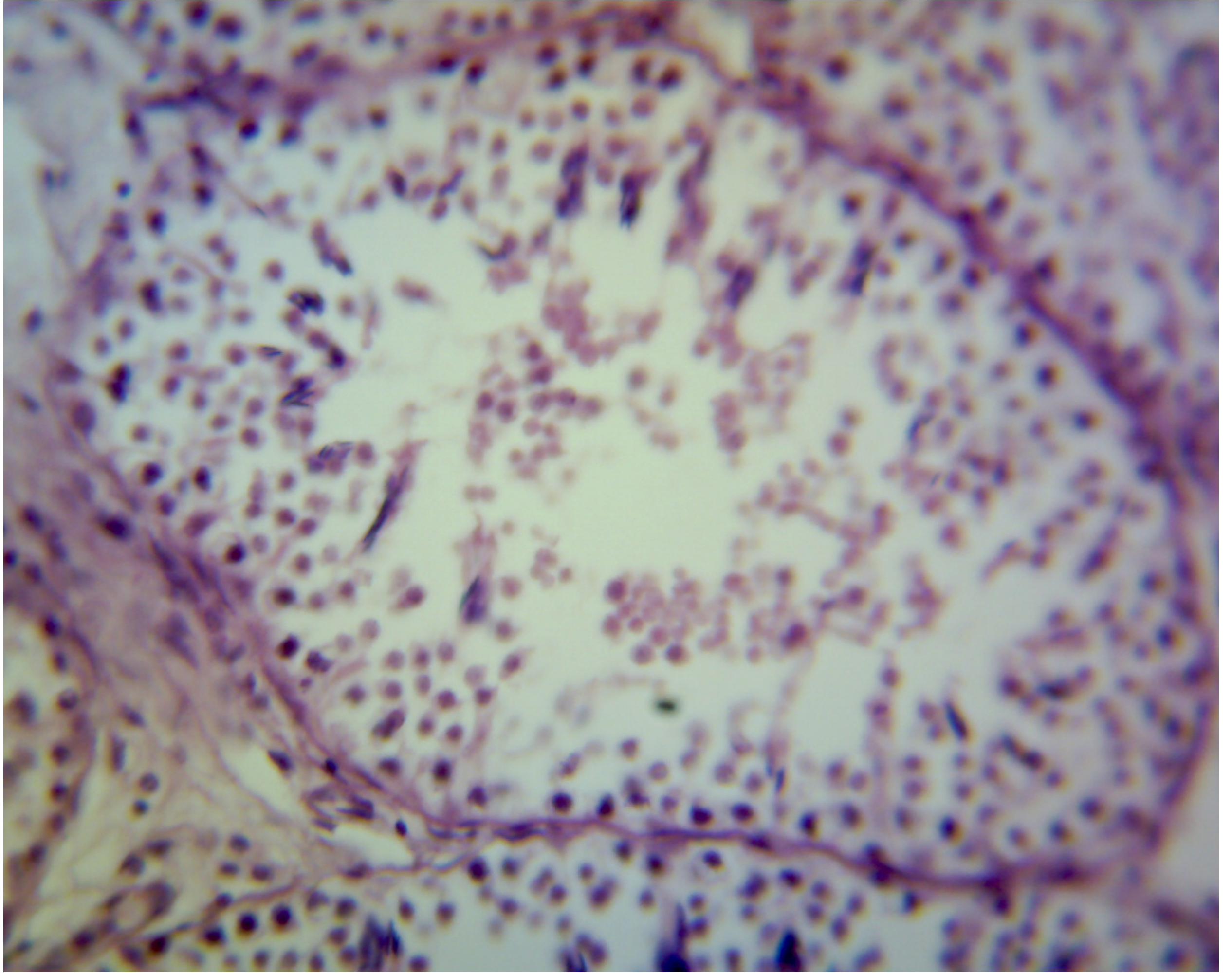

Figure 5.16: Seminiferous tubules of the testis of thirteen months old West AfricanDwarf bucks H & E $\times$ 400

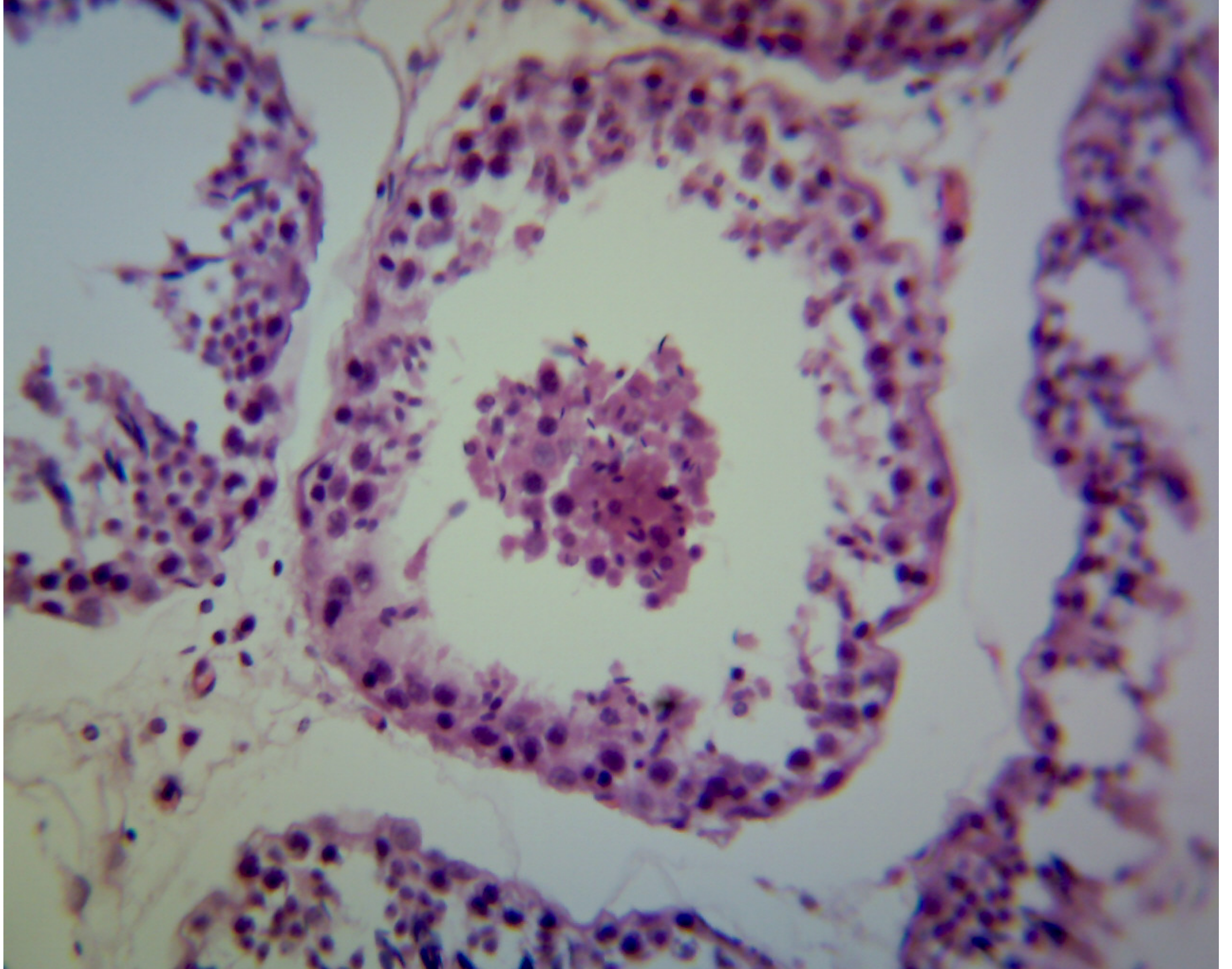

Figure 5.17: Seminiferous tubules of the testis of fourteen months old West AfricanDwarf bucksH & E × 400

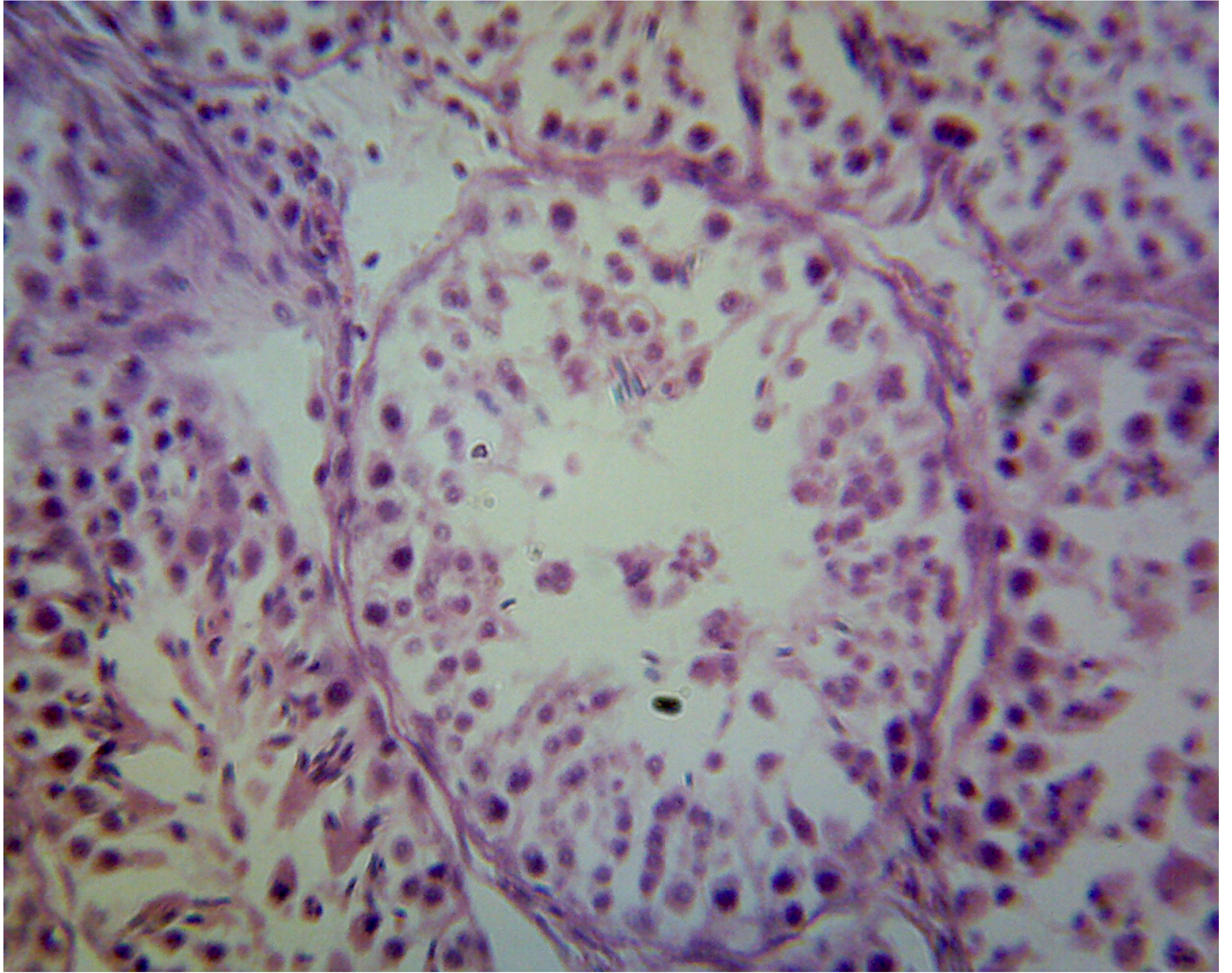

Figure 5.18: Seminiferous tubules of the testis of fifteen months old West AfricanDwarf bucksH & E $\times$ 400

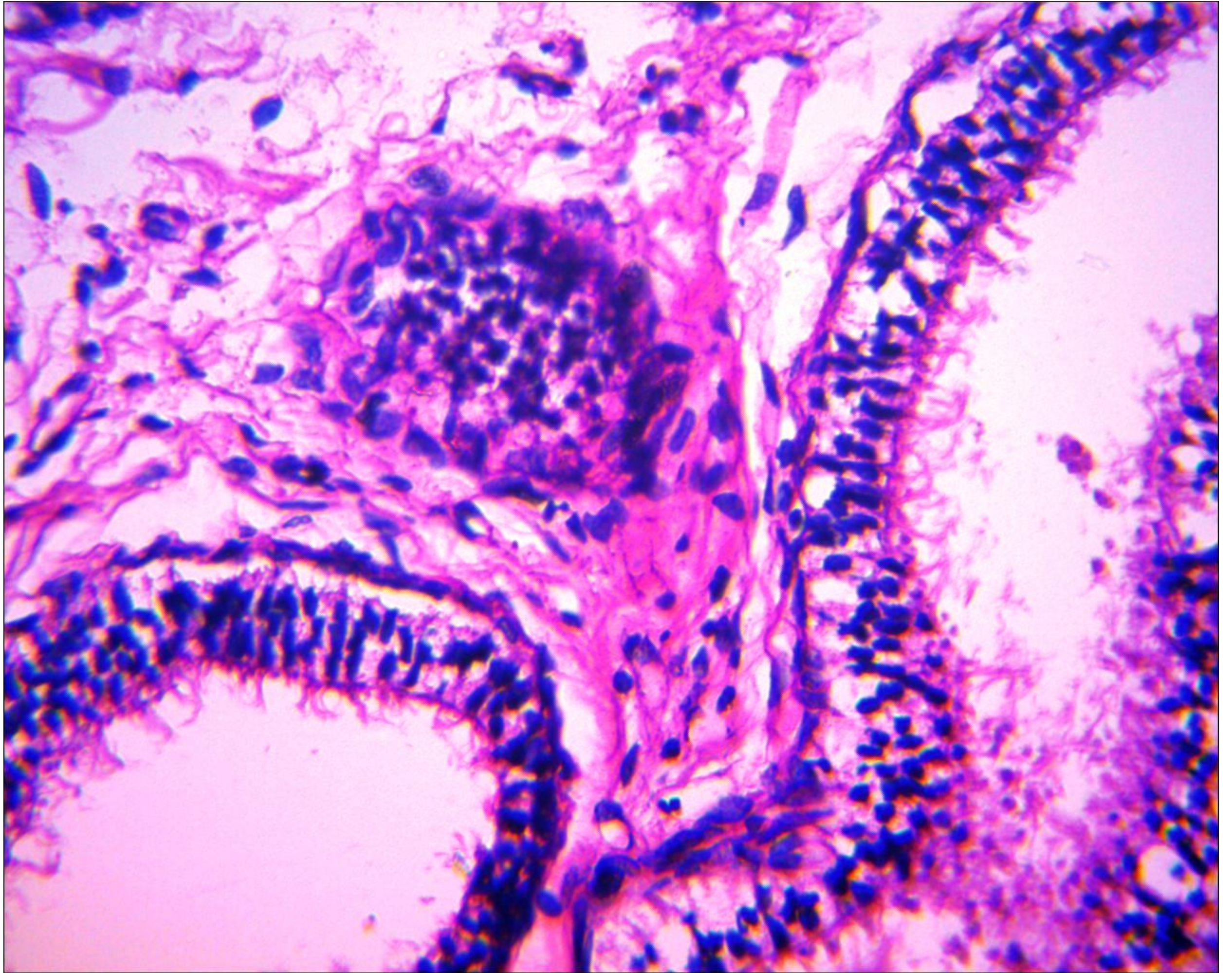

Figure 5.19: The head of epididymis of one month old West African Dwarf Buck H & E x 400

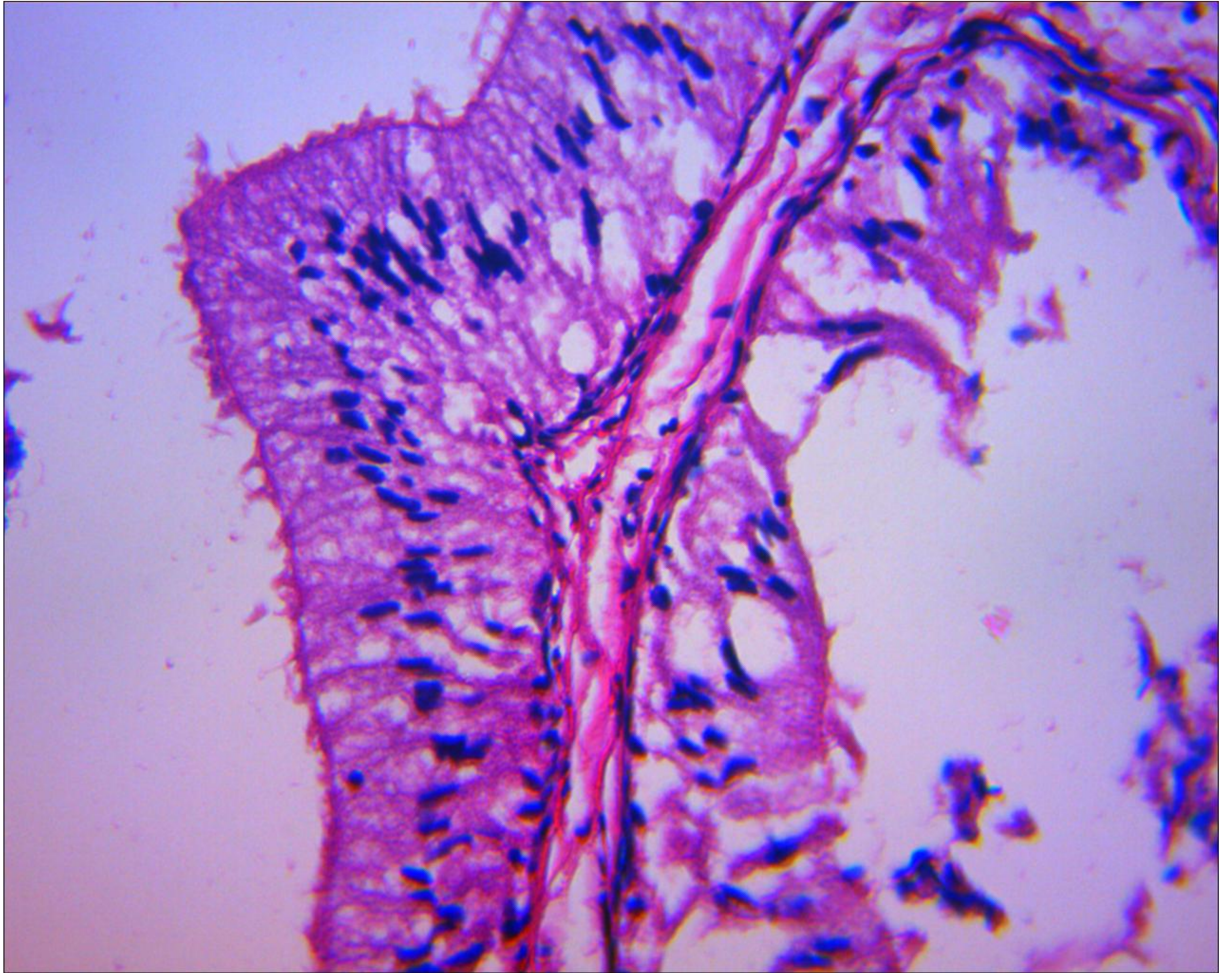

Figure 5.20: The body of epididymis of one month old West African Dwarf Buck H & E x 400

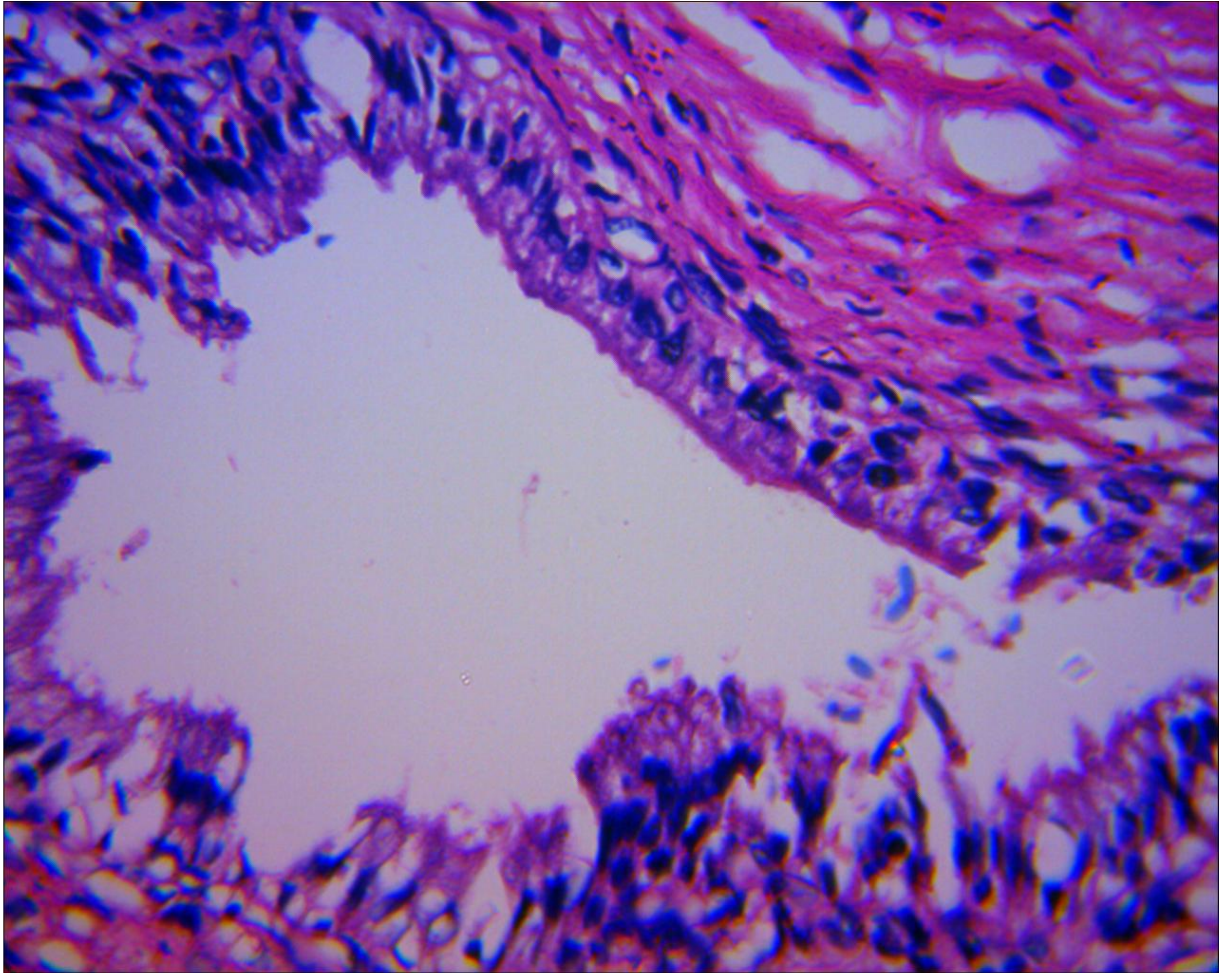

Figure 5.21: The tail of epididymis of one month old West African Dwarf Buck H & E x 400

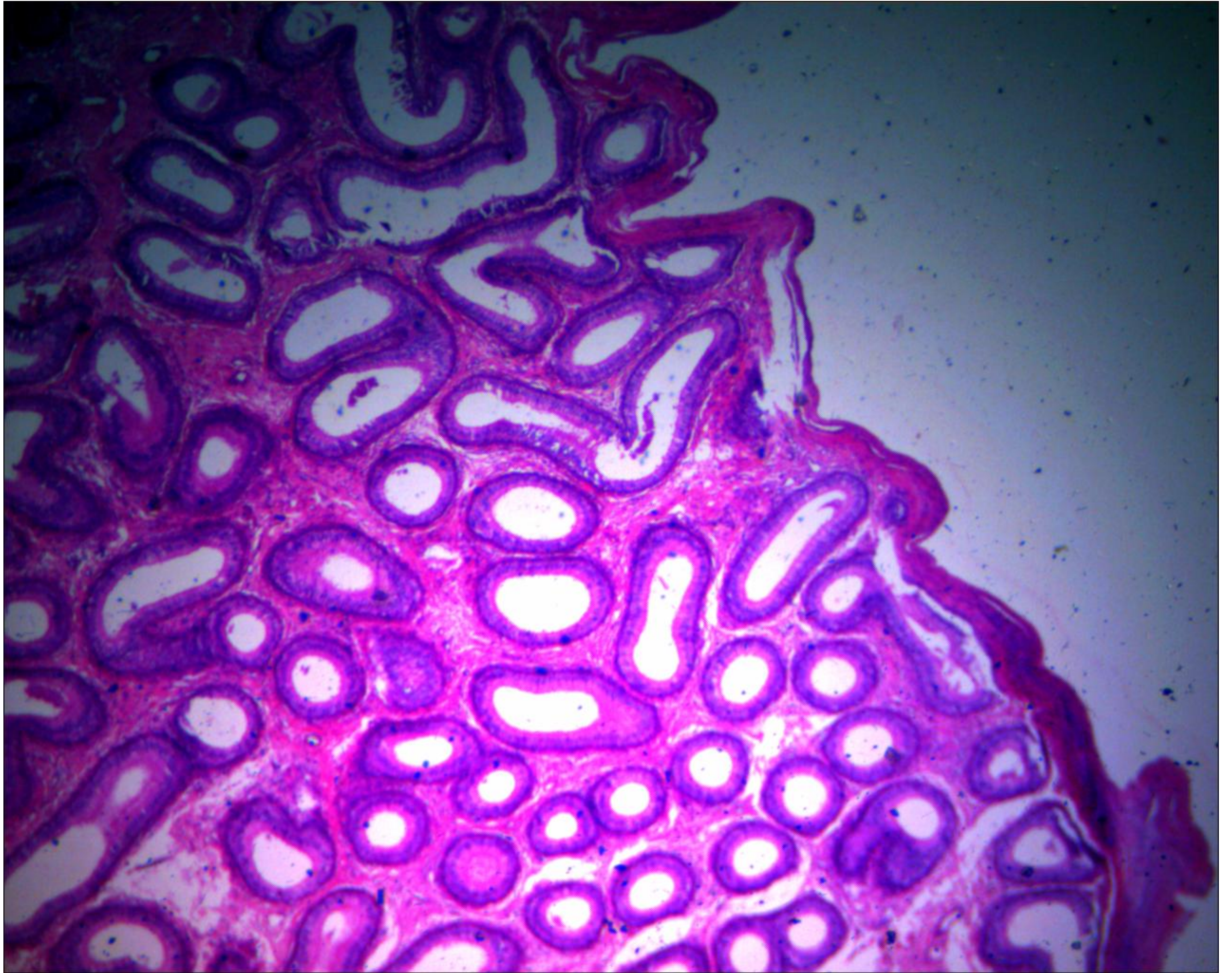

Figure 5.22: The head of epididymis of two months old West African Dwarf Buck H & E x 100

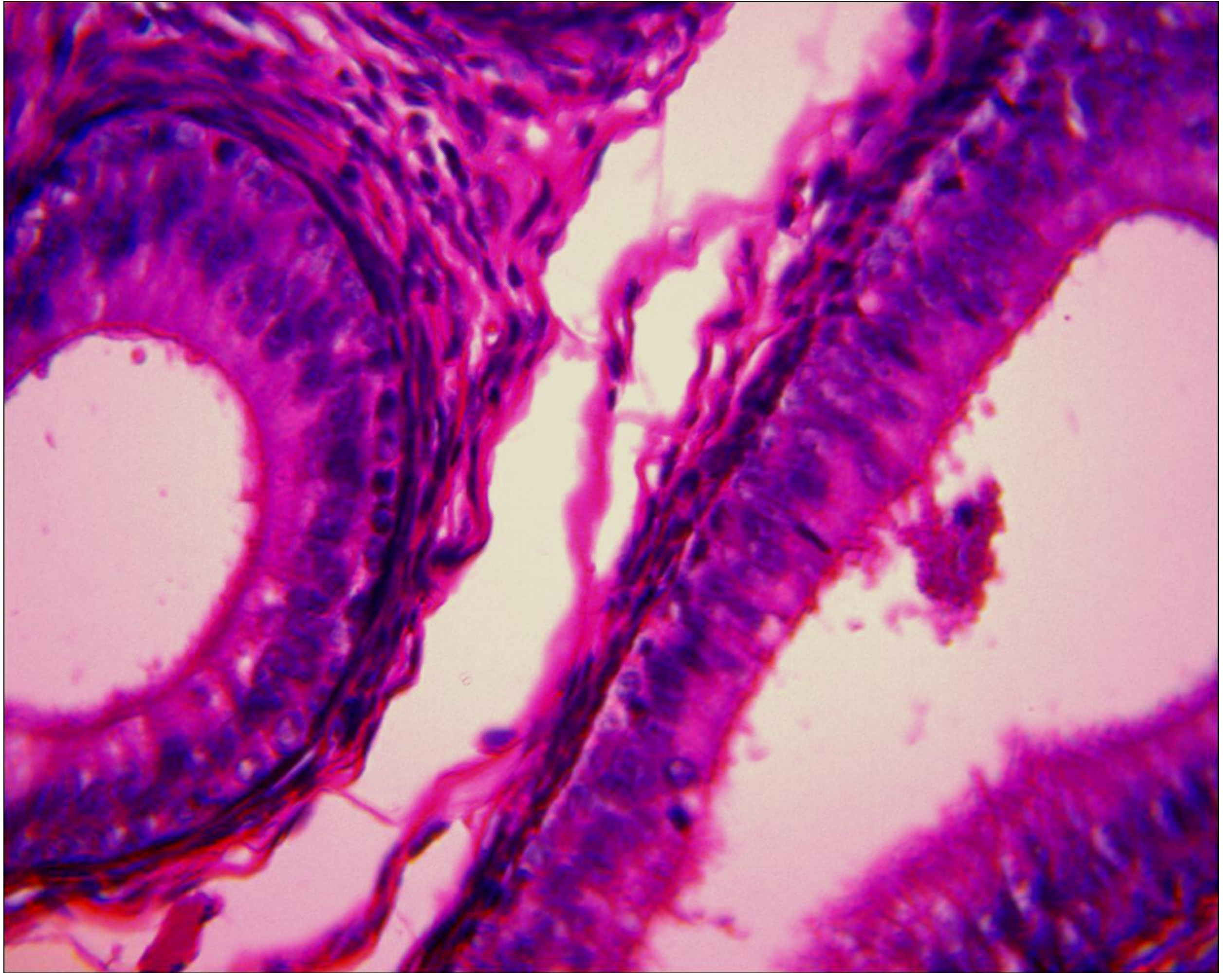

Figure 5.23: The body of epididymis of two months old West African Dwarf Buck H & E x 400

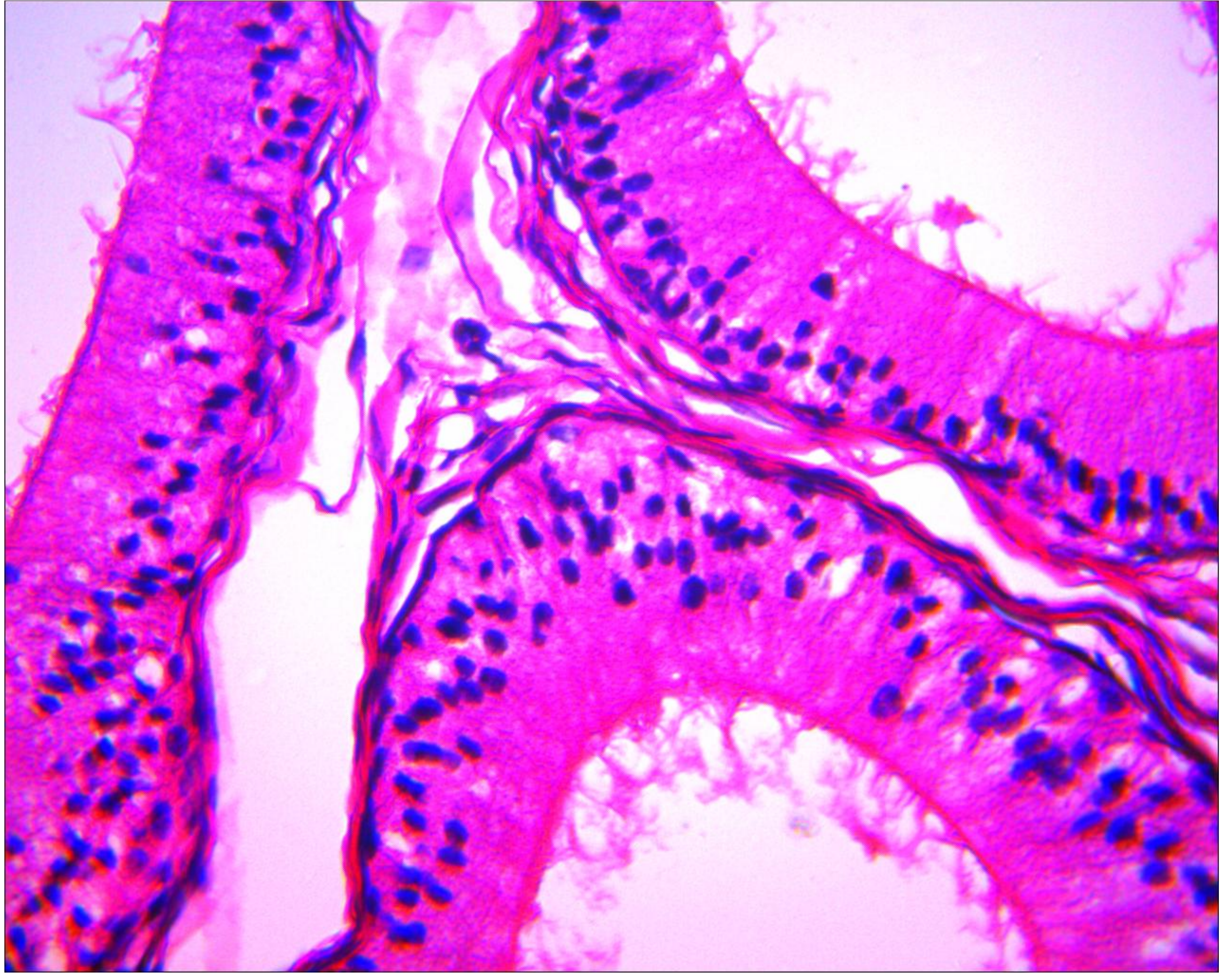

Figure 5.24: The tail of epididymis of three months old West African Dwarf buck H & E x 400

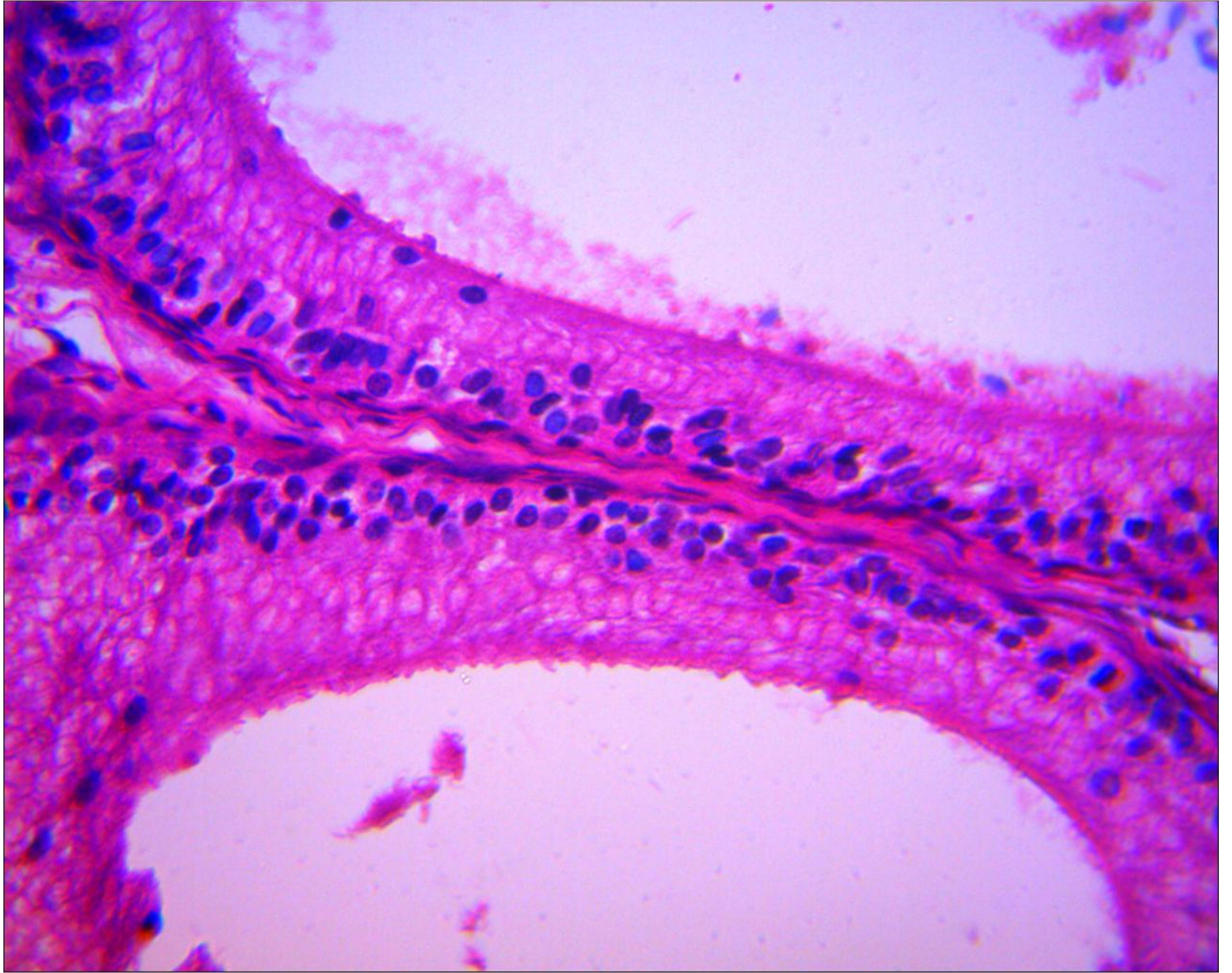

Figure 5.25: The head of epididymis of four months old West African Dwarf buck H & E x 400

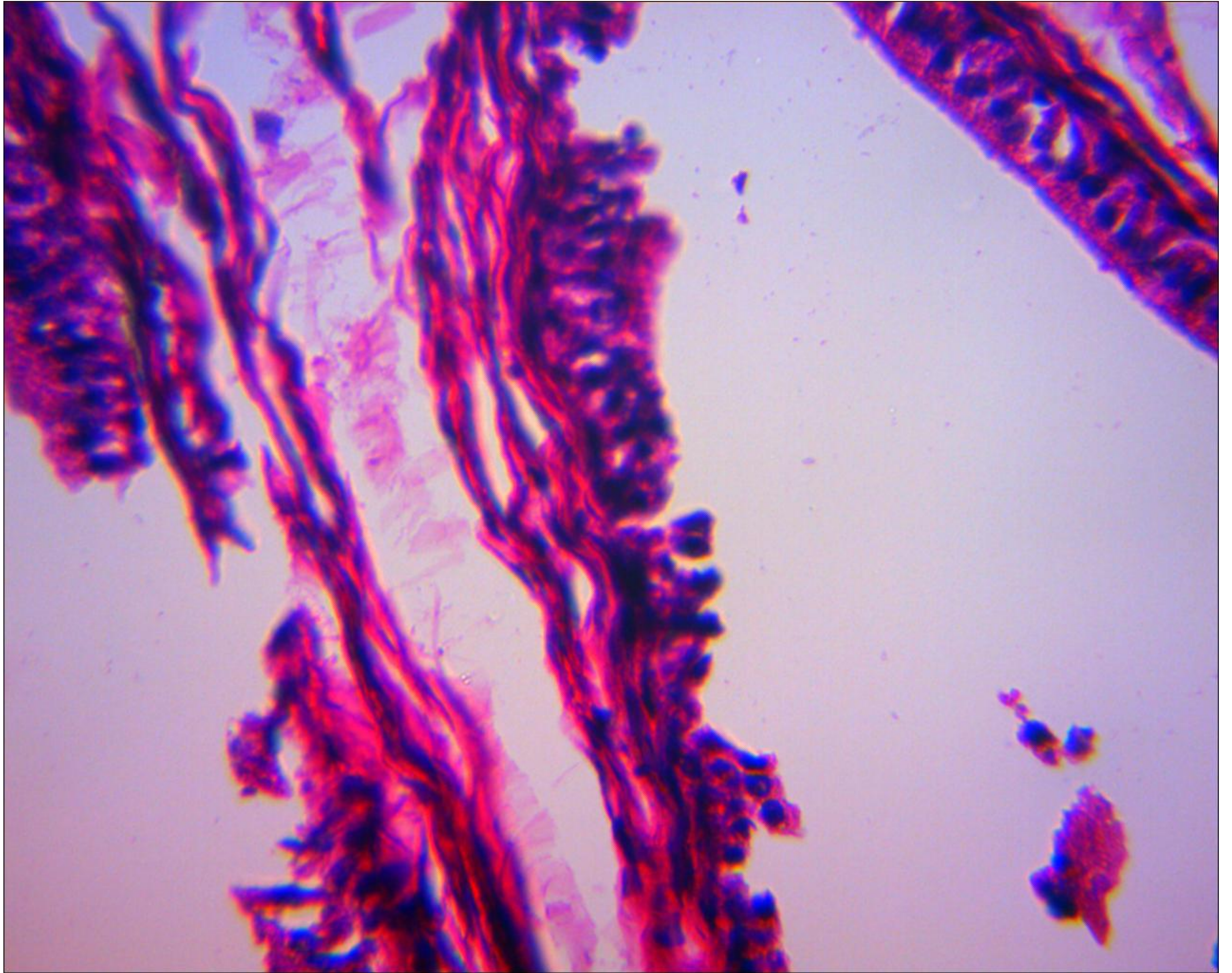

Figure 5.26: The tail of epididymis of four months old West African Dwarf buck H & E x 400

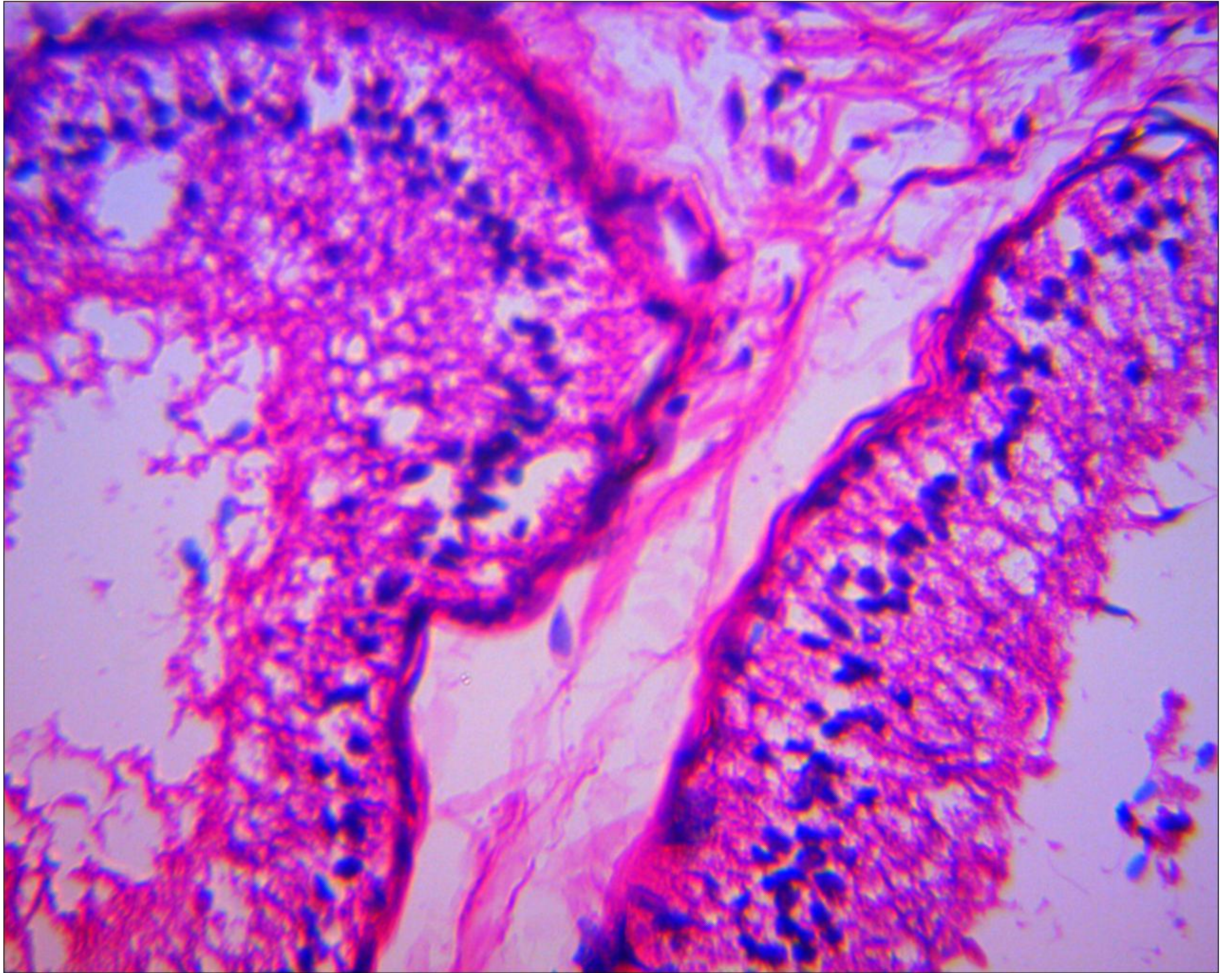

Figure 5.27: shows the head of epididymis of five months old West African Dwarf buck H & E x 400

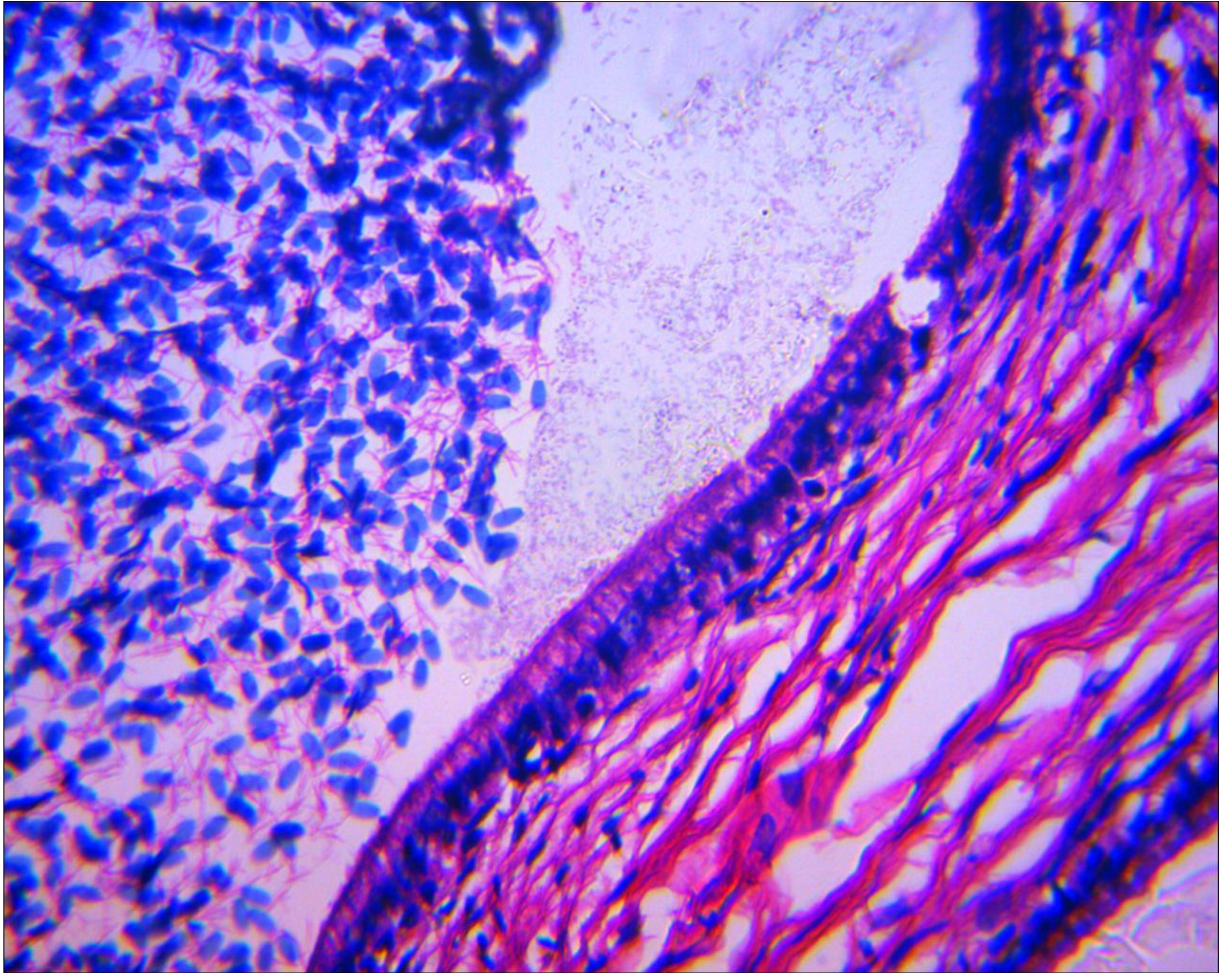

Figure 5.28: The tail of epididymis of six months old West African Dwarf buck H & E x 400

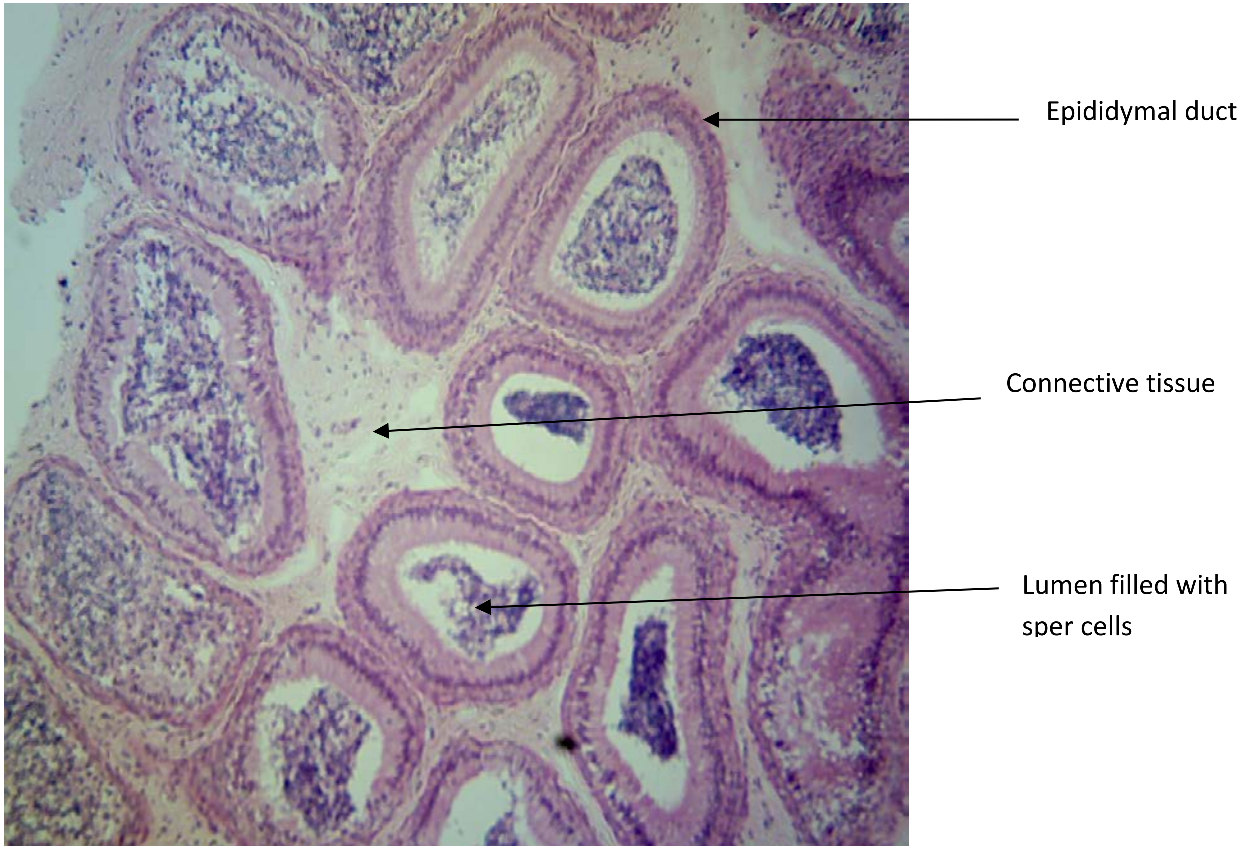

Figure 5.29: The caput epididymis of eight months West African Dwarf buck H & E × 100

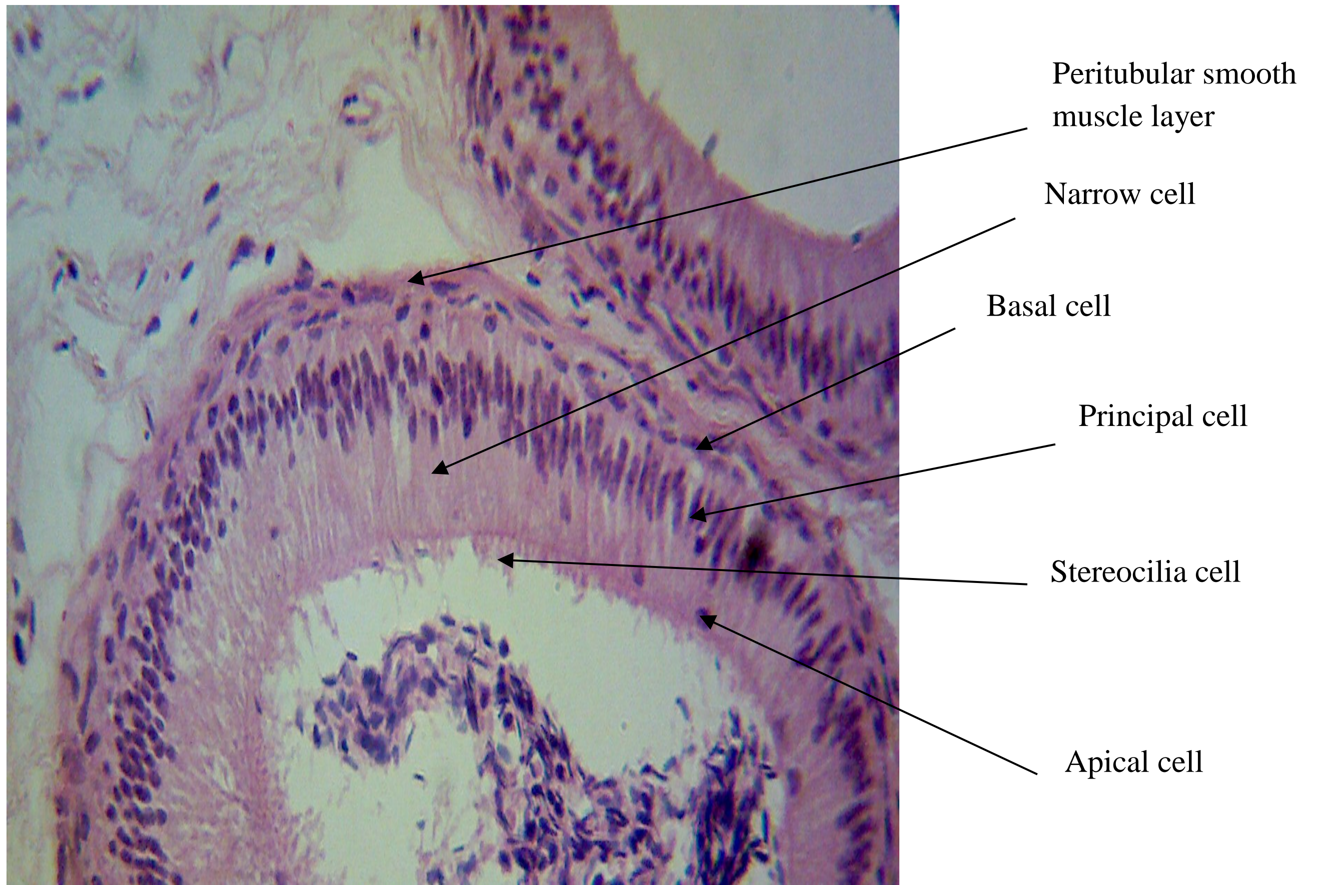

Figure 5.30: The caput epididymis of eight months West African Dwarf buck H & E × 400.

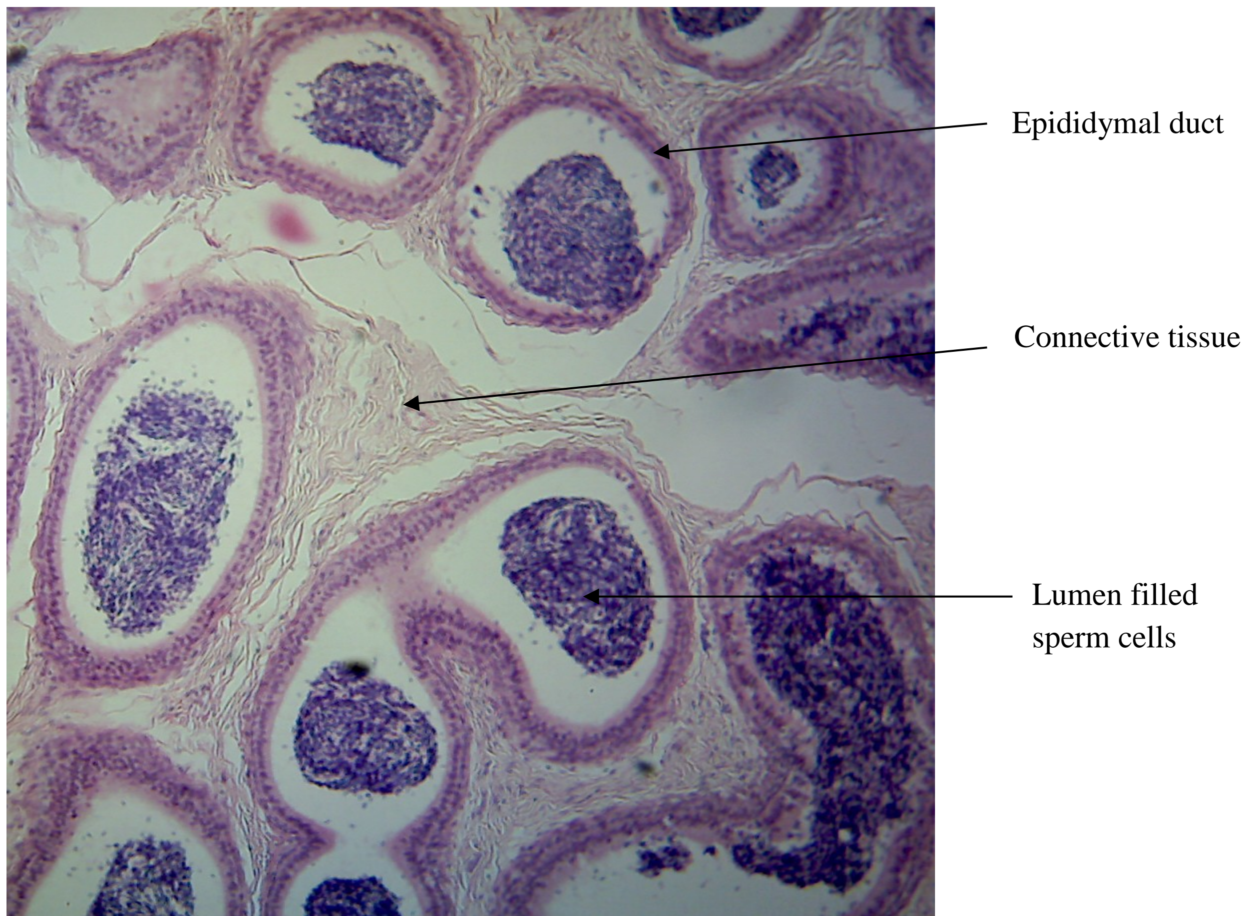

Figure 5.31: The corpus epididymis of eight months West African Dwarf buck H & E × 100.

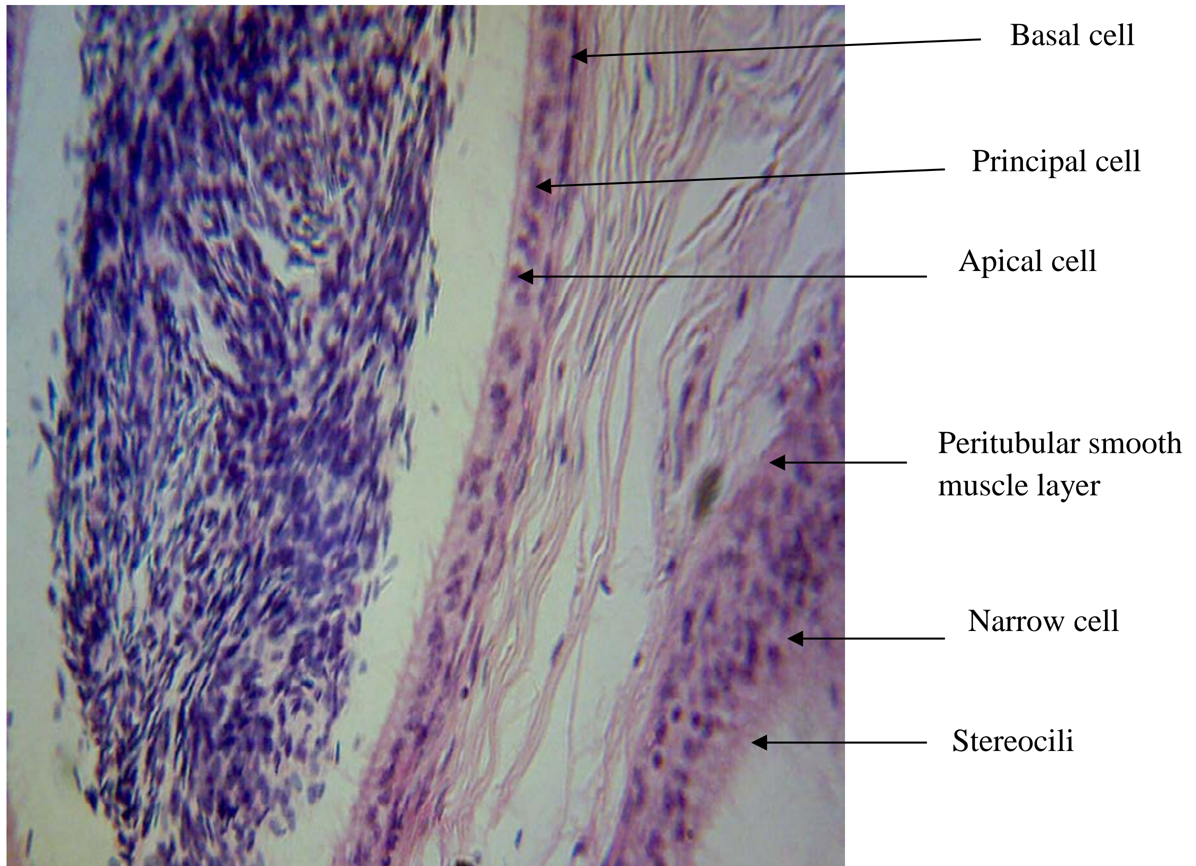

Figure 5.32: The corpus epididymis of eight months West African Dwarf buck H & E × 400.

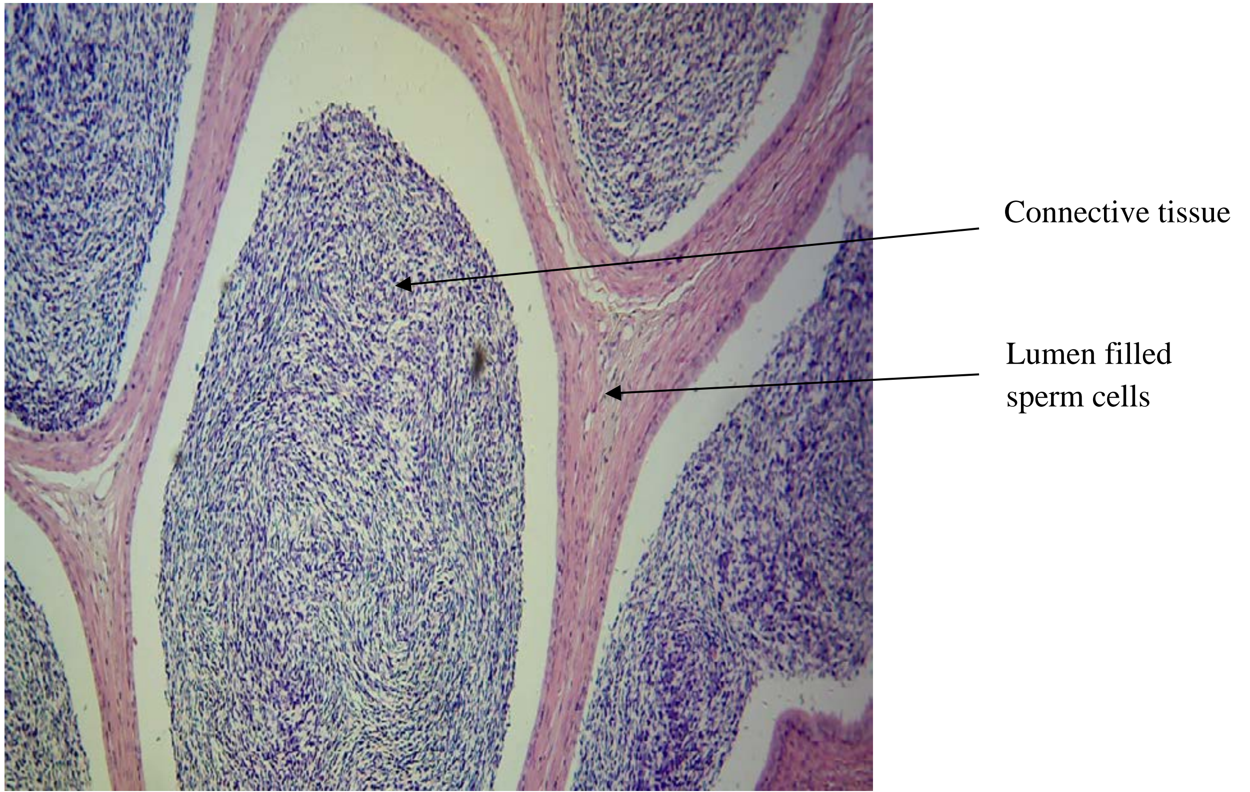

Figure 5.33: The cauda epididymis of eight months old West African Dwarf buck H & E × 100.

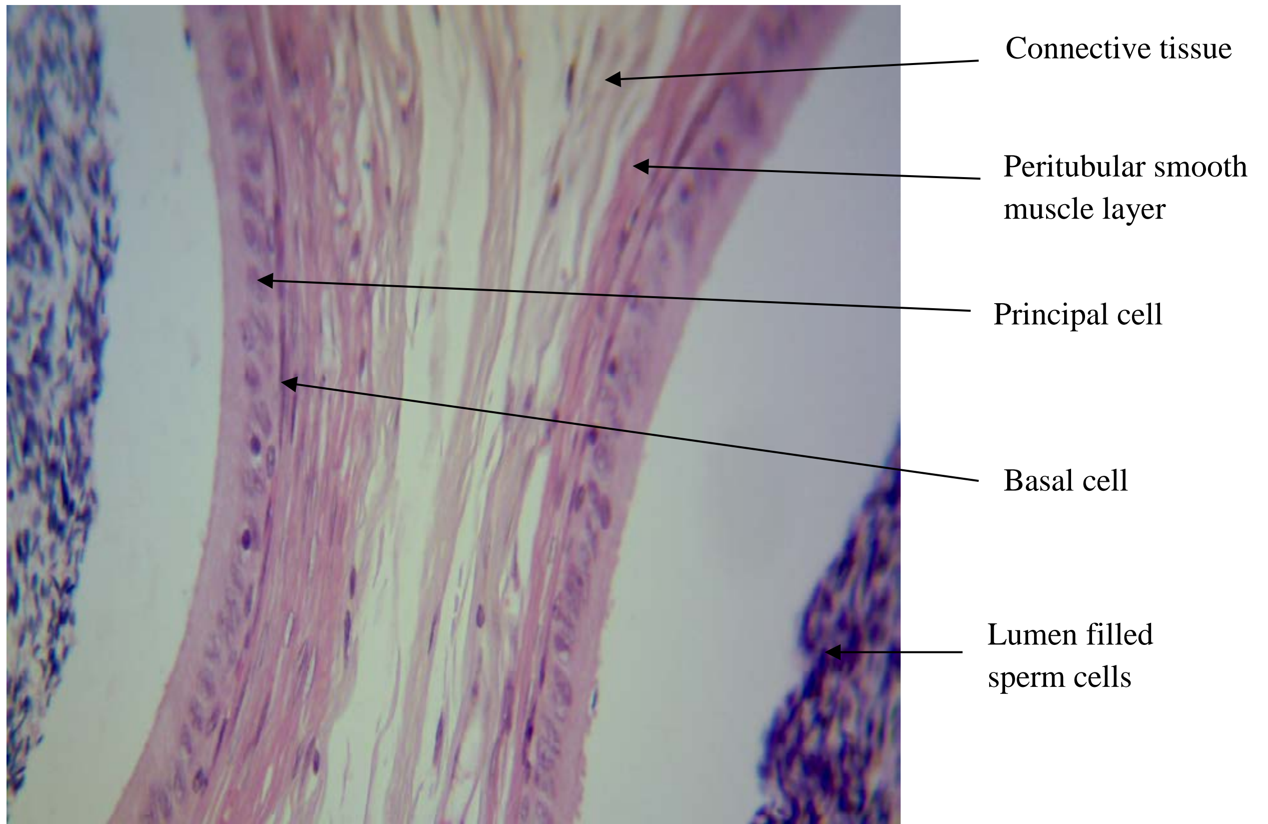

Figure 5.34: The cauda epididymis of eight months old West African Dwarf buck H & E × 400.

5.4.0 DISCUSSION

This study highlighted the morphological and morphometric characteristics of the testes and epididymides of one to fifteen months old WAD bucks. The first appearance of spermatozoa in the seminiferous tubules (ST) was in the six months old WAD bucks. At this age, the spermatozoa were scanty in the lumen of the ST. However at eight months, the ST lumen was densely packed with spermatozoa and was observed up to the age of fifteen months in the WAD bucks. Each of the testes was oval or ellipsoidal in shape and covered by a layer of dense irregular connective tissue called *tunica albuginea* and a network of finely arranged blood vessels of arteries and veins. On sagittal incision, the mediastinum testis appeared as a densely white longitudinal structure at the centre of the testis. These gross morphological characteristics of the WAD buck testis observed in this study were similar to those reported in Indian goat bucks (Nimase *etal.*, 2009; Mohammed *etal.*, 2011; Archana *etal.*, 2014). This suggests that there are no distinct testicular morphological variations amongst these breeds of goats. Gross morphometric results revealed that there were no significant differences in the weight, length, height and width of the right and left testes. These findings are similar to those reported by Bitto and Egbunike (2006) and Ugwu (2009) with the exception of the testicular height taken in this study which was not documented by these previous authors. From this additional parameter, calculation of the volume of each testis was done using the three different formulae (as described above). Of these formulae, the volumes of testes calculated from the PEF was the closest to the volumes obtained using the WDM. This corroborates the report in study two (i.e chapter four) that this formula is the best of the three in calculating the volume of the testis of WAD bucks. However, in contrast, the Lambert formula has been reported to be the best for calculating testicular volume in human (Sakamoto *etal.*, 2007; Sotos and Tokar, 2012). Further studies should be conducted to determine the best formula that would be most suitable for calculating testicular volume in other breeds of goats and species of animals. As regards using the water displacement method, it was also observed that, the weight of the WAD buck testis was approximately the volume of normal saline (water) it displaced (constituting its own volume). This implies that the heavier the testis, the more the volume of water it displaces, hence the higher its volume. Since testicular volume is an index of spermatogenesis (Kollin*et al.*, 2006; Sakamoto *et al.*, 2008), this implies that the heavier

the weight of the testis, the more the seminiferous tubules the testis contains, hence the more spermatozoa it will produce. The testicular weight and volume of the testes observed in this study was similar to those reported by Ugwu (2009).

Histological studies revealed that each testis comprised of numerous mostly rounded and some irregularly shaped ST which were arranged into lobules. Each of the ST was lined by a layer of basement membrane over which lied the germinal cell layers of spermatogonia, spermatocytes and spermatids in chronological order towards the lumen which is filled with sperm cells. Within these cells were the supporting cells (sertoli cells). In between adjacent testes were found the leydig cells. This is similar to earlier reports in Indian goats (Nimase *et al*., 2009); Northern great grey Kangaroo (*Macropus giganteus giganteus)*(Khamas *et al*., 2014) but the *tunica albuginea* was thicker and there were more numerous leydig cells between the ST in these animal; greater cane rat (Adebayo and Olurode, 2010) and African sideneck turtle (Olukole *et al*., 2014). Histomorphometry biometrics revealed that there were no significant differences between the right and left testes STD, SLD and GEH. This is similar to the report by (Olukole *et al*., 2014) in African sideneckturtle (*Pelusios castaneus*) but the biometric parameters were higher in values in the WAD bucks presumably because of species differences. However, Gofur (*et al.*, 2008) reported that the STD of the left testis was significantly wider than that of the right testis in the *Bos indicus* (an indigenous bull in Bangladesh). Probably, this may be due to species differences.

It was also observed that the epididymis lied on the cranio-dorsal to the caudo-dorsal aspect of the medial aspect of the testis. The epididymis was made up of three regions namely the head, body and tail regions. The head of the epididymis was flat and irregularly oval to polygonal in shape at the caudo-dorsal aspect of the testis. The body of the epididymis was a slender long cylindrical tissue lying on the medial aspect of the testis. The tail of the epididymis was a raised short stocky firm tissue extending a little beyond the caudo-dorsal aspect of the testis.The lumen of the head, body and tail of epididymis were filled with sperm cells in increasing order respectively. These were first noticed in the six months old WAD bucks with an increasing order in the volume of spermatozoa in the lumen across all of the regions of the epididymal ducts up to eight months of age. The head of the epididymis was made up of tall columnar epithelium with basal nuclei and scanty sperm

cells. The free ends of the surface of these cells possessed stereocilia. The body was characterized by low columnar epithelium with prominent stereocilia and small groups of sperm cells within the lumen. The tail was made up of cuboidal epithelia but without stereocilia and large amount of spermatozoa were observed within the lumen. These findings on the epididymis of WAD goat bucks were similar to those reported in the Indian goats *(Capra hircus*) (Sharma *et al*., 2014), in dromedary camel (Alkafafy *et al.*, 2011) and domesticated adult great cane rat (Olukole and Obayemi, 2010). However, the entire epididymal regions (head, body and tail) in this case were lined by pseudostratified columnar epithelium with stereocilia and large amount of sperm cells within the whole length of the epididymis (Khamas *et al*., 2014).

In conclusion, the study highlighted the characteristic features of the testes and epididymides at the prepubertal (one to five months), pubertal (six months) and OBA (i.e. eight months) in WAD bucks. These findings suggest that although WAD bucks semen may be capable of producing semen at six months; however their best semen production capability commences at eight months. Also, the morphological and morphometric data observed for the testes and epididymides can stand as references for these WAD bucks as well as for regional comparative reproductive anatomy studies of goat bucks.

CHAPTER SIX

6.0 TESTOSTERONE, LUTEINIZING HORMONE AND FOLLICLE STIMULATING HORMONE PROFILES IN RELATION TO OPTIMUM BREEDING AGE IN WEST AFRICAN DWARF BUCKS

6.1 INTRODUCTION

Reproductive hormones such as testosterone, Follicle Stimulating Hormones (FSH) and Luteinizing Hormones (LH) play significant roles in goat buck reproduction (Grasselli *et al*., 1992; Amrane *et al.*, 2013) similar to what occurs in other male animals. Testosterone plays key roles in the development of the testes, prostate and production of spermatozoa. Apart from this, it also increases libido and frequency of erection as well as secondary sexual characteristics amongst other numerous roles in the goat bucks (Daramola *et al*., 2006; Leite-Browning, 2009). The LH (Interstitial Cell Stimulating Hormone - ICSH) is produced by gonadotroph cells in the anterior pituitary and is responsible majorly for the stimulation of the production of testosterone by the leydig cell acting synergistically with the FSH. Its production is under the influence of Gonadotropin Releasing Hormone (GnRH) which is produced from the hypothalamus (Mckeown *et al.*, 1997; Ochiogu *et al.*, 2015). The FSH also synthesized and secreted by the anterior pituitary, stimulates the proliferation and secretory activities of the sertoli cells. Sertoli cells function to aid spermatogenesis, move developing sperm cells to the lumen of the seminiferous tubules and to reduce motility and capacitation of sperm cells to maintain viability (Hafez and Hafez, 2000; Mullen, *et al.*, 2013). Therefore, this study was conducted to evaluate testosterone, LH and FSH concentrations in relation to semen production, attainment of puberty and OBA in WAD bucks.

6.2.0 MATERIALS AND METHODS

6.2.1 Animals

For this study, six bucks were selected per age group (of one to fifteen months old) from the450 WAD bucks raised semi-intensively used in study one (i.e chapter three). All bucks used were all in good reproductive and health conditions based on the criteria from study one.

6.2.3 Blood sample collection and handling

Blood were collected at 8.00 a.m in the morning twice a week from the bucks via the jugular vein into non-heparinized sample bottles. The blood samples were allowed to clot then centrifuged at 3000 revolutions for 30 minutes to separate serum from the whole blood. The serum samples were carefully decanted into plain bottles and stored in a deep freezer at - 40^0C until used. These serum samples were assayed for serum concentration of testosterone, FSH and LH level using Enzyme-Linked Immunosorbent Assay (ELISA) technique. The hormonal assay was done at the Laboratory of Reproductive Physiology and Developmental Programming (LRPDP), Department of Physiology, Faculty of Basic Medical Sciences and the Laboratory of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, both of the University of Ibadan. ELISA kit was used for the immunoassay. Basically, the procedure for running the assays was the same.

6.2.4 Hormonal assay procedure

0.50ml of calibrator, control serum and samples were pipetted with new disposable tips into appropriate wells. The first six wells of the assay kits (Testosterone, FSH and LH) were filled with their hormones in protein based buffer that was provided together with the kits (with known concentrations). The next wells were filled with the control serum and 0.1ml (100µl) of conjugate was pipetted into each well. The micro-plate was gently swirled for 20-30 seconds to mix and then covered. This was followed by incubation. The FSH and LH were incubated for 90 minutes at room temperature.Testosterone was incubated on a thermo-shaker (approximately 500-800 rpm) for 30 minutes at 37°C. The content of the micro-plate was discarded by decantation and it was blotted with absorbent paper. 0.3ml

(300µl) of washing solution was added to each micro-plate and it was decanted and blotted. The washing procedure was repeated for four more times. 0.1ml (100µl) of Tetramethylbenzidine (TMB) substrate was pipetted into each well at timed intervals. The plate was incubated for 15-20 minutes for FSH and LH but for 25 ± 5 minutes for testosterone at room temperature in the dark. 0.15ml (150µl) of stopping reagent was added into each well at the same time interval. It was gently mixed for 5-10 seconds. The plate was read off in a micro-plate reader at 450nm within 20 minutes after addition of the stopping reagent. The mean of the optical density of each duplicated calibrator was calculated. The optical density of each unknown duplicate was calculated. The best-fit curve was drawn through the plotted points on the linear graph with the mean optical densities on the Y-axis and the calibrator concentration on the X-axis. To determine the concentration of LH, for an unknown, the average absorbance of the duplicate for each unknown on the vertical axis of the graph was located and the concentration was read (in mIU/ml) for the horizontal axis of the graph. The ELISA reader calculates the result automatically. This same procedure was repeated for testosterone and FSH concentration in the samples.

6.2.5 Statistical analysis

Data were expressed as Mean ± SD and analyzed using one way analysis of variance (ANOVA) followed by a post hoc test; Duncan multiple range test at $\alpha_{0.05}$ using SPSS version 20.

6.3.0 Results

6.3.2 Hormonal assay results

The results for hormonal studies are as shown on Table 6.1. The testosterone concentrations [$F_{1, 5}$=4636.4] increased significantly from one month (0.1±0.0 ng/ml) to eight months (4.7±0.2 ng/ml) without further significant increase up to 15 months. Similarly, the LH concentrations [$F_{1, 5}$=50066.3] increased significantly from one month (4.7±0.1 ng/ml) to eight months (13.6±0.2 ng/ml) without further significant increase up to 15 months. Also, the FSH concentrations [$F_{1, 5}$=1808.9] increased significantly from one month (9.5±0.1 ng/ml) to eight months (13.1±0.2 ng/ml) without further significant increase up to 15 months.

Table 6.1: Testosterone, Luteinizing hormone and Follicle stimulating hormone profiles of West African Dwarf goat bucks.

Age (months)	**N**	**Testosterone (ng/ml)**	**LH (ng/ml)**	**FSH (ng/ml)**
1	6	0.1±0.0*afh	4.7±0.1* afh	9.5±0.1* afh
2	6	0.2±0.1*	5.8±0.1*	10.2±0.1*
3	6	0.4±0.1*	7.0±0.2*	10.7±0.2*
4	6	0.8±0.1*	8.6±0.2*	11.3±0.2*
5	6	1.4±0.2*	9.8±0.2*	12.1±0.2*
6	6	2.6±0.2**fah	12.3±0.1** fah	12.6±0.1* fah
7	6	3.3±0.2*	13.2±0.1*	12.7±0.2
8	**6**	**4.7±0.2**haf**	**13.6±0.2** haf**	**13.1±0.3* haf**
9	6	4.3±0.2	13.3±0.2	13.0±0.1
10	6	4.2±0.2	13.2±0.2	12.8±0.2
11	6	4.3±0.2	13.1±0.2	12.7±0.2
12	6	4.3±0.2	13.2±0.2	12.7±0.2
13	6	4.3±0.2	13.0±0.2	12.6±0.2
14	6	4.3±0.2	13.1±0.2	12.6±0.2
15	6	4.2±0.1	13.0±0.1	12.5±0.3

*Significant at $\alpha_{0.05}$ i.e when row afh – row a is compared to those of f and h, etc.

6.4.0 DISCUSSION

This study revealed that reproductive hormones (especially testosterone) were valuable in determining the OBA in WAD bucks. The testosterone concentrations increased significantly in WAD kids (i.e from one to five months) to six months in pubertal WAD bucks and up to the eights months old WAD bucks of the OBA; after which there appeared not to be further significant increase up to the fifteen months old WAD bucks. This increase was particularly significantly higher at puberty and especially at the OBA when compared to the other age groups of WAD bucks studied; probably because of the onset and increase in spermatogenesis. Studies on reproductive hormones such as testosterone in relation to puberty and OBA in WAD bucks are very scarce. However, Daramola *et al*., (2007) observed similarly to this study, increase in testosterone concentrations in WAD kids of the control group in an experiment where they induced puberty in WAD kid bucks of two to three and half months old using melatonin. Although, there was slight variations in testosterone concentrations observed which may be due to the difference in management of the WAD bucks; in this study semi-intensively raised WAD bucks were used compared with the experimentally or intensively raised WAD bucks they used.

The trend for LH was similar to that of testosterone in terms of increase in concentration, also at puberty and OBA. Research works on LH in relation to puberty and OBA in WAD bucks are very scarce. However, similarly but with a slight variation to the report in this study, Chakraborty *et al*., (1989) reported increase in LH concentration in Nubian goats from birth to twenty weeks of age (i.e before puberty which they determined to be 32.4±0.9 weeks) and then significantly declined to fourty four weeks of age. This variation may be due to breed and study condition differences.

There was no significant increase in FSH at puberty and OBA as observed for testosterone and LH. This corroborates earlier reports that LH and testosterone are more importantly required than FSH for spermatogenesis (Sanford *et al*., 1977; Amrane *et al*., 2013); certainly not undermining the role of FSH in spermatogenesis.

In conclusion, this study also corroborates earlier observations in studies one, two and three (i.e chapers three, four and five of this study) that puberty occurred at six months while the

OBA was at eight months in WAD bucks raised semi-intensively. However, it should be stated that hormone concentration should always be combined with other correlates in determining OBA in WAD bucks. It is therefore suggested that the best time to introduce WAD bucks raised semi-intensively for breeding is eight months when maximum semen production capability is attained.

CHAPTER SEVEN

7.0.GENERAL DISCUSSION

Protein is the building block of life. Almost all of the important cells, tissues and organs are protein dependent structurally and functionally. Antibodies which are required for body defence against diseases are proteins. Spermatozoa which are the most important products from the male animals are protein. Insulin is a small stable protein that is required for the regulation of blood sugar levels. These are just few examples of invaluable protein materials and their importance to the survival of man and animals (Hoffman and Falvo, 2004; Naser, 2016).

The deficiency of protein is a major problem and challenge of the 21st century especially in the developing countries. Its devastating effects cannot be over-emphasized often ultimately leading to death in men, women and especially the young ones. Correcting these anomalies is even a greater challenge. This has been attributed mainly to lack of affordable protein, particularly animal protein (Odebode and Odebode, 2005; Kanayo, 2014).

The West African Dwarf (WAD) goats are excellent sources of animal protein in terms of meat and milk. They are in high demand and are very affordable; in fact they are popularly referred to as the "cattle of the poor" (Devendra, 1999; Oyeyemi, *et* al., 2002). However, supply of animal protein from these WAD goats is very limited. There is need to improve on the production of these special breeds of goats.

The WAD buck has a major role to play in this challenge because of its importance in WAD goat production. It can sire many does on the farm; hence improving the quality of its service/role will go a long way in improving WAD goat production (Nolte, 2012). This will ultimately lead to increase animal protein supply and help in meeting up with the challenges of protein deficiency. However, the ability of the WAD goat buck to perform this important role in increasing WAD goat production largely depends on the age at which

it reaches puberty and most importantly the optimum breeding age (OBA). At puberty, the male animal's reproductive capability begins but is not fully developed until it reaches the OBA (Osinowo and Williams,2008).

At puberty, the first set of spermatozoa are ejaculated (and depending on the species or breed of animal) may or may not be adequate enough in terms of quality and quantity to give the best reproductive results desired in the female animal in relation to fertilization, conception and production or number of offsprings. For instance, in cattle production (particularly in the developed countries), the bulls are usually allowed to reach the OBA after reaching puberty before being put to use in terms of mating and reproduction (Barth, 2000). Also, this is the common practice in rabbit (Proverbs and Quintyne, 1992).

In this study, puberty and OBA in WAD bucks were established. In this study, the best age to introduce a WAD buck for breeding was determined using the SC in correlation with spermatogenesis. SC values for the different age groups of WAD bucks were also observed. With these, a standard for BSE can be established and this will ensure that the best bucks at the right ages are used for breeding. With these, reproductive wastage in terms of using sub-fertile or even non-fertile WAD bucks for breeding will be prevented.

This study also highlighted the value and importance of Testicular Ultrasound (TU) particularly in taking an important biometric parameter i.e Testicular Volume (TV) which was used in determining the OBA in the WAD buck of which the PEF was the most valuable in achieving this. This non-invasive method of measuring TV could be used to replace the invasive technique (that requires the removal of the testes) currently being used. The introduction of these into the BSE and breeding programmes of these valuable sources of protein animalswill improve production and increase protein animal supply. This would go a long way in meeting up with challenges of protein deficiency now and in the future particularly in the developing countries like Nigeria.

Also, the morphologic and morphometric characteristics of the testes and epididymides were valuable in determining puberty and OBA in WAD bucks. Spermatozoa were first noticed in the lumen of the seminiferous tubules of the six months old WAD bucks. However, the fullest lumens of the seminiferous tubules were observed from eight months

to fifteen months old bucks. Similar trend was observed for the caput, corpus and caudal epididymides. The STD and GEH increased from one month to eight months without further significant increase up to fifteen months. The GEH is an index of spermatogenic activities within the testes. The higher the GEH, the more the spermatogenic activities that will take place within the testes. In this study the highest spermatogenic activities were observed from eight months old WAD buck testes when GEH peaked.

This study also corroborated earlier observations of the importance of reproductive hormones to sexual maturity and spermatogenesis in male hormones. The LH, FSH and especially testosterone profiles studied were valuable in determining puberty and OBA in WAD bucks. However, it is suggested that hormone profiles should be combined with other correlates in determining the OBA and not in isolation. The testosterone and LH concentrations increased significantly from one month and rapidly increased at puberty (six months) and OBA (eight months) without further significant increase up to fifteen months in the semi-intensively raised WAD bucks studied. The FSH concentrations also increased significantly from one month to fifteen months without any noticeable rapid increase at puberty and OBA.

It will be worthy of mentioning that the United States of America only started domestic goat farming in 1992. Today, they are the leading goat farmers in terms of dairy and meat production. They have indeed advanced to the extent that they issue Certificates of Breeding Soundness for their livestock goats. Also, of particular interest is the immense value that is placed on WAD goats in the USA. The WAD goat milk is rated as the best in terms nutritional value and taste. But quite unfortunately is the fact that the value placed on these animals, is almost the direct opposite in the countries where these animals originated from (Keith *et al.*, 2009; Ford *et al.*, 2009). However, it is hoped that the contributions of this study will stimulate the improvement and standardization of WAD goat farming to bring it to the enviable status these animals have attained in the advanced countries such as the USA.

7.2 Conclusion

In this study, SC values for one to fifteen months age groups of WAD bucks were highlighted. This was used in correlation with semen production in determining the OBA for these WAD bucks. This will be valuable in the standardization of BSE in these animals. Also, TU was observed to be a valuable non-invasive method for determining the OBA using TV estimated by PEF and also as a BSE and diagnostic tool in WAD bucks. The testicular and epididymal morphological and morphometric characteristics were valuable in determining puberty and OBA in WAD bucks. The FSH, LH and particularly testosterone profiles were also valuable in determining the OBA of semi-intensively raised WAD bucks.

It is hoped that the contributions of this study will stimulate the standardization of WAD goat farming and ultimately lead to increase of animal protein supply that is required for human survival.

7.3 Contributions to knowledge

In this study, the age at puberty and the OBA in the semi-intensively raised WAD bucks were determined through spermiogram in correlation withimportant BSE parameters such as the SC and TV; morphologic and morphometric characteristics of the testes and epididymides; and FSH, LH and especially testosterone profiles.

Also, the values for the SC and TV of semi-intensively raised WAD bucks between the ages of 1-15 months were provided;hence, BSE standards (which has been lacking) can be set and routinely carried out on this invaluable source of protein animals for improved production purposes.

Furthermore, this study revealed the great values of TU as a biometric and BSE tool in WAD bucks; by TU a non-invasive and easier approach to measuring TV of WAD buck was determined using PEF. This could be used in place of the invasive method of WDM (which had always required the permanent removal of testes) that had been used in measuring TV (a very important BSE parameter).

Again, morphological and morphometric data on the testes and epididymides, particularly in relation to puberty and OBA, of semi-intensively raised WAD bucks were revealed in

this study. The first time matured spermatozoa appeared and when they were most densely populated in the lumen of the seminiferous tubules were highlighted. These were highly correlated with when spermatozoa were first observed and of the best quality in terms of volume, motility, morphology, livability and concentration, and noticed in the WAD bucks' ejaculate.

Finally, this study highlighted the FSH, LH and especially the testosterone correlates particularly in relation to puberty and OBA. There was increase in the concentrations of these hormones with a distinct rapid increase in the concentrations of LH and testosterone at puberty and OBA in the semi-intensively raised WAD bucks.

7.4 Recommendations

The WAD bucks of eight months old with at least SC of 17.6 ± 0.2 cm, BS of 3.2 ± 0.5 and TV of 28.3 ± 0.2 cm^3 (by TU) could be used to breed does successfully on the farm. The PEF should be adopted and used for estimating TV of WAD bucks grossly and by ultrasound. TU should be adopted in the BSE of WAD buck goats.

7.5 Suggestions for further studies

Further studies on SC beyond 15 months of age should be carried out in order to have an age standard for SC for WAD bucks. This can also be done in other breeds of Nigerian goats and livestock in efforts to standardizing our livestock system of production. Morphological and morphometric studies of the testes and epididymides of the other Nigerian breeds of goats and livestock can also be done for a more comprehensive regional reproductive comparative anatomy. Further testicular ultrasound and biometric studies on WAD buck and other breeds of goats and livestock should be carried out.This will be very valuable in setting a BSE standard for these animals which is a prerequisite to improved livestock production. Also, further studies on reproductive hormonesincluding Gonadotropin Releasing Hormones (GnRH) in relation to puberty and OBA can be carried out for better understanding of the endocrinology of WAD bucks.

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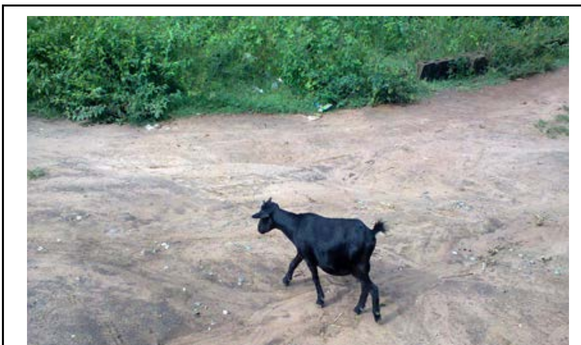
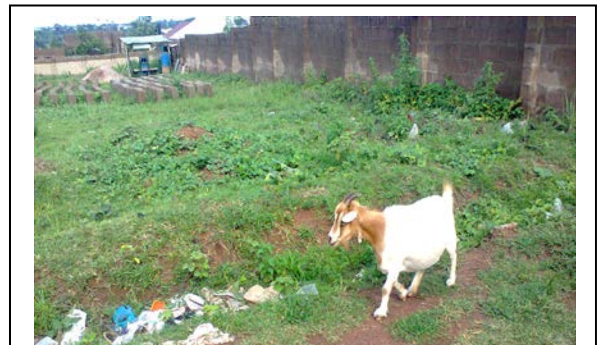
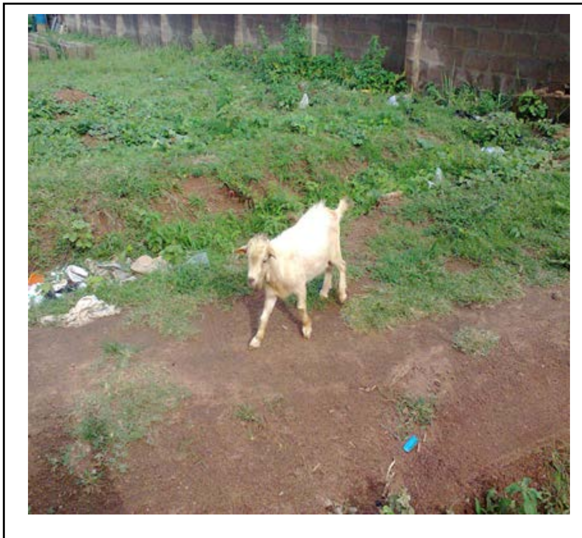
PLATES

Plate 1: West African Dwarf goats raised under the semi-intensive system of production.

Plate 2: A West African Dwarf Buck with excessive split







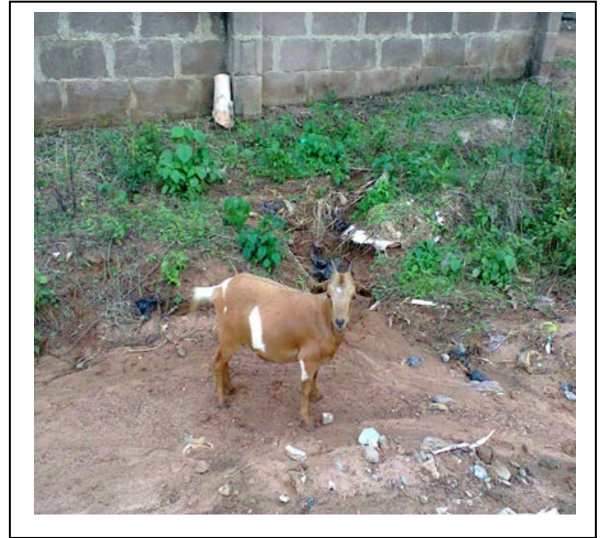


Plate 3: Different colors of the West African Dwarf goat.

Plate 4: Testicular ultrasound of West African Dwarf bucks

Plate 5: Right and left testes of four months old West African Dwarf buck.

Plate 6: Right and left testes and epididymides of five months old West African Dwarf buck.

Plate 7: Right testis of six months old West African Dwarf buck.

Plate 8: Right and left testes and epididymides of seven months old West African Dwarf buck.

Plate 9: Testicular weight of eight months old West African Dwarf buck.

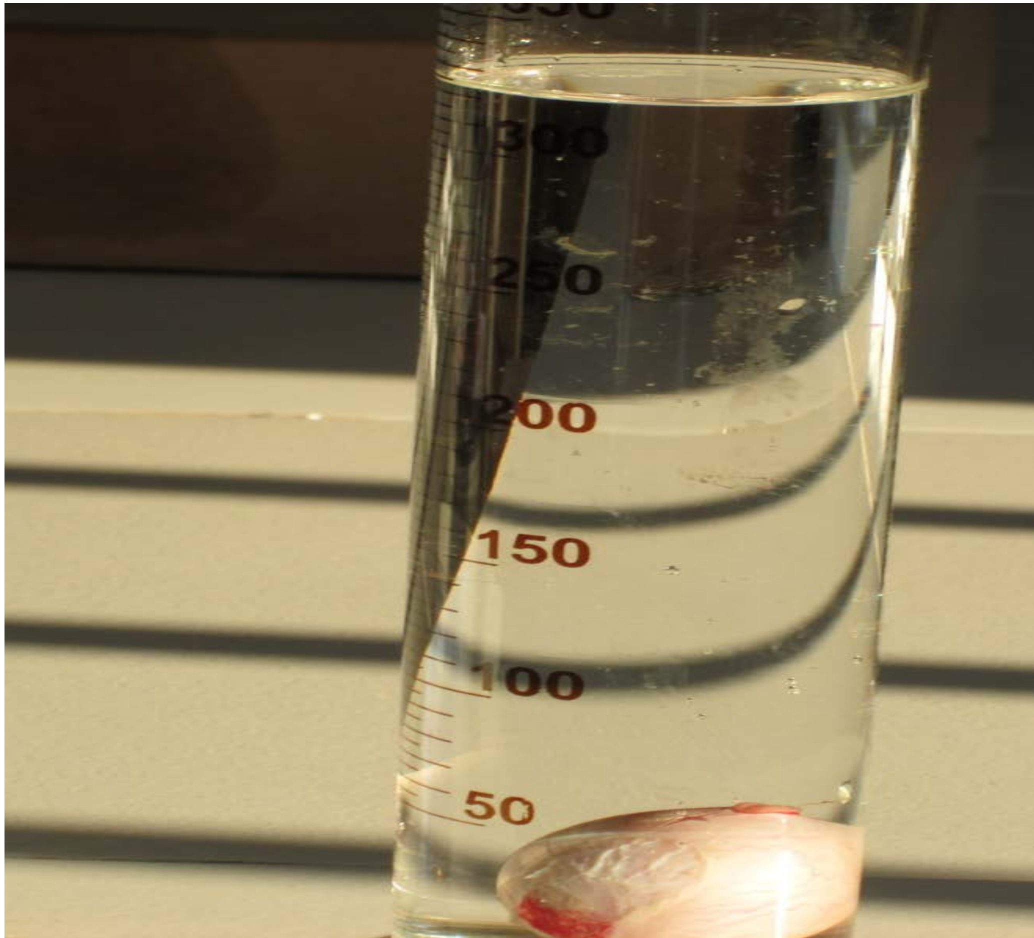


Plate 10: Measurement of true testicular volume of a West African Dwarf buck testis by water displacement method

Plate 11: Taking blood sample fromthe jugular vein of a West African Dwarf

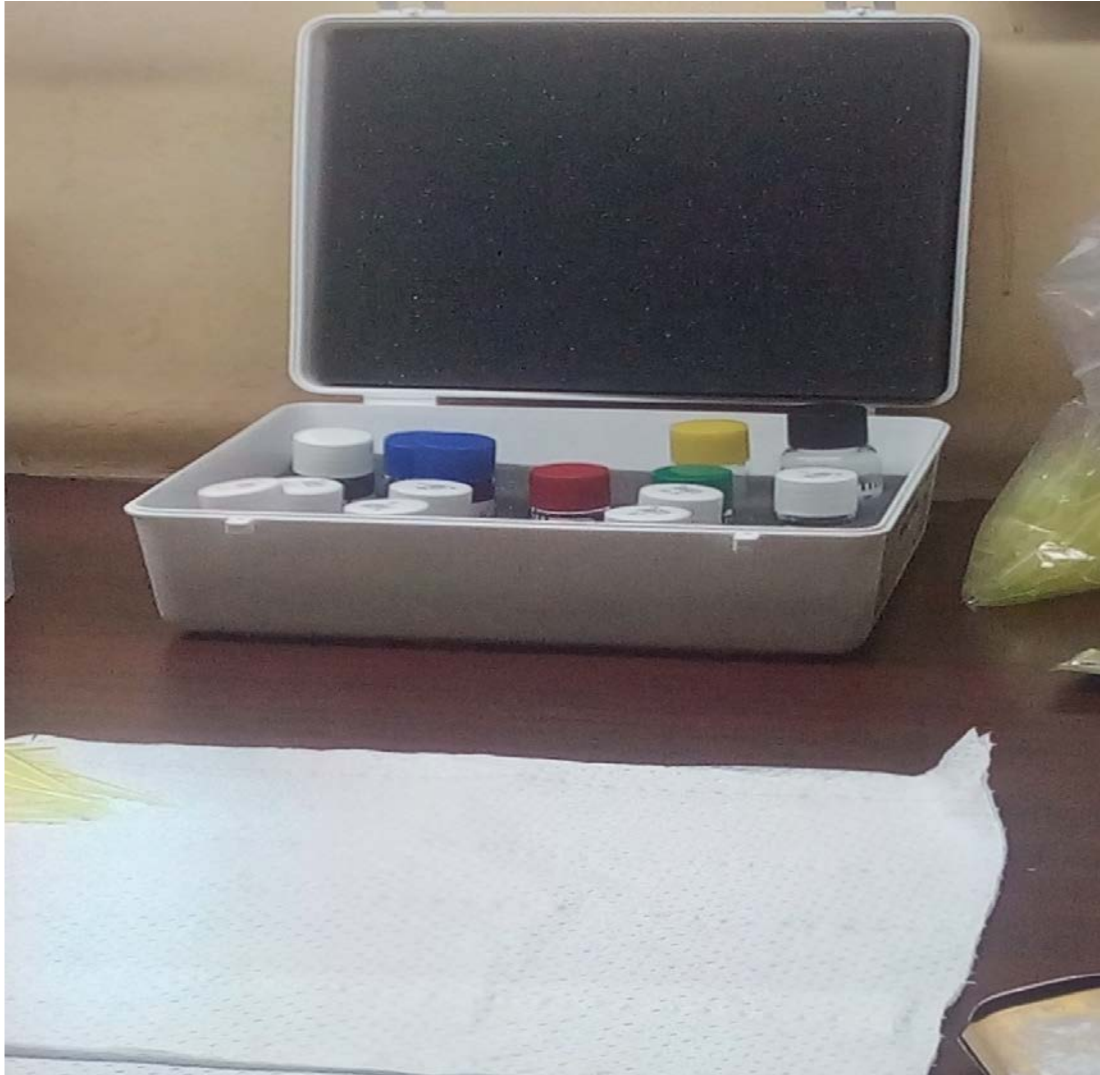


Plate 12: ELISA kit for hormonal assay

Plate 13: ELISA reader

Plate 14: ELISA hormone standards and plate

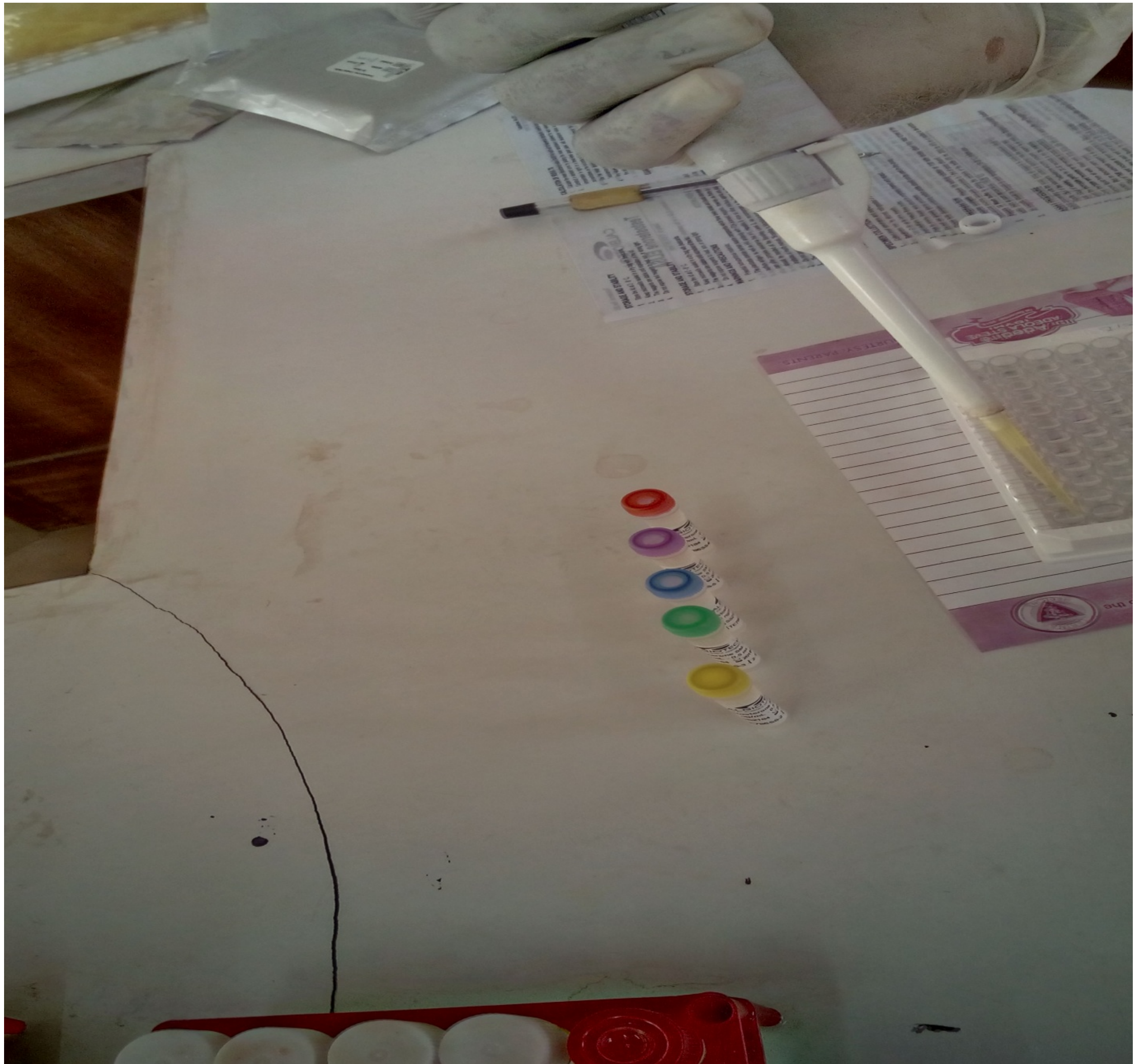


Plate 15: Addition of the hormone standards to the wells on the plate using a pipette

Plate 16: Addition of serum samples to the standards in the microtiter wells on the plate using a pipette

Plate 17: ELISA microplate reader