EVALUATION OF SOME SONOGRAPHIC PARAMETERS OF THE TESTES COMPARED WITH SPERMOGRAM AND SOME HORMONAL PROFILES IN CLINICALLY HEALTHY YANKASA RAMS

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AHMADU BELLO UNIVERSITY,

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN VETERINARY DIAGNOSTIC IMAGING

DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY,

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MAY, 2017

DECLARATION

I declare that the work in this dissertation entitled "**EVALUATION OF SOME SONOGRAPHIC PARAMETERS OF THE TESTES COMPARED WITH SPERMOGRAM AND SOME HORMONAL PROFILES IN CLINICALLY HEALTHY YANKASA RAMS**" has been performed by me in the Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, under the supervision of Prof. A. Z. Hassan and Dr N. D. Chom. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

MOHAMMED ABDULRAUF<u>OBALOWU</u> _____ _____

Name of Student Signature Date

CERTIFICATION

This dissertation entitled "**EVALUATION OF SOME SONOGRAPHIC PARAMETERS OF THE TESTES COMPARED WITH SPERMOGRAM AND SOME HORMONAL PROFILES IN CLINICALLY HEALTHY YANKASA RAMS**" by**MohammedAbdulrauf OBALOWU**, meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof. A. Z. Hassan Chairman Supervisory committee	Signature	Date
Dr. N. D. Chom Member, Supervisory committee	Signature	Date
Dr. S. T. Fadason Head of Department	Signature	Date
Prof. S.Z. Abubakar Dean, School of Postgraduate Studies, A.B.U. Zaria	Signature	Date

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ABSTRACT

The aim of the present study was to establish the normal sonographic parameters for the Yankasa ram's testes and epididymides; and to observe their relationship with the semen parameters and reproductive hormones (testosterone, luteinizing and follicle-stimulating hormone LH and FSH, respectively). Fifteen clinically healthy Yankasa rams were acquired from reputable livestock markets (Makarfi, Makarfi LGA and Tudun Sebu, Soba LGAs Kaduna State, Nigeria. The testicular length (TL), testicular width (TW) and testicular circumference (TC) were measured by using a vernier caliper and flexible tape after clinical assessment for breeding soundness. B-mode Sonostar ultrasound scanner with a 5MHz probe was used to scan the testisticular and epididymal echo texture. Semen samples were collected from each ram using electro-ejaculator and two mls, of blood sample was collected via jugular puncture every hour from each ram over a period of 6 hours (6:00 am to 11:00 am) on the day of sampling for reproductive hormone assay. The mean values of ultrasound measurement of the left and right testicular length were 130.4 ± 2.73 mmand 125.5 ±2.57 mm respectively, the left and right testicular breadth were 42.15 ±2.53 mm and 44.85 ±2.14 mm respectively, the left and right heights were 52.25 ± 2.10 mm and 53.02 ± 2.20 mm respectively, while the volumes were 280,340 ± 12,780 mm^3 and 296,432 ± 17,549 mm^3. The mean value for the left epididymal head volume using ultrasound was 6,480.0 ± 620mm^3 while that of the right was 4,490.0 ± 420 mm^3 and the mean value for the left epididymal tail volume was 2,830.0 ± 300 mm^3, while that of the right was 3,010.0 ± 500 mm^3. The mean left testicular length (15,700.0 ± 550 mm) and that of the right (15,900 ± 610mm) using vernier caliper was greater than the value obtained with ultrasound machine (13,040.0 ± 270mm and 12,550.0 ± 270mm respectively). Similarly, the mean left and right testicular volumes obtained using vernier caliper were 1,482,000 ± 64,370 mm^3 and 1,503,000 ± 74,270 mm^3 respectively, and were also higher than the ultrasound measurements. The testicular parenchyma of Yankasa rams was an isoechoic structure (midgray echo texture) with uniform echogenicity which was surrounded by an hyper-echoic structure (*Tunica albugina* and *Tunica vaginalis*). The mediastinum testis was seen as a point of hyperechoic area in the center of testicular parenchyma when scanned in the transverse plane, while the echogenicity of the epididymides was similar to that of the echogenicity of the testes, but with some hypoechoic area of echogenicity in the epididymal head. From the normal Doppler evaluation of testicular

arteries, the velocity, pressure and resistance to blood flow in the left testes of Yankasa rams were higher than in the right testes. The sperm concentration, reaction time and normal sperm cells correlated positively(r = 0.05) and insignificantly with the testicular volume using vernier caliper or ultrasound, while the sperm motility correlated negatively(r = -0.05) with the left and right vernier caliper measurements of the testicular volumes but positively with the left and right ultrasound measurements of the testicular volumes (r = 0.05). The testosterone and luteinizing hormone correlated negatively and insignificantly, with the right and left testicular length, circumference, volumes and weight (r= -0.05). None of the reproductive hormones studied had effects on the epididymides of Yankasa rams. Evaluation of echotexture and biometry of the Yankasa rams testes and epididymides using ultrasound is essential for maximum and rational utilisation of the breeding stock and can be used to predict future semen parameters.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

There is constant economic pressure for highly efficient lamb production, produced through natural breeding and artificial insemination (AI). Research has shown that, for over three decades, the ram is responsible for nearly 87.5% of the genetic contribution to a flock (Falconer, 1976). Ultrasonographic examination of the testes, epididymides and accessory sex glands has proved to be a valuable, non- invasive technique for the assessment of genital macroscopic morphology and pathology in several mammalian species of veterinary interest, (Kahn, 2004), including the ram (Gouletsou *et al.,* 2003). Accessory sex glands are all found along the length of the pelvic urethra (Anonymous[1], 2013). They produce secretions containing different components which facilitate movement of the sperm as well as providing a physiological buffer against the acidic environment of the female genital tract. One of the most important substances of the secretion is fructose which is an energy source for sperms (Anonymous[1], 2013).

Ultrasonographic imaging has been used in measuring size and structure of the testis in bulls and rams (Cartee *et al.,* 1990; Ahmad *et al*., 1991; Chandolia *et al*., 1997a). Several studies have been used to determine the changes in structure of the testis using ultrasound imaging in pathological conditions (Ahmad and Noakes 1995_a; Karaca *et al*., 1999) and clinically healthy animals (Ahmad *et al*., 1991; Evans *et al*., 1996; Aravindakshan *et al*., 2000a, 2000b; Gouletsou *et al*., 2003).

Dramatic advances within the last two decades in sonographic technology and instrumentation have been responsible for the emergence of ultrasound as an indispensable diagnostic tool for evaluation of scrotal pathology (Langer, 1993). High resolution real-time ultrasonography has demonstrated a high degree of accuracy and sensitivity in the detection, characterization, and localization of intrascrotal abnormalities. The advent of colour Doppler imaging, a technique that provides both excellent morphologic detail and physiologic information concerning testicular perfusion, has led to ultrasound's rapid emergence in the evaluation of acute scrotal pathology (Langer, 1993).

Trans-scrotal scanning allows visualisation of tissue interfaces within the scrotum, as well as the accurate measurement of scrotal circumference (Love, 1992). Trans- scrotal scanning also allows assessment of both palpable and non-palpable testicular lesions (Ahmad and Noakes, 1995_b). In addition, the technique is convenient and non-invasive, making it ideal for on-farm situations. Diagnostic ultrasound has been found to be the best technique for the evaluation of scrotal contents, which are ideally, suited for examination because of their general superficial location (Ali *et al*., 2011). It is very useful in confirming the presence or absence of a mass, as the homogeneous echo pattern of the normal testis serves as a good background for the detection of small intratesticular focal lesions; the size of which can also be measured accurately to aid in giving a tentative diagnosis. With this technique, it is also possible to differentiate between solid and cystic enlargements and may also be used as a guide for a definitive biopsy or surgical exploration (Ali *et al*., 2011)

Reproductive evaluation may be used to eliminate sub-fertile sires from breeding programmes (Chapwanya *et al*., 2008). Though the most conclusive evidence of fertility is

made on the basis of pregnancy rate in the females served, breeding soundness evaluation of the male is used to predict expected performance which enhances overall herd productivity (Chapwanya *et al*., 2008). Diagnostic methods of assessing the health of the bull and ram testes and related structures include manual palpation, measurement of scrotal circumference, testicular diameter and evaluation of semen. Other methods like thermography, tonometry and biopsy could not prove their worth in this context (Ali *et al*., 2011).

1.2 Statement of Research Problems

Since scrotal ultrasonographic technique is done without discomfort or risk to the patient and can be readily performed, it has gained acceptance in a wide variety of clinical applications including evaluation of scrotal enlargement or masses, occult testicular neoplasms, scrotal trauma, and screening of patients with previous history of testicular tumors such as lymphoma, and leukemia (Langer, 1993). To the best of our knowledge, there is no comparative report on the testicular and epididymal biometry with the semen and hormonal profile of Yankasa rams. Hence, this research could give base- line data in evaluating reproductive health status of Yankasa rams and be applied to predict the fertility of such flock, and detect salient genital problems that could reduce or prevent fertility.

1.3 Justification of the Research

Echo texture of testes, epididymides and likewise testicular biometry had been studied by Ali *et al*., (2011) and Srinivas *et al.,* (2012). Since there is no study of such in Nigerian Yankasa rams, this research therefore becomes imperative. There is also need for improved productivity to meet up with the Nigerian population demand for small ruminants by

elimination of sub-fertile sires from breeding programs. Ultrasonographic examination of palpable scrotal abnormalities can provide much useful information, particularly in the diagnosis of epididymitis, orchitis and testicular atrophy (Scott, 2012). Trans-scrotal ultrasonography allows assessment of palpable and non-palpable testicular lesions and is a useful, non-invasive and non-harmful method in selecting good breeding bulls (Chapwanya *et al*., 2008). It is therefore, important to explore its use in Yankasa rams.

1.4 Aim of the Study

The aim of this study is to establish normal ultrasonographic parameters for the Yankasa ram's testes and epididymides.

1.5 Objectives of the Study

i. To determine testicular and epididymal biometry of Yankasa rams using ultrasound.
ii. To determine scrotal vernier caliper measurements of Yankasa rams.
iii. To evaluate the echo texture of testes and epididymides of Yankasa rams.
iv. To evaluate the relationship between testicular and epididymal biometry compared to the semen quality.
v. To evaluate relationship between testicular and epididymal biometry compared to the reproductive hormones testosterone, FSH and LH.

1.6 Research Questions

i. Is there any difference between testicular biometry of Yankasa rams taken with ultrasound and vernier measurements?
ii. Can testicular or epididymal biometry taken with ultrasound be used for diagnosis of reproductive diseases in Yankasa rams?

iii. Can ultrasonography be employed as a diagnostic technique for the morpho-physiological evaluation of future reproducers?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Anatomy of Genital System of Rams

2.1.1 The Male Reproductive System

The male reproductive system comprises, scrotum, testes, epididymides, ductus deferens, urethra, the penis and the accessory sex glands (vesicular gland, prostate gland, ampulla and bulbourethral gland) (Barth *et al*., 2008).

2.1.2 Scrotum

The scrotum is a fibromuscular pouch divided by a median septum (raphe) forming 2 compartments, each of which contains a testis, epididymides and part of the spermatic cord (Zachary, 2013). Scrotal circumference (width of the testicles at the widest point) gives a goodn indication of a ram's breeding ability. Lambs and adult rams with a scrotal circumference of less than 30 cm should be exempted from breeding because evidence suggest that rams with larger testicles sire more prolific ewes (Suchana, 2013).

2.1.3 Testes

The testes are the primary male reproductive organs, suspended in the scrotum by the dartos muscle and spermatic cord and responsible for the production of testosterone and sperm (Zachary, 2013). The normal adult testisof the small ruminants is ovoid in shape and measures approximately 2 to 3 cm in width and 3 to 5 cm in length. Hafez and Hafez (2000) reported that testicular sizes do vary throughout the year in seasonal breeders like rams. The testisticular size is known to be significantly affected by age of the animal, being smaller in young than the adult or old animals (Younis *et al*., 2003; Srikar, 2008).

The testicles are situated in the prepubic region, enclosed in a fibromuscular pouch divided by a median septum (raphe) forming 2 compartmentseach of which contains a testis,epididymides and part of the spermatic cord (Zachary, 2013). The testes shape, size and location vary from one species to another but the vital structures of the organ are the same (Khan *et al*. 2014). Grossly, testis consists of two surfaces, two borders and two extremities, medial and lateral surfaces of testis are convex and smooth (Raji and Ajala, 2015; Khan *et al*., 2016). Epididymal borders are at cranial and caudal positions of the testis. Dorsal and ventral extremities are rounded in shape (Khan *et al*., 2016). The testicles are covered by outer *Tunica vaginalis*, *Tunica albugenia* and *Tunica vasculosa* layers (Khan *et al*., 2016). Morphologically, there is little difference between the right and left testicle within a group (Nimase *et al*., 2008).

2.1.4 Epididymides

Grossly, the epididymides is intimately attached with the testis border (Khan *et al*., 2016). It is a C-shaped structure lying intimately along the posterior border of each testis (Zachary, 2013). It measures approximately 6 to 7 mm in length and consists of a head, body and tail (Srikar, 2008). The body is fine, slender and it lies beside unattached lateral border of the testicle while the tail is long and directly attached at the ventral extremity of the testicle. The colour of the epididymides is pale to yellowish white (Kishore *et al*., 2012; Wares *et al*., 2013; Khan *et al*., 2016). Transport of sperms through epididymides takes 9-13 days (Hafez and Hafez, 2000). The environment of sperm in the caudalepididymides provides factors that enhance fertilizing ability. It contains about 75% of the total epididymal spermatozoa (Hafez and Hafez, 2000). Sperm from this region give higher fertility than those from the corpus epididymides (Amann, 1987). The special ability of the caudall

epididymides to store sperm depends on low scrotal temperatures and on the action of male sex hormone (Foldesey and Bedford, 1982).

2.1.5 Ductus deferens (Vas deferens)

The ductus or vas deferens is the continuation of the epididymides it conveys sperm to the ejaculatory ducts. The convoluted portion of the ductus deferens becomes straighter (2-3-mm diameter) as it travels posterior to the testis and medial to the epididymides. Subsequently, the ductus ascends on the posterior aspect of the spermatic cord until it reaches the deep inguinal ring where it participates in the formation of the spermatic cord and loops over the inferior epigastric artery (Zachary, 2013).

2.1.6 Spermatic cord

The spermatic cord extends from the deep inguinal ring, through the inguinal canal to the testis. The layers of the spermatic cord include (from without to inward): external spermatic fascia (derived from the deep fascia of the external abdominal oblique muscle), cremasteric fascia (derived from the internal oblique muscle), and internal spermatic fascia (derived from the transversalis fascia. The structures that form the spermatic cord include: (i) the ductus deferens and associated vasculature and nerves (posterior wall of the cord), (ii) the testicular artery, (iii) the pampiniform plexus, ultimately forming the testicular vein, and (iv) the genital branch of the genito-femoral nerve (Zachary, 2013).

2.1.7 Urethra

The urethra runs over the pelvic floor from the bladder to the tip of the glans penis. It serves as a passage for urine and semen. The prostatic urethra extends vertically from the

bladder neck, through the prostate before becoming the membranous urethra and before penetrating the perineal membrane (Zachary, 2013).

2.1.8 Penis **.**

The penis is made up of an attached rootat the ischial arch and a pendulous body extending from its root to the glans penis. The root consists of two crura and the bulb. The body (corpus penis) has a sigmoid flexure in the bovine specie that is caudaltothescrotum.(Zachary, 2013).

2.1.9 Seminal vesicles

The 2 seminal vesicles are located between the bladder and the rectum and measure approximately 5 cm in length (Zachary, 2013). They lie laterally to the terminal parts of each ductus deferense. In ruminants, they are compact lobulated glands (Hafez and Hafez, 2000).

2.1.10 Bulbourethral glands

These are dorsal to the urethra near the terminal portion of its pelvic portion (Hafez and Hafez, 2000). In ruminants, the ducts of the bulbo-urethral gland open in the urethral recesses (Garea, 1987).

2.1.11 Prostate

A distinct lobulated external part of its body stretches along the length of the uretra dorsal to the lumen outside the thick urethral muscle and a second external or disseminated part surrounds the pelvic urethra. The prostate extends caudally as far as the ducts of the bulbo-urethral glands (Hafez and Hafez, 2000).

2.2 Spermatogenesis in the Ram

Spermatogenesis is a process of formation and liberation of spermatozoa from undifferentiated germ cells on the seminiferous tubules of the testis (King, 1993). It involves the production of spermatozoa from male primordial germ cells by way of mitosis and meiosis (Barth *et al*., 2008). During spermatogenesis, spermatogonia differentiate into spermatocytes and spermatids following mitosis and meiosis respectively (Barth *et al*., 2008). The spermatozoa undergo maturation in the epididymides where they are stored until ejaculation takes place (Bearden and Fuquay, 1997).

2.2.1 The Process of spermatogenesis

Spermatogenesis is divided into two distinct phases; the first is spermatocytogenesis, which involves production of spermatids from spermatogonia. The second is spermiogenesis, which involves metamorphosis of spermatids to spermatozoa (Barth *et al*., 2008). The entire process is completed in 46 to 49 days in rams (Harder *et al*. 1995; Ortavant, 1990). As spermatogenesis proceeds, the developing gametes migrate within the seminiferous tubules from the basement membrane toward its lumen (Harder *et al*., 1995).

2.2.2 Spermatocytogenesis

Two types of cells are located along the basement membrane of the seminiferous tubules, Sertoli cells, which are larger and less numerous, and somatic cells which play a supporting role during both spermatocytogenesis and spermiogenesis (Bearden and Fuquay, 1997). Spermatogonia located along the basement membrane of the seminiferous tubules, are the small, rounded, more numerous cells, which are the potential gametes. After migrating from basement membrane into the embryonic testes, primordial germ cells will undergo a number of mitotic divisions before forming gonocytes (Barth *et al*., 2008). After puberty

gonocytes will differentiate into A1 and A2 spermatogonium, the A2 spermatogonium will divide, forming a dormant (A1) spermatogonium and an active (A3) spermatogonium and starting a new generation of developing germ cells (Bearden and Fuquay, 1997). The active spermatogonium will undergo four mitotic divisions in bulls and rams, eventually forming 16 primary spermatocytes. In rams, these mitotic divisions are completed in 15 to 17 days. The primary spermatocyte will undergo a meiotic division forming two secondary spermatocytes; with this division, the chromosome complement in the nucleus is reduced by half so that nuclei in secondary spermatocytes contain unpaired (n) chromosomes. This step requires approximately 15 days in the rams and bulls (Bearden and Fuquay, 1997). Within a few hours after their formation, each secondary spermatocyte will again divide, forming two spermatids. Thus, four spermatids form from each primary spermatocyte, or 64 from each active (A3) spermatogonium in bulls and rams. Since A1 spermatogonium divides by mitosis to form A2 spermatogonium, the potential yield of spermatids is higher than is actually realized. Degeneration of spermatogonia during mitotic divisions accounts for this loss.

Although A0 spermatogonia (reserve stem cells) will occasionally divide, forming new A0 and A1 spermatogonia, formation of dormant spermatogonia from A2 spermatogonia is the key for maintaining the continuity of spermatogenesis and thereby not diminishing the supply of potential gamates within the testes (Rodriguez-Martinez, 2006).

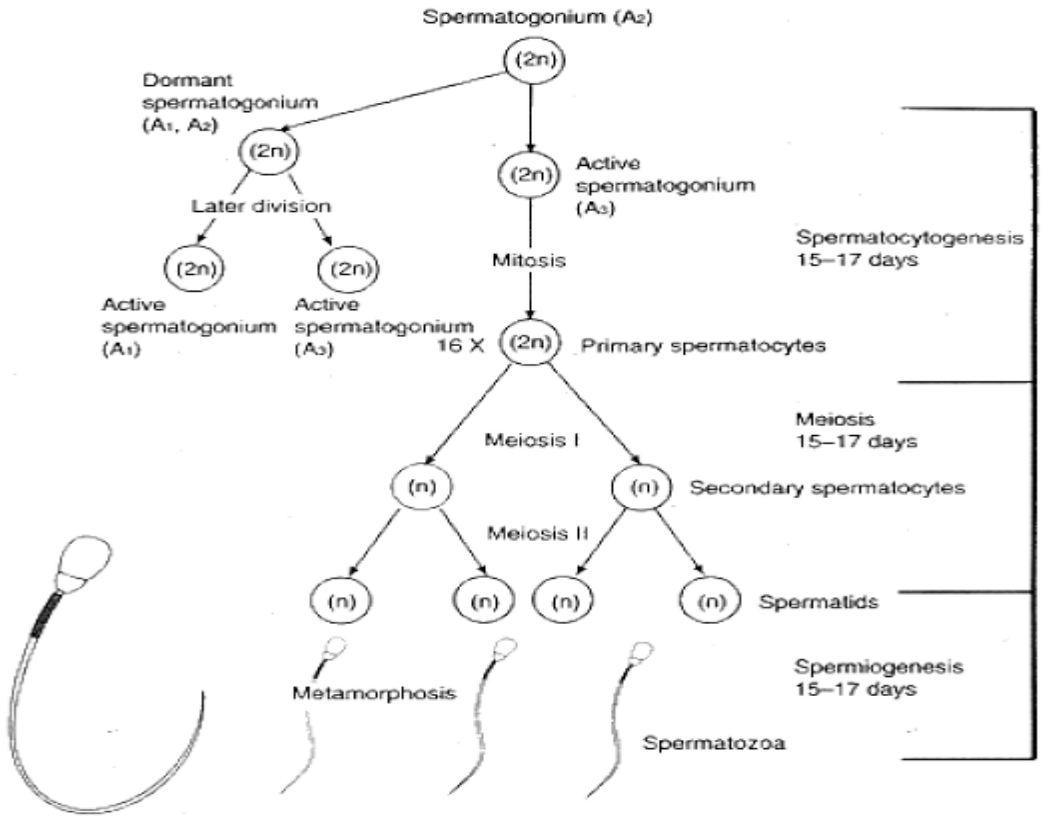


Plate I. Sequence of events and time involved in spermatogenesis in the ram (adapted from Bedford and Hoskins, 1990).

2.2.3 Spermiogenesis

During spermatogenesis spermatids are attached to Sertoli cells, each spermatid undergoes a metamorphosis forming a spermatozoon (Bearden and Fuquay, 1997). During this metamorphosis, the nuclear material will compact in one part of the cell, forming the head of the spermatozoa, while the rest of the cell elongates, forming the tail (Rodriguez-Martinez, 2006). The spermatid will form the acrosome, a cap around the head of the spermatozoa (Bearden and Fuquay, 1997). Spermatozoa are unique cells that have no cytoplasm, as the cytoplasm from the spermatid is cast off during formation of the tail, a cytoplasmic droplet will form on the neck of the spermatozoon. The mitochondria from the spermatid will be arranged in a spiral form around the upper one sixth of the tail, forming the mitochondrial sheath (Harder *et al*., 1995). Newly formed spermatozoa will then be released from the Sertoli cell and forced out through the lumen of the seminiferous tubules into the rete testis (Bearden and Fuquay, 1997).

Transit of sperm through the efferent ductules and the epididymides is associated with significant maturation changes such as gaining the capacity for progressive motility, final condensation of the nucleus and further modification of the form of the acrosome, alteration of plasma membrane surface (release, modification and adsorption of proteins and lipids) and migration of cytoplasmic droplet from a proximal to distal mid-piece position (Rodriguez-Martinez, 2006). Sperm taken from the corpus and caudalepididymides already have the potential to fertilize (Harder *et al*., 1995; Barth *et al*., 2008).

Seminal fluids secreted by accessory sex glands (ampulla, seminal vesicles, prostate, and bulbourethral glands) added during ejaculation serve as a vehicle, stimulate the metabolism of sperm and provide the energy requirements for passage through the uterus (Ortavant, 1990; Barth *et al*., 2008). The semen of the ram is creamy-white in colour, 0.3 to 1.0 ml in volume with normal concentration varying from 1.6 x 10^6 to 6.0 x 10^6 per ml with an average of 3.6 x 10^6 per ml (Oyeyemi *et al*., 2009). Following maturation in the epididymides, the specialized cells are available for delivery to the female reproductive tract (Rodriguez-Martinez, 2007). The physical and chemical properties of semen are largely determined by the seminal plasma which constitutes the bulk (Hoogenbozem, and Swanepoel, 2000). The seminal plasma contains some unusual organic compounds such as fructose, citric acid, sorbitol, inositol, glyceryl, phosphorylcholine and ergothioneine, which are not found anywhere in the body and they serve as source of nutrients for the spermatozoa (Ortavant, 1990).

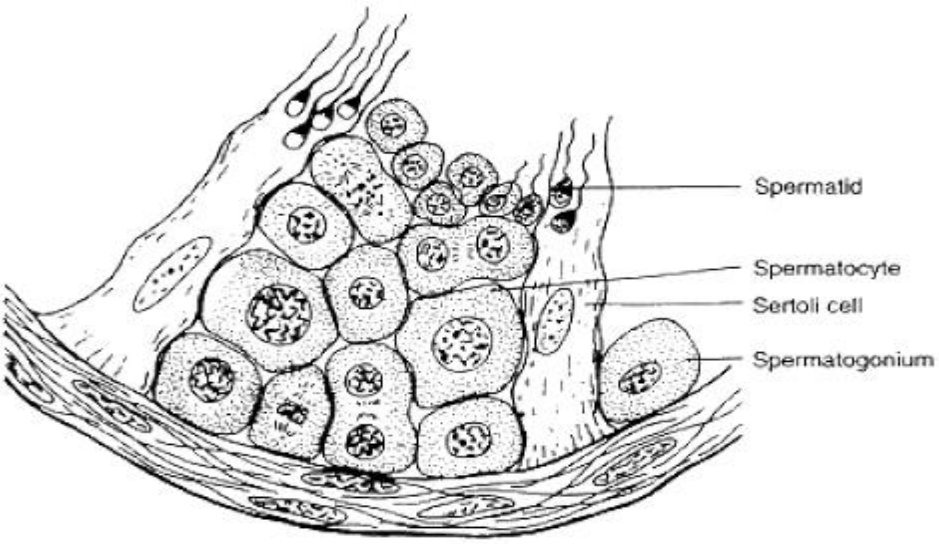


Plate II. Section of small segment of an active seminiferous tubule. The developmental stages that occur during spermatogenesis were expressed along with the concentric layers of spermatogonia, spermatocytes, and spermatids progressing from the wall of the seminiferous tubules to the lumen. (Adapted from an original drawing by Brian Satchell, 1990).

2.3 Endocrine Control of Reproduction

The reproductive functions of animals are controlled by a coordinated and efficient neuroendocrine system (Sekoni, 1991). The hormones involved in reproduction originate from three principal structures, hypothalamus, pituitary and the gonads (Sekoni, 1991). Normal testicular function is dependent upon a functional pineal gland and hypothalamic-pituitary-testicular (HPT) axis (Ortavant, 1990; Sekoni, 1991). Melatonin is the hormone of the pineal gland which is essentially produced during period of darkness and it is recognized to reach almost all bodily cells and organs regulating their function, especially the reproductive organs (Mohammed *et al*., 2016). The pineal gland secretes melatonin that acts on the hypothalamus to regulate gonadotropin-releasing hormone (GnRH) output. GnRH stimulates secretion of the pituitary hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn acts at the level of the testis (Johnson *et al*., 1994). LH binds to receptors on testicular Leydig cells to stimulate production of testosterone and which in turn stimulates the germinal cells and maintains spermatogenesis (Hafez and Hafez, 2000). Testosterone has a negative feedback effect on the hypothalamus and anterior pituitary, high concentrations of testosterone will inhibit the release of GnRH, FSH and LH, whereas low concentrations permit their release. It has been demonstrated that prostaglandin-2α (PGF2α) will stimulate the release of LH and testosterone (Wada *et al*., 2009). Therefore, PGF2α may be involved in the feedback regulation between the hypothalamus, anterior pituitary, and testes (Johnson *et al*., 1994).

Differentiation of spermatids to spermatozoa requires FSH and probably testosterone (Wada *et al*., 2009). The FSH stimulates the testes and causes a significant increase in the diameter of the seminiferous tubules (Gabor *et al*., 2011).

Hypophysectomy in the rat, dog and ram causes a decrease in the weight of the testes and degeneration of the seminiferous epithelium; the process can be restored or reversed by giving gonadotrophin (Gabor *et al*., 2011). This implies that, one or both of the gonadotrophin are concerned either directly or indirectly with the maintenance of the seminiferous epithelium and production of spermatozoa (Gabor *et al*., 2011). In the rat, both FSH and LH stimulate spermatogenesis after hypophysectomy but the effect of LH may be due to androgen released from the interstitial cells since the injection of high doses of testosterone was shown to partially maintain spermatogenesis provided the germinal epithelium had not completely atrophied (Wada *et al*., 2009; Gabor *et al*., 2011).

2.3.1 Hormonal control of spermatogenesis

The testis is the main genital male organ that possesses both exocrine and endocrine gland roles (Mohammed *et al*., 2016). A significant correlation between testisticular volume and serum testosterone concentration has been indicated after injection of GnRH in Holstein- Friesian bulls (Gabor *et al*., 1995). Though spermatogenesis is under the control of pituitary gland (Bearden and Fuquay, 1997), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone are necessary for maintenance of spermatogenesis (Salisbury *et al*., 1978; Bearden and Fuquay, 1997). LH stimulates the release of testosterone from the Leydig cells which in turn acts through the cells in seminiferous tubules to stimulate spermatogenesis. FSH is necessary for the establishment of normal population of Sertoli cell and the stimulation of production of androgen-binding protein from the Sertoli cells (Kilgour *et al*., 1984). The androgen-binding protein binds

with the testosterone making it available for its function in spermatogenesis (Bearden and Fuquay, 1997).

2.3.2 Testosterone production, regulation and function in ruminants

More than 90% of the body's testosterone is secreted by the Leydig cells, whose structure and function depend on the functional maturity of the hypothalamus-pituitary-Leydig cell axis (Koonjaenak, 2006). When the hypothalamus detects the need for more testosterone, it secretes gonadotropin-releasing hormone GnRH (Martins *et al*., 2006). The gonadotropin-releasing hormone makes its way over to the pituitary gland, and causes it to start producing follicle-stimulating hormone (FSH) and luteinizing hormone (LH), the FSH and LH get to the testicles via the bloodstream (Martins *et al*., 2006). When the FSH and LH reach the testicles, the FSH binds with receptors in Sertoli cells and stimulates spermatogenesis through a complex process (Alexander and Stimson, 1988). The LH in the testicles' Leydig cells converts cholesterol into testosterone (Alexander and Stimson, 1988). Leydig cells get most of what they need to produce

testosterone by simply absorbing the cholesterol floating around in the blood, if there isn't enough cholesterol in the blood, the testicles can produce a bit of it so that the Leydig cells can convert it to testosterone (Alexander and Stimson, 1988). But relying too much on cholesterol produced by the testicles can actually inhibit the Leydig cells from producing testosterone (Martins *et al*., 2006). Once testosterone is produced, it is sent back into the bloodstream, most of the testosterone binds to sex hormone binding protein (SHBP) and albumin, becoming biologically inert leaving a small percentage that remains free and unbound which circulates in the blood stream (Al-Qarawi *et al*., 2004). The body maintains a homeostatic level of testosterone through the negative feedback effects of testosterone on

the pituitary gland or the hypothalamus or both (Steinberger, 1977). Testosterone levels may be affected by the levels of FSH and LH (Martins *et al*., 2006).

Testosterone is essential for production of seminal plasma, epididymal maturation of sperm, development and maintenance of secondary sex characteristics and libido (Amann, *et al*., 1998). It has been reported that this hormone participates in sexual (O'Connor *et al*., 2011), social (Gabor *et al*., 2011) and spatial (Spritzer *et al*., 2011) behaviours.

Testosterone levels can be measured in serum, plasma or saliva using a variety of commercially available kits. The most commonly used are ELISA kits, which are already well established (Gabor *et al*., 2011).

2.3.3 Metabolism and testosterone half-life in blood

Testosterone is the most important androgenic-anabolic steroid (Van Eenoo and Delbeeke, 2006). About 4-10 mg of testosterone produced by a ram per day is biosynthesized in the testes and 0.5 mg per day is produced in the adrenaline cortex of sheep (Van Eenoo and Delbeeke, 2006). Testosterone is converted to two other important hormones, it is reduced to dihydrotestosterone in skin and prostate; and it is oxidized to estradiol. In rams this oxidation mainly takes place in adipose tissue and in the testes while in ewes the biosynthesis of estradiol takes place in the ovaries (Van Eenoo and Delbeeke, 2006).

The metabolism of 90 % of testosterone and dihydrotestosterone takes place in the liver. The reductases and dehydrogenases catalyse the reactions of the D4-double bond, the C3-carbonyl group and the C17-hydroxyl group. Finally the hydroxyl groups are converted to glucuronic acid or sulphate, followed by excretion via urine (Van Eenoo and Delbeeke, 2006).

2.3.4 Diseases affecting endocrine function and reproduction in ruminants

Any disease that causes pituitary lesions may interfere with the production of gonadotropic hormones (Sekoni, 1991; Gabor *et al*., 2011). In ruminants, this has been studied extensively with trypanosomiasis (Sekoni, 1991). Male animals infected with trypanosomes show loss of libido, severe epididymal degeneration, deterioration of semen characteristics and aspermatogenesis (Anosa and Isoun, 1984; Sekoni *et al*., 1990) as well as reduction in testosterone levels (Masake 1980, Wada *et al*., 2009). High environmental temperature, other conditions such as fever, orchitis, periorchitis, hydrocele, scrotal haemorrhage, scrotal edema, scrotal dermatitis and improper scrotal descent can interfere with the thermoregulatory mechanism necessary to cool the testis and allow normal spermatogenesis (Johnson *et al*., 1994).

2.4 Factors Affecting Semen Quality in Rams

2.4.1 Testicular weight, size and volume

Vidament *et al.* (2007) reported a positive correlation between the testicular weight and sperm concentration while Palasz *et al.*, (1994) reported a correlation between testicular size capacity for sperm production, number of sperms ejaculated and sperm reservoirs. Testicular size and circumference also have a direct correlation with spermatozoa output as observed by several researchers (Osinowo, 1979; Shinobi and Kiyomi, 2006). In 2013, Sharath *et al.*,reported that testicular volume has a direct correlation with semen profiles.

2.4.2 Scrotal circumference

Scrotal circumference had a positive linear regression ($P < 0.04$) with the percentage of progressively motile sperm, a negative linear regression ($P < 0.1$) with the incidence of

primary sperm defects, and a positive linear regression ($P < 0.0001$) with epididymal sperm reserves (Suchana, 2013).

2.4.3 Epididymal effects

Spermatazoa are stored in the epididymides. The body of the epididymides transports the mature sperm to the tail, or caudal, where it is stored (Jordan, 2013). Goovaerts, (2006) has shown that epididymal sperm has a lower motility and moves less straight with higher amplitude than ejaculated semen. The epididymal straight line velocity is lower, but the curvilinear velocity is higher than those of ejaculated semen (Suchana, 2013).

2.5 Ultrasonography of Reproductive System of a Ram

2.5.1 Ultrasound and ultrasonography

Ultrasonography is a non-invasive diagnostic method and the examination can be carried out quickly and easily (Peter *et al*., 1992). It is based on the ability to reflect transmitted high-frequency sound waves by tissue (Kumari *et al*., 2016). Ultrasound waves are generated by the piezoelectric effect in a suitable medium such as lead zirconate (Kumari *et al*., 2016). Echoes of ultrasound depend on relative density of tissue (Pierson and Adams, 1995). A, B, and M modes are the three basic forms of ultrasound used in soft tissue imaging. A-mode ultrasonic imaging is a one-dimensional display of echo amplitudes versus distance; B-mode ultrasonic imaging produces an accurate two-dimensional (2D) cross-sectional image of soft tissues while the M-mode ultrasonic imaging is an adaptation of B-modes to evaluate moving structure of the heart (Kumari *et al*., 2016). Diagnostic ultrasound frequencies range from 2 to 15 MHz and are inaudible to the human ear (Kumari *et al*., 2016). The ultrasonogram is essentially an image of a thin slice of tissue, and therefore, enables serial examinations to monitor the progression of the condition, response

to treatment and to practice scanning techniques (Hornbuckle and Kleine, 1980). One of the drawbacks of diagnostic ultrasound however is that it requires a great deal of skill and experience (Kumari *et al*., 2016).

Ultrasound has been used for the diagnosis of several types of testicular abnormalities in human medicine. In veterinary medicine it has been used mostly for pregnancy diagnosis and monitoring ovarian lesions and morphological changes in the female reproductive tract (Arthur *et al.,* 1982). Ultrasonography is one of the most widely used diagnostic tools in modern medicine used to visualize many internal organs, their size, structure and any pathological lesions with real time tomographic images, (Ahmed *et al*., 2012). Transcutaneous ultrasonography has been used to demonstrate the ultrasonic morphology of testes of bulls (Pechman and Eilts, 1987; Eilts and Pechman, 1988; Yimer *et al.*, 2011) rams and boars (Cartee *et al.,* 1986). Ultrasonographic techiniques have also been used in different species of animals; female dromedary camel (Tibary and Anouassi, 1997; Pasha *et al*., 2011), equine (Pierson *et al*., 1988; Fontijne and Hennis, 1999), bovine (Kastelic *et al.*, 1988; Pierson and Ginther, 1988), caprine and ovine (Buckrell, 1988; Ahmad *et al.*, 1991; Gouletsou *et al*., 2003; Andrade *et al*., 2014; Raji *et al.,* 2016) and dogs (Camela *et al*., 2014).

2.5.2 Ultrasonographic appearrance of testis and epididymides

The visceral and parietal layers of the tunica are visualized as one echogenic stripe (Srikar, 2008). The normal testis has mid-gray or medium-level echo-texture and is homogenous in appearance as shown in plate III. The echogenicity of the testis is similar to that of the liver or the thyroid gland while the epididymideshas similar or slightly increased echogenicity. The mediastinum testis is seen as a linear echogenic band running

craniocaudallly or parallel to the epididymides. The appendix testis and appendix epididymides are small ovoid hyperechoic protuberances found at the superior pole of the testis, normally hidden by the epididymal head. They are difficult to find on ultrasound and only seen when outlined by fluid from a hydrocele. The spermatic cord appears as multiple hypoechoic linear structures in the longitudinal plane and circular hypoechoic structures in the transverse plane (Promes, 1997; Blaivas, 2003; Akin *et al.*, 2004; Blaivas and Brannam, 2004).

The testicular parenchyma appeared homogeneous with a coarse medium echo-pattern. (Gouletsou *et al.*, 2003) . The tail of the epididymides is a heterogeneous structure that is clearly visible as hypoechoic compared to the testicular parenchyma plate. The epididymal body is not visible, while the epididymal head is consistently partially imaged. The pampiniform plexus is clearly seen as a dome-shaped structure masking the upper part of the head of the epididymides. The scrotal septum is seen in lateral sonograms as a highly echogenic line between the testicles while the scrotal skin formed a thick hyper-echoic peripheral structure (Suchana, 2013).

2.5.3 Use of testicular and epididymal ultrasonography in breeding soundness examination

Ultrasonographic imaging of testes appeared as homogeneous hypoechoic parenchyma with centrally located hyperechoic mediastinum testis and surrounded by distinct hyperechoic tunics. The epididymal tail in the ultrasonographic image appears as heterogeneous and less echogenic than the testis (Ahmed *et al.*, 2012). Ultrasonographic examination of the testicles can provide an accurate measure of sperm production by the testicle (Lopate *et al.*, 2003), though it can be affected by many sources of error (Chenier, 2009). *In vivo* ultrasonography of testicles has been used to determine the testicular sperm production in

the ram (Cartee *et al.,* 1990), stallion (Love *et al.,* 1991), Bull (Bailey *et al.,* 1998) and boar (Clark *et al.,* 2003). Chenier, (2009) also reported that testicular measurements by ultrasonography has been an important part of the physical examination of the breeding soundness examination in stallions.

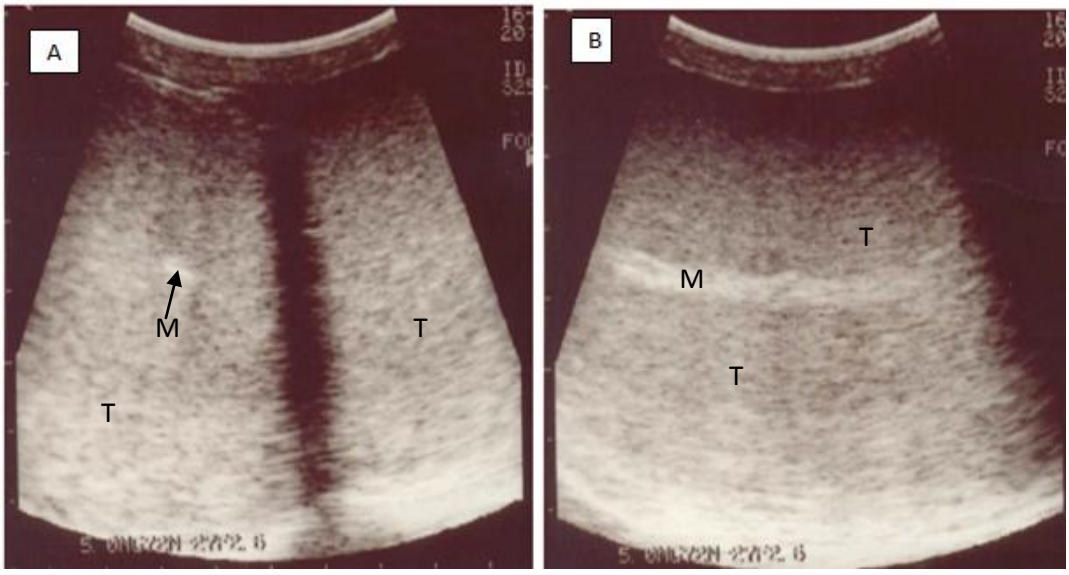


Plate III.Sonograph of testis. A: Transverse sonograph of a paired testis with homogenously isoechoic testicular parenchyma (T) and a hyperechoic circular area in the middle representing mediastinum testis (M). B: Longitudinal sonograph of normal testis showing a hyperchoic line in the middle representing mediastinum testis (M). Source: (Ali *et al.*, 2011).

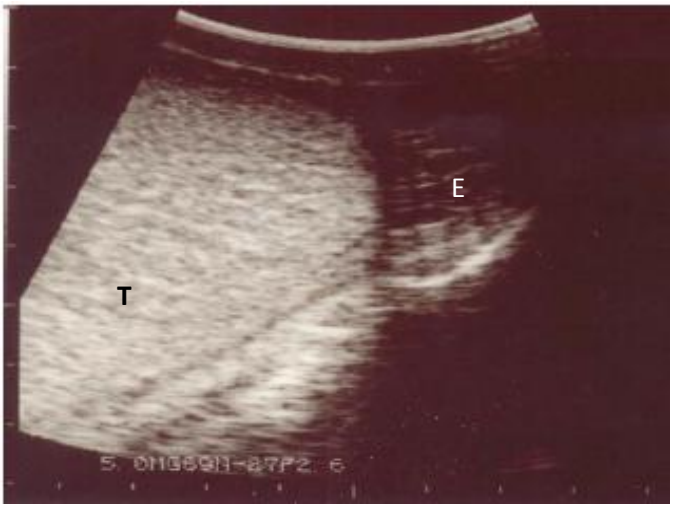


Plate IV. Longitudinal sonograph of a normal testis (T) and epididymal tail (E). Source: (Ali *et al.*, 2011).

Mean testicular echogenicity is negatively correlated with the percentage of morphologically normal live spermatozoa (more echogenic testes were associated with fewer normal sperm) but not with any other semen quality measure (Moxon *et al*., 2015). Moxon *et al*. (2015) reported that there is no relationship between either mean prostatic echogenicity or mean prostatic heterogeneity and any semen quality measure. He further reported that there was no relationship between age and any testicular or prostatic parameter; bodyweight was however significantly correlated with total testicular volume, total epididymal tail volume and total prostatic volume

Studies on testicular measurements show that the width of testicle is the parameter that mostly correlates with testicle weight (Palmer *et al.,* 1998). Palmer *et al.* (1998) found that the testicular width is highly correlated with its weight (r = 0.83) than by the length (r = 0.80), or by the height (r = 0.78). Najjar *et al.* (2012) also reported that testicular width can be measured quickly by ultrasonography and allows the assessment of testicular sperm production.

2.5.4 Use of ultrasound in the diagnosis of testicular and epididymal diseases

Diagnostic methods of assessing the health of the ram testis and related structures include manual palpation, measurement of scrotal circumference, testicular diameter and evaluation of semen. Diagnostic ultrasound has been used for the diagnosis of several types of testicular and epididymal lesions; and the extent of damage to a testis can also be determined (Ali, *et al*., 2011). Such conditions include orchitis (Bird and Rosenfield, 1984) occult neoplasms (Hendry *et al.*, 1984), cystic lesions (Leung *et al.*, 1984)and epididymitis (Yin and Trainor, 2009). It is also helpful to aid diagnosis in testicular torsion, cryptorchidism, scrotal hernia, varicocoele and follow-up of testicular microlithiasis

(Karmazyn, 2010). In animal reproduction, it is almost exclusively used in the female for early pregnancy diagnosis (Anwar *et al*., 2008), monitoring morphological and physiological changes of the ovaries and tubular genital tract, as well as for the diagnosis of pathological lesions of these organs (Hinkeldey and Hopkins, 1996). Technical advances of ultrasound applications and post processing developments have enabled new aspects in the structural and functional analysis of testicular tissue and hence, male fertility (Schurich *et al.,* 2009).

Diagnostic ultrasound seems to be the best examination technique of male fertility assessment and is ideally suited for the evaluation of scrotal contents because of their general superficial location. It is very useful in confirming the presence or absence of a mass, as the homogeneous echo pattern of the normal testis serves as a guide for the detection of small intra-testicular focal lesions; the size of which can also be measured accurately. In many cases, it can give a specific diagnosis (Suchana, 2013). With this technique, it is also possible to differentiate between solid and cystic enlargements. It may also be used as a guide for a definitive biopsy or surgical exploration.

Evaluation of the testis and accessory glands of rams using ultrasound provides new tools for breeding soundness evaluation (Camela *et al*., 2014). Testicular biometry can be used as an auxiliary tool (normality reference) to diagnose pathologies in rams (Camela *et al*., 2014).

The reproductive capacity of breeding is accurately measured by andrological examination (Unanian, 2000). It is also possible to diagnose asymptomatic andrological changes by conventional ultrasound examinations (Teixeira *et al*., 2012).

2.5.5 Testicular abnormalities

2.5.5.1 Infertile bulls*:*

Ali *et al*. (2011) reported various abnormalities in the scrotal echo texture of infertile bulls. An infertile bull may be presented with hard consistency of the testes and abundance of hyperechoic areas with acoustic shadowing scattered in the parenchyma of both testes which were assumed to be mineralization in the testes (Ali, *et al*., 2011). Ahmad *et al.* (1993_a); Ahmad and Noakes (1995_a) also observed similar hyperechoic areas in the testicular parenchyma of infertile goats.According to Lenz and Giwercman (2008), such bright echogenic spots were representing microlithiasis or microcalcifications.

Infertile bulls may also be presented with bigger testes, pulpy consistency with many anechoic areas (about 3.5cm) within the testis and the epididymal head, demarcated from the rest of the ultrasonographic measurement (Ali, *et al*., 2011). These lesions, which were assumed to represent cysts (spermatocoele), were also reported in dogs (England, 1991). Hyperechogenic mass, along with anechoic areas, was also seen below the scrotal skin. Ahmad *et al.* (1999) described a case of hydrocoele in a male goat following ligation of testicular artery. The affected testis was encircled by anechoic fluid with hyperechoic fibrin strands within the fluid. In other studies, Ahmad and Noakes, (1995b); Ahmad *et al.* (2000) observed anechoic areas, representing sperm granulomata within the epididymal head and the tail of infertile rams.

In Brucella affected infertile bulls, the tail of the epididymides may be enlarged with few hyperechoic areas and acoustic shadowing, presumably representing chronic epididymitis

(Ali, *et al*., 2011). Similar observations have been described in a ram (Ahmad *et al.*, 1991), in which the tail of epididymides revealed a few hyperechoic areas.

2.5.5.2 Hydroceles*:*

This may be unilateral or bilateral and can be seen as an isolated finding or in conjunction with other acute or chronic pathology. Many of these fluid collections are congenital. Acquired hydroceles are associated with infections, tumors, trauma, torsion and radiation therapy. Hematoceles and pyoceles are complex hydroceles. Sonographically, a simple hydrocele is seen as an anechoic dark fluid collection surrounding the testicle, whereas a complex hydrocele may contain internal echoes with septations and loculations. A chronic hydrocele may also demonstrate internal echoes from cholesterol crystal formation. (Blaivas *et al.,* 2001; Blaivas and Sierzenski, 2001; Blaivas, 2003; Blaivas and Brannam, 2004).

2.5.5.3 Varicocele*:*

These are collection of tortuous and dilated veins within the pampiniform plexus of the spermatic cord. They are found in approximately 15 % of adult males and can result in infertility secondary to decreased sperm motility and count. They are due to incompetent valves in the testicular vein. The vast majority of varicoceles are located on the left side and only 1 % are bilateral. The left sided predominance of varicoceles is thought to be due to the long course and angle of entry of the left testicular vein as it empties into the left renal vein. The right testicular vein is shorter and empties directly into the inferior vena cava. Varicoceles are much more apparent when the patient performs a Valsalva maneuver or is standing. Hence, ultrasound scans are performed in both supine and standing positions.

Sonographically, they appear as multiple anechoic serpiginous tubular or curvilinear structures of varying sizes (larger than 2 mm in diameter) in the region of the epididymides. Power Doppler should be used to confirm flow in the varicocele (Promes, 1997; Blaivas, 2003; Akin, 2004).

2.5.5.4 Orchitis:

This is an acute infection of the testicle usually following epididymitis. Orchitis often presents with a tender and inflamed testicle. On gray-scale ultrasound, orchitis is seen as an enlarged testicle with heterogeneous echogenicity. This appearance is nonspecific and can be seen in many other conditions such as tumors, metastasis, infarction and torsion (Blaivas *et al.,* 2000; Blaivas and Sierzenski, 2001; Blaivas, 2003).

2.5.5.5 Testicular torsion:

This is a urologic emergency. Prompt diagnosis and early treatment are essential as time is critical for testicular salvage. The majority of testicular torsions result from anatomic defects that lead to redundant spermatic cord and anomalous suspension of the testes in the scrotum. Sonographic findings can be variable depending on the duration of torsion and extent of vascular compromise. The testicle can appear enlarged and hypoechoic and the parenchyma of the testicle will become less homogenous when compared with the unaffected testicle. Unfortunately, ultrasound is sometimes not helpful, as sonographic findings may be subtle early in the course of a condition (Blaivas *et al.,* 2000; Blaivas *et al.,* 2001; Blaivas and Sierzenski, 2001).

2.5.5.6 Blunt trauma to the scrotum:

This can lead to damage of the testicle and adjacent structures. Injuries to scrotum include laceration, hemorrhage, or contusion of the testicle. Sonographic findings suggestive of testicular injury include irregular outline and an inhomogeneous echo texture from hemorrhage or infarction. A discrete fracture line is seen by ultrasound in only 17 % of ruptures (Blaivas*et al.,* 2000; Blaivas *et al.,* 2001; Blaivas and Sierzenski, 2001).

2.5.6 Epididymal abnormalities

Epididymitis is the most common cause of acute scrotal pain in post-pubertal males. Classically, the patient is presented with a painful tender scrotum, dysuria, and fever. Retrograde spread of infection from the bladder or prostate is usually the underlying etiology with the head of the epididymides most commonly involved. Gray-scale findings of acute epididymitis include an enlargedepididymides with decreased echogenicity. Often, a reactive hydrocele is noted as well. A chronically inflamed epididymides becomes thickened and has focal echogenicity with areas of calcification. With Doppler sonography, increased blood flow secondary to epididymal inflammation is noted. The presence of normal or increased blood flow in the affected testicle when compared to the contralateral side differentiates epididymitis from testicular torsion (Blaivas, 2000; Blaivas *et al.,* 2001; Blaivas and Sierzenski, 2001; Blaivas, 2003).

2.6 Ultrasonographic Measurement of the Testes and the Epididymides

Cartee *et al.* (1989) stated that there was no significant difference in physical and ultrasonographic measurements of exposed testicles (in vitro), while such difference was observed when the scanning was made through the intact scrotal wall. Ali, *et al*. (2011),

observed a significant positive correlations between ultrasonic and calipers measurements for the testicular diameter ($r = 0.992$; $P<0.01$) and diameter of the mediastinum testis ($r = 0.979$; $P<0.01$) as was similarly observed by Bailey *et al.* (1998) while measuring the testicular length and width using calipers and ultrasonographic imaging. Hence, both techniques were found to be quite reliable (Ali, *et al*., 2011). However, Ali, *et al*., (2011) suggested that some value (of about 3 mm) should be added to the ultrasonic measurement to compensate the decrease caused due to pressure effect of the transducer on the organ.

Ultrasonically, the testis of fertile buffalo and cattle bulls appeared as a homogenously hypoechogenic structure. The mediastinum testis was represented by an almost circular hyperechoic area in the middle of testis in transverse images, while it was seen as a hyperechoic line in longitudinal images. These observations are similar to those described for boar (Cartee *et al.*, 1986), dog (England, 1991), bull (Cartee *et al.*, 1989), rams and male goats (Ahmad *et al.*, 1991) and camels (Pasha *et al*., 2011).

Ahmad *et al*. (2011) also recorded an increase in the echogenicity of testicular parenchyma with advancement in age of the bull; the mean number of pixels of testicular ultrasonograms increased significantly up to 24 months of age and then leveled off as the age increased. The diameter of the mediastinum testis, irrespective of the side or measuring technique, varied from 2.2 to 4.3 mm. These findings are supported by those of Cartee *et al.* (1989), who observed that diameter of mediastinum testis of cattle bulls ranged from 2.0 to 4.0 mm. However, the diameter of mediastinum of canine testis was 2.0 mm (Pugh *et al*., 1990), which reflects specie differences.

Teixeira *et al*. (2011), studying sheep ultrasonography established an analytical method of testicular stromal echogenicity through its gray scale and correlated it with testicular morphometry and seminal evaluations. In 2012, Teixeira *et al*. also reported the ultrasound echogenicity of the testicular stroma in prepubertal sheep to be homogeneous, with echogenicity 53.95 ± 6.5%, 55.70 ± 6.4% and 55.68 ± 6.4% for the right, left and the testis mean, respectively and also reported a high correlation between echogenicity and scrotal circumference (r= 0.80).

2.7 Breeding Soundness Examination

Breeding soundness evaluation (BSE) is the primary assessment of the ram's capacity for serving and impregnating ewes (Fitzgerald, 2007). It consists of an overall physical examination, with special emphasis on the reproductive organs and semen evaluation. Semen for evaluation is usually obtained by electroejaculation (Bahareh *et al*., 2012). The BSE cannot be used frequently to evaluate semen because repeated collections may affect the sperm output and recurrent electroejaculations may be traumatic (Bahareh *et al*., 2012).

At present, there is no reliable method to predict changes in semen quality in the ram hence ultrasonographic examination of the testes, epididymides and accessory sex glands have proved to be a valuable, noninvasive technique for the assessment of genital macroscopic morphology and pathology in several mammalian species of veterinary interest (Ka¨hn, 2004) including the ram (Gouletsou *et al*., 2003). Computerized image analysis of ultrasonograms can provide further information on the structure and function of the tissues (Evans *et al*., 1996; Giffin *et al*., 2009). The echo texture, or the ultrasonographic appearance of a tissue, can be assessed by determining the pixel intensity and uniformity (Liu *et al*., 2008). Each pixel represents the ability of a discrete unit of tissue to transmit or

refract high frequency ultrasound beams, which in turn is dependent upon the physical properties of the acoustic tissue interfaces (i.e. cellular composition, water and macromolecular content (Pierson and Adams, 1995).

Bahareh *et al*. (2012) reported that scrotal ultrasonography combined with computer-assisted analyses of epididymal and testicular echo texture in the ram is a valuable method for determining certain current and future semen parameters, which would help in breeder selection.

2.7.1 Semen characteristics of normal ram

Visually, ovine semen is evaluated for volume, colour, density, gross motility and presence or absence of foreign material. Ejaculate volume varies from 0.3 to 1.5 ml, with an average of 0.5 to 1.0 ml in most rams when semen collection is done with an artificial vagina (Salisbury *et al*., 1978). Ejaculate volume may be influenced by duration of stimulation when semen collection is done by electro ejaculation (Zemjanis, 1970). Semen superiority can be affected by heat stress, poor nutrition, overfeeding and diseases like foot abscess and foot rot, diseases of the reproductive tract, including ovine brucellosis and *Actinobacillus seminis* infection (Zemjanis, 1970).

2.7.2 Volume of ejaculate

The volume of ejaculate is easily measured with a calibrated tube which may be incorporated into the collection receptacle (Mickelsen *et al*., 1991). Marked species variation and the method of collection greatly influence the volume obtained, its gross appearance and spermatozoa concentration. Usually, the ejaculate volume is larger but sperm concentration is lower when collected by electro ejaculation than when collected by

artificial vagina (Mickelsen *et al*., 1991). Further repeated ejaculations whether associated with semen collection or sexual activity decreases the volume and concentration of semen. Semen volume tends to be greater if collection is preceded by a period of sexual arousal (Mickelsen *et al*., 1991). The approximate average ejaculate volumes are about 1-1.5 ml in the ram though ejaculate volume does not correlate with fertility. In general, sperm volume, motility and morphology are better guides to fertility (Brito *et al*., 2002).

2.7.3 Gross appearance of ejaculate

The opacity and colour of the samples are usually recorded. Opacity subjectively reflects the concentration of the sperm. Categories include thick, creamy, opaque, opalescent, milky and watery white (Brito *et al*., 2002). Bucks, bulls and rams usually have opaque, creamy white semen due to high sperm concentration; and as the density of sperm decreases, the semen becomes more translucent and "milkier" in appearance. Contaminants especially intact or degenerated erythrocytes can cause discolouration of the semen (Brito *et al*., 2002).

2.7.4 Sperm motility

Sperm motility is assessed subjectively and depends on careful handling of the sample for meaningful results (Brito *et al*., 2002). Motility is correlated with fertility; however, improper semen handling adversely affects its quality. Wave motion is a subjective assessment of the gross motility of sperm. Four general classifications are used; very good, good, fair and poor based on the amount of swirling activity observed in a drop of semen on a microscope slide at low power magnification (x40). These categories roughly correspond to distinct vigorous swirling, moderate slow swirling, and barely discernable swirling or

lack of actual swirling but with motile sperm present which may cause the sample to have an irregular oscillating experience. (Brito *et al*., 2002).

2.7.5 Sperm morphological abnormalities

Species differences exist with respect to the fine points of sperm morphology, but, all sperm have the same basic structure (Koonjaenak, 2006). Abnormalities are conveniently divided into those of the head, mid-piece and tail, and are often categorized as primary or secondary defects.

Primary defects occur during spermatozoal production and caused by pathological processes in the seminiferous epithelium (Johnson, 1994). Primary defects are therefore testicular in origin and include such defects as nuclear vacuoles, heads that are double, too large, too small or oddly shaped (pear-like, round, twisted, knobby); mid pieces that are swollen, kinked, twisted, double or eccentrically attached to a head (abaxial), and tails that are coiled. Primary abnormalities are considered more serious than secondary defects (Amann, 1998). Secondary defects can occur at any time from storage in the epididymides until the smear is made (Amann, 1998). Secondary abnormalities are created after leaving the seminiferous tubules, the maturing sperm pass through the rete testis, the ductuli efferentes, the head of the epididymides, the body of the epididymides, and into the tail of the epididymides, which serves primarily as a storage site until ejaculation. Failure of maturation and abnormal epididymal function may result to secondary defects (Acott and Hoskins, 1989). Secondary defects include tailless heads, protoplasmic droplets on the mid piece and bent or broken tails or heads (Amann, 1998). Tertiary abnormalities are developed *invitro* as a result of improper semen collection or handling procedures (Verstegen *et al*., 2002). Using the traditional classification system the assortment and its

interpretation can be incorrect because the origin of some spermatozoal morphologic abnormalities is unknown and some defects can be either primary or secondary (Amann, 2000). Proximal droplets may result due to disturbance of spermatogenesis (primary) or disturbance of epididymal function (secondary). Detached heads may be due to a defect of the basal plate which connects the sperm head to the mid piece (primary), or it may be due to abnormal epididymal function (secondary) (Barth *et al*., 2008), or it can be artifact produced by smearing the semen (tertiary).

Primary defects are more deleterious to fertility than secondary defects (Amann, 1998). Furthermore, since adverse conditions that cause both types of abnormalities can affect epididymal function and spermatogenesis simultaneously, primary and secondary defects are equally important as indicators of disturbance of testicular function (Barth *et al*., 2008).

Verstegen *et al*. (2002) also reported defects to be classified as major or minor according to their importance to fertility. Major defects include abnormal heads, mid pieces, proximal droplets, double forms, which are thought to have a greater impact on fertility (Amann, 1998). The major defects are mostly those that have been associated with a presumed disturbance of spermatogenesis. Minor defects such as distal droplets or simple bent tail have an unknown role or no consequence for fertility (Capell and Paulsen, 1972). Although minor defects are of less importance with regard to fertility, they may cause a serious reduction in fertility when present in very high numbers (Barth *et al*., 2008). Generally less than 20 % of sperm abnormalities are usually accepted but high percentages of abnormal spermatozoa may compromise fertility (Sekoni *et al*., 1991; Amann, 1998).

2.8 Other Imaging Modalities Used in the Evaluation of Male Reproductive System

Other diagnostic imaging modalities used to evaluate the clinical disorders of the male reproductive system include Doppler Ultrasound, Contrast-enhanced transrectal ultrasound, Sonoelastography, computed axial tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT) and positron emission tomography (PET) (Futterer *et al.*, 2008). SPECT and PET are nuclear medicine procedures (Bernardo-Filho *et al.*, 2005).

2.8.1 Doppler Ultrasound (DUS)

2.8.1.1 Power Doppler US and colour Doppler US*:*

Power Doppler US is more sensitive (97%) than colour Doppler US (88%) for the detection of intratesticular blood flow as a result of increased depiction of intratesticular vessels (Luker and Siegel, 1996; Barth and Shortliffe, 1997). In the diagnosis of testicular torsion using Doppler ultrasound, the presence of blood flow does not exclude torsionbecause some false negative results with colour Doppler US are attributed to an incomplete torsion of the spermatic cord when the systolic value still is recorded whereas the diastolic one is absent or reduced. Reactive hyperemia of the *Tunica vaginalis* can also be wrongly interpreted as blood flow into the capsular arteries (Berman *et al.*, 1996).

2.8.1.2 Doppler trans rectal ultrasound:

Since prostate cancer has an increased microvessel density compared with healthy prostatic tissue Doppler visualization of streaming blood within the vasculature may aid in detecting and localizing prostate cancer (Futterer *et al.*, 2008). Cancer grade correlates positively with the degree of Doppler signal (Tang *et al.*, 2003). Few studies have compared Doppler-

guided and systematic biopsies directly (Heijmink *et al.*, 2007), with one study achieving a detection rate of 40% (Okihara *et al.*, 2000). A study performed on Doppler TRUS staging revealed a sensitivity of 59% for detecting locally advanced disease (Sauvain *et al.*, 2003). While addition of Doppler imaging to gray-scale TRUS seems to aid in differentiating fibrotic tissue from recurrence (Sudakoff *et al.*, 1996; Tamsel *et al.*, 2006).

2.8.2 Contrast-enhanced transrectal ultrasound

Visualization of the cancer microvasculature can be improved by means of contrast-enhanced TRUS (Futterer *et al.*, 2008). New contrast agents are constantly being developed; however, not all are approved by the Food and Drug Administration or European Medicines Agency and few have been applied in prostate research. The contrast agents used most widely in prostate cancer are Levovist (Schering, Berlin, Germany) and SonoVue (Bracco, Milan, Italy). Contrary to magnetic resonance imaging (MRI) contrast agents, these 2 mm to 8 mmmolecules remain within the vasculature, thus acting as blood pool agents. Several signal reception techniques may however be applied to the contrast agents (Futterer *et al.*, 2008). For example, conventional Doppler imaging may be enhanced by the micro-bubbles. This increases the signal received from the vasculature. The main disadvantage, however, is the high-energy output by the US probe (the high mechanical index [MI]). Micro-bubbles remain intact at low MIs but at higher MIs, the bubbles expand beyond their limits and are destroyed. Therefore, scanning with high MIs decreases the half-life of the contrast agent (Futterer *et al.*, 2008).

2.8.3 Sonoelastography

US signal transmission and backscattering can also be used to analyze small displacements of tissue during application of compression (Mitterberger *et al.*, 2007). From these data, the

compression characteristics of the prostatic tissue can be determined. Malignant tissue has lower compression ability (Pelzer *et al.,* 2005) and cancer foci are more solid. Few studies of prostate sonoelastography are published. In a study by (Heijmink *et al.,* 2006) on a population of 404 men at risk of prostate cancer, the detection rate by means of real-time sonoelastography targeted biopsies was 37.4%. With the inclusion of systematic biopsy in the analysis the sensitivity increases to 84.1% of all positive cases. A preliminary study comparing sonoelastography imaging with systematic biopsy revealed a sensitivity and specificity of localizing prostate cancer of 86% and 72%, respectively (Jakobsen *et al.*, 2005).

2.8.4 Magnetic Resonance Imaging (MRI)

MRI is useful for both detection and characterisation of prostatic cysts detected on a TRUS and evaluation of the vas deferens, seminal vesicles and ejaculatory ducts (Ammar *et al*., 2012). MR imaging is a powerful method to image the prostate with high spatial resolution, which provides excellent soft tissue contrast (Futterer *et al.,* 2008). It is a valuable tool for the detection of cancer, evaluation of extra prostatic extension of cancer, and treatment. MR imaging of the prostate preferably is performed with an endo rectal coil. On T2-weighted images the zonal anatomy is distinguished clearly. The peripheral zone displays hyper intense signal compared with the central gland because the latter is more in homogeneous because of benign prostatic hyperplasia (BPH). Prostate cancer commonly occurs as an area of low signal intensity within the brighter peripheral zone (Futterer *et al.,* 2008).

Since MR imaging is costly and time-consuming it plays no role as a screening imaging modality in patients who have suspected prostate cancer. In patients who have at least two negative TRUS-guided biopsy sessions, however, MR imaging plays an important role

(Beyersdorff *et al.,* 2002). T2-weighted MR imaging obtained accuracies of 67% to 72%, with relatively high sensitivities (54% to 81%), whereas specificities were low (46%–61%) in localizing tumors (Jager *et al.,* 1996; Scheidler *et al.,* 1999; Haider *et al.,* 2007; Testa *et al.,* 2007). Functional MR imaging techniques, such as dynamic contrast-enhanced MR imaging (DCE-MRI) and proton magnetic resonance spectroscopic imaging (MRSI) can be applied to increase the localization performance.

2.8.4.1 Dynamic contrast-enhanced MR imaging (DCE-MRI): DCE-MRI is a method that provides information about micro vascularity and angiogenesis. It is an effective tool in visualizing the pharmacokinetics of gadolinium uptake in the prostate. Therefore, data reflecting tissue perfusion, micro vessel permeability, and extracellular leakage space can be obtained. Insights into these physiologic processes are obtained qualitatively by characterizing kinetic enhancement curves or quantitatively by applying complex compartmental modeling techniques (Futterer *et al.,* 2006).

2.8.4.2 Proton magnetic resonance spectroscopic Imaging (MRSI):

MRSI is a unique method that provides information on prostate metabolism. It provides quantitative data based on the citrate, choline, and creatine levels and the ratios between these metabolites (Futterer *et al.,* 2008). A high level of citrate is an important marker in healthy or benign prostate tissue. Thus, reduced levels of citrate and increased levels of choline are indicative of prostate cancer. A commonly used marker for cancer tissue is the ratio of (choline+creatine)/ citrate. This allows MRSI to be used for tumor localization, characterization, treatment planning, and therapy evaluation. The addition of MRSI to T2-

weighted MR imaging increases the localization specificity up to 91% (Scheidler *et al.*, 1999).

2.8.4.3 Diffusion-weighted imaging: Diffusion is a 3-D physical process important in many physiologic functions. This technique has been introduced in prostate MR imaging as a result of the introduction of ultrafast echo-planar sequences. The apparent diffusion coefficient (ADC) values of malignant tissue are lower than in the healthy peripheral zone probably caused by increased cellular density (Anderson *et al.*, 2000). The ADC value of the healthy peripheral zone also is higher compared with the central gland because of the higher water content in the prostatic luminal space.

2.8.5 Nuclear medicine procedures

Nuclear medicine imaging demonstrates physiology rather than anatomy and it is considered as a metabolic (functional) image. Hence, these techniques are valuable in the early diagnosis of various diseases (Bernardo-Filho *et al*., 2008). PET and SPECT procedures basically involve the administration of a radiopharmaceutical (radiobiocomplex) (Bernardo-Filho *et al.*, 2005) that is selected for its ability to be localized with high uptake in the specific tumor site or target tissue. The radiobiocomplexes are molecular and cellular structures labeled with a specific radionuclide and are applied to patients after appropriate quality control (Perkins, 2007). The image is formed by detection of the gamma rays or characteristic X-rays (SPECT) or the photonic energy emitted due to the annihilation of the positron (PET) (Saha, 2004, Perkins, 2007; Fanti *et al*., 2007).

The characteristics of an ideal PET or SPECT tracer for imaging prostate cancer (PC) include; availability, ability to be up taken and exhibited at the high target tissues, identify

both local and distant lesions, formulated by rapid and simple radiochemical syntheses, labeled with a radionuclide with favorable decay characteristics and should have physical half-life of sufficient duration to allow performance of the clinical examination (Saha, 2004, Jana and Blaufox, 2006, Fanti *et al*, 2007).

2.8.5.1 Single photon emission computed tomography (SPECT): The gamma rays or characteristic X rays are emitted by the radionuclide that is fixed in the radiobiocomplex. These rays are detected by a gamma camera and an image of the localized radioactivity is produced. In SPECT, the gamma camera rotates around the body of the patient and the image is reconstructed to produce image slices similar to a CT scan (Bernardo-Filho *et al*., 2008). SPECT images may be recorded following conventional planar imaging without increasing the amount of radioactivity administered to the patient (Perkins, 2007). The main radionuclides used to label radiopharmaceuticals used in the SPECT are shown in the Table 2.1

2.8.5.2 Positron emission tomography (PET):

PET scanning uses a positron emitting tracer fixed in the radiobiocomplex (Bernardo-Filho *et al*., 2008). The pair of photonic energy is detected by the PET scanner, recorded and tomographic images of the tracer distribution in the body are reconstructed using mathematical algorithms in a computer (Bernardo-Filho *et al*., 2008). Positron emitting radionuclides are produced in a cyclotron optimized for routine clinical use and they have a relatively short halflife (Table 2.2). PET images are a volumetric set of data that can be displayed as tomographic images in the transaxial, coronal, or sagital planes. A limitation

of PET is the lack of an anatomical reference frame whereas CT is an excellent morphological imaging modality with anatomical resolution (Bernardo-Filho *et al*., 2008).

The usefulness of PET scanning with 18-fluorine– labeled deoxyglucose (FDG) in detecting prostate cancer is compromised by the low uptake of FDG by prostate cancer cells and significant overlap with the marker uptake by (benign prostatic hyperplasia) BPH, fibrosis, and inflammation (Futterer et al., 2008). Generally, FDG-PET is not recommended for evaluation of the prostate. Carbon-11–labeled choline (^{11}C-choline) accumulates in prostatic cells and has an advantage over FDG, in that it is not excreted via the urinary tract and thereby does interfere with the visualization of the prostate. More so, the prostate is the only organ in the pelvis to accumulate ^{11}C-choline. Drawbacks are the high costs of ^{11}C-choline and its short 20-minute half-life (Futterer et al., 2008).

Table 2.1 - Main radionuclides used to label radiopharmaceuticals in SPECT

Radionuclide	Decay	Main photon energy (keV)	Physical half-life
Technetium-99m (99mTc)	Gamma	140	6 hours
Indium-111 (111In)	Gamma	173 and 247	2.9 days
Iodine-123 (123I)	K capture	160	13 hours
Iodine 131 (131I)	Beta minus and gamma	360	8 days
Thalium-201 (201Tl)	K capture	78	73.1 hours

Source: (Perkins and Frier, 1999; Perkins, 2007)

Table 2.2 - Half-life of radionuclide positron emitters used to PET scan.

Radionuclide	Physical half-life
Fluorine-18 (18F)	110 minutes
Carbon-11 (11C)	20 minutes
Nitrogen-13 (13N)	10 minutes
Oxygen-15 (15O)	122 seconds

Source: (Perkins and Frier, 1999; Perkins, 2007; Bouchelouche and Oehr, 2008)

2.8.5.3 Radiopharmaceuticals used in the clinical evaluation of the testes:

Nuclear medicine studies have been used to evaluate the acute scrotum **(**testicular torsion and epididymoorchitis) and testicular tumors (Jana and Blaufox, 2006; Futterer *et al.*, 2008). The radiopharmaceuticals used in the clinical evaluations of disorders of the testes include; 99mTc-pertechnetate (Kim *et al*, 1993; Jana and Blaufox, 2006); Gallium-67 citrate (Willan *et al*, 1987; Warren, 1995) and 18F-FDG (Ganjoo *et al*, 1999).

2.8.5.4 Radiopharmaceuticals used in the clinical evaluation of the prostate:

Images of the prostate are highly relevant because of their involvement in many male disorders. However, a number of problems have prevented success in its exploration, such as the size and its location deep in the pelvis; and proximity to the urinary bladder (Bernardo-Filho *et al*., 2008). Most of the radiopharmaceuticals used are primarily excreted by the kidneys, resulting in bladder activity that obscures the prostate (Jana and Blaufox, 2006, Futterer *et al.*, 2008). Radiopharmaceuticals used in the clinical evaluations of disorders of the prostate include; 99mTc-colloid (Zuckier *et al*, 1990; Jana and Blaufox, 2006); 99TcMDP (Gerber and Chodak, 1991); 11C-Acetate (Kotzerke *et al*, 2002); 11C-Choline (Kotzerke *et al*, 2000; Roivainen *et al*, 2000); and 18F-Choline (Jana and Blaufox, 2006).

2.8.5.5 Radiopharmaceuticals used in the clinical evaluation of the seminal vesicles, urethra, penis and pelvic floor muscles:

Radiopharmaceuticals like 99mTc ciprofloxacin and 99mTc sulphur colloid (Choe *et al*, 2003; Orhan *et al*, 2008) have been used for imaging the seminal vesicles; 18F-FDG, 99mTc- nanocolloid, 99mTc-labeled Red Blood Cells (Halac *et al*, 2007; Valdés Olmos *et*

al, 2001) have been used for Penis while 18F-FDG (Stelzner *et al*, 2005) has been used for imaging the muscle activity in the pelvis.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of Research

This experiment was carried out in the Department of Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, North-Western Nigeria with geographical coordinates of Zaria; $11^0$4'0'' North and $7^0$42'0'' East.

3.2 Materials and equipment

3.2.1 Equipment used

A Sonostar S600 ultrasound Machine with a 5.0M Hz linear probe, a sony thermal printer, digital camera with 32 mega pixel, laptop computer, ultrasound table, Tiger electric generator, electric plug (extension box), clinical laboratory coat, meter rule, razor blade, electric clipper, rope, examination table, flexible measuring tape, digital vernier caliper, sample bottles, table centrifuge, deep freezer, 23G needles and syringes.

3.2.1.1 Equipment used for semen collection and analysis:

Electro ejaculator, semen collector, collecting tubes, slides and cover slips, funnel, light microscope, and bucket.

3.2.1.2 Equipment used for Testosterone Assay:

Pipette, Adjustable dispensers, Microplate washer, Microplate Reader (450nm and 620nm wavelength), Absorbent paper, Microplate cover, Vacuum aspirator and Timer.

3.2.1.3 Equipment used for Follicle Stimulating Hormone and Luteinizing hormone Assay:

Precision pipette, disposable tips, ELISA reader (450nm), paper towel and Graph paper

3.2.2. Consumables

3.2.2.1 Consumables used:

Thermal printing paper, aqua sonic gel, tissue paper, cotton wool, disposable gloves, feeding materials, toilet soap, spirit, dettol antiseptic solution, soap and petrol for fueling the generator.

3.2.2.2 Consumables used for semen analysis:

Cotton wool, petroleum gel, test tube rack, test tube, pH papers and clean water.

3.2.2.3 Consumables used for Testosterone Assay:

Testosterone assay kit, Testosterone Calibrators, Testosterone Enzyme Reagent, Steroid Conjugate Buffer, Testosterone Biotin Reagent, Streptavidin Coated Plate, Wash Solution Concentate, Substrate A, Substrate B and Stop Solution.

3.2.2.4 Consumables used for Follicle Stimulating Hormone and Luteinizing hormone Assay:Distilled water, microwell coated with streptavidin, FSH standard, FSH Enzyme conjugate, LH standards, LH enzyme conjugate, TMB substrate, Stop solution and wash concentrate 20X.

3.2.3 Type and source of study animals

The study animal was the Yankasa ram. Fifteen rams were acquired from reputable livestock of marketsin Makarfi and Tudun Sebu in Makarfi and Soba Local Government areas respectively of Kaduna state, Nigeria.

3.2.4 Ethical Committee permission

The animal care and handlingwere carried out according to the guidelines and principles in the care and use of experimental animals in Nigeria. The protocols used were approved by Ahmadu Bello University's Committee on Animal Use and Care (ABUCAUC).

3.2.5 Inclusive factors and Exclusive factors

Only clinically healthy Yankasa rams between the age of 12 months and 2years were used in the studies. Female animals**,** lambsand sick animals were excluded from the studies.

3.3 Methodology

3.3.1 Study design

Adopting the method by Ahmadi *et al.,* (2012), fifteen adult apparently healthy Yankasa rams were used in this study. They were kept in five pens under zero grazing method, fed with hay, groundnut leaves/straw '*harawa*' and wheat offal with water supplied *ad libitum.* The animals were rested and allowed to adjust to their new environment and feeding regimen for a period of four weeks. Blood and faecal samples were collected and analysed at the hematology and parasitology laboratories respectively and all the animals were dewormed with Albendazole irrespective of the laboratory result. The study was carried out between February and June, 2016. A detailed history, age (Plate V) and weight of each ram were obtained as shown in table 3.1. A thorough physical and clinical examination was also

carried out on each ram. Body condition scores were recorded as per the method described by Nicholson and Butterworth, (1986).

3.3.2 Vernier caliperic measurements

The scrotal circumference was measured in centimeters (cm) by using a measuring tape (McGowan *et al., 1995) (Plate VI)*. The testicular length, width and depth were measured by caliper to the nearest 0.1 cm as described by Abdel-Razek and Ali, (2005). Care was taken to exclude the epididymides while measuring the length.

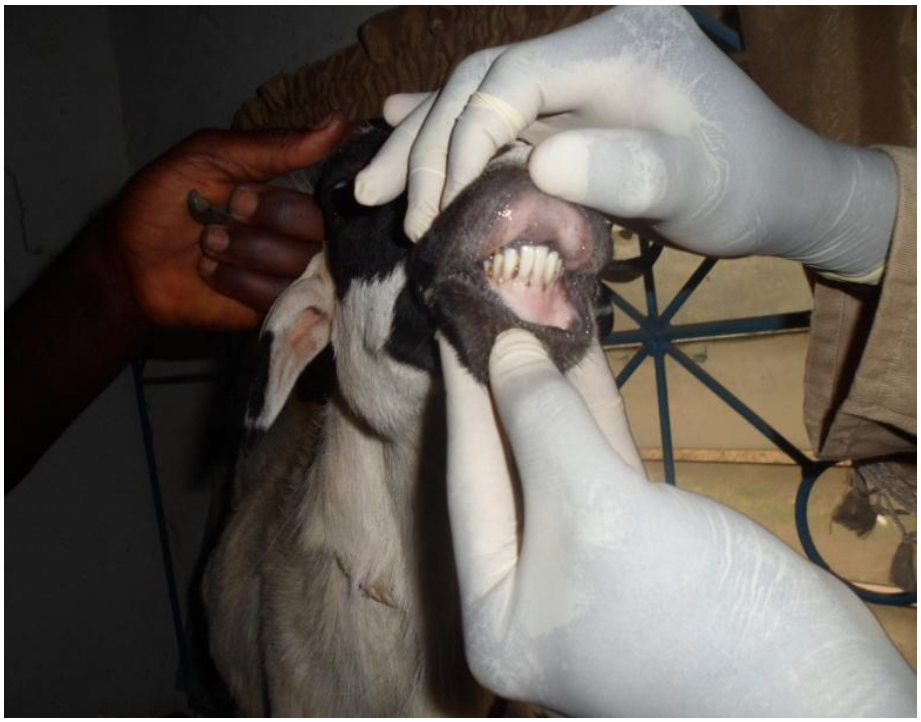


Plate V: Aging of the animals using dentition

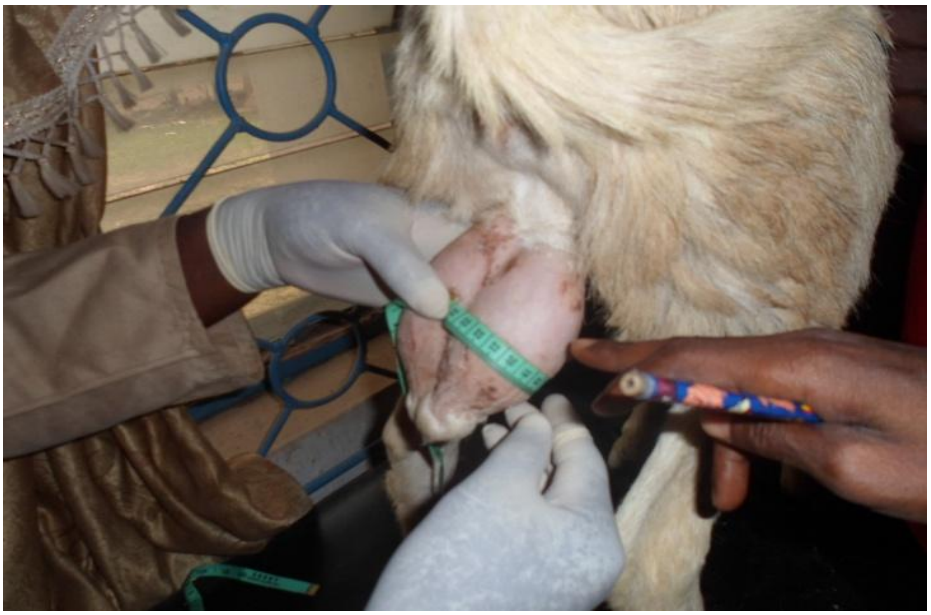


Plate VI: Using a measuring tape to measure testicular circumference of the testes

Table 3.1. Demographic data (age, weight and reproductive history) of the fifteen Yankasa rams Date:- 26/02/2016

Tag No	Age (months)	Weight (Kg)	Body length (cm)	Girth (cm)	Height (cm)	Conformation
1	18	33	88	75	77	Normal
2	30	30	89	84	69	Normal
3	18	22	80	82	65	Normal
4	18	22.5	86	75	73	Normal
5	18	30	81	90	73	Normal
6	18	18	78	70	66	Normal
7	18	29	87	76	74	Normal
8	30	25	85	76	75	Normal
9	30	37.4	106	104	66	Normal
10	18	32.9	86	97	66	Normal
11	24	53.3	105	101	77	Normal
12	30	54.4	110	107	81	Normal
13	18	25	81	78	74	Normal
14	12	31.9	95	97	72	Normal
15	18	22.9	89	87	67	Normal

Testicular length (TL) was measured with flexible tape in centimeters as the distance along the caudal surface of the scrotum, from its point of attachment to the tip of the scrotum (Plate VII); testicular circumference (TC) was taken as the point of maximum dimension around the pendulous scrotum (Akpa *et al*., 2006) while the testicular width (TW) was taken as the division of the testicular circumference by two (Plate VI).

After recording the actual testicular length and width their averages were calculated and the predicted volume (cm^3) was calculated by the formula for a prolate spheroid volume $0.5236(L)(W)^2$ while the predicted testicular weight (gm) was calculated by the formula weight $0.5533(L)(W)^2$ as per the method of Bailey *et al*., (1998). Where L is the Testicular length (TL); W is the testicular width (TW)

3.3.3 Pre-ultrasound scanning consideration

The testicular region to be scanned was liberally shaved after which it was thoroughly cleaned (Plate VIII). The animal was restrained with minimal force on left lateral recumbency on an examination table.

3.3.4 Ultrasonographic procedure and measurements of testes and epididymides

Adopting the method used by Kandiel *et al.,* (2012), an ultrasound examination of Yankasa rams was done using 5.0 MHz linear probe with the B-mode Sonostar ultrasound scanner per cutaneous to examine the testis and epididymides. The testis was scanned transcutaneously in transverse (X) and longitudinal planes (Plate IX and XI). While the thickness of *Tunica albugenia* and mediastinum testis were examined from a longitudinal plane. The tail of epididymideswas visualized from a diagonal plane near the distal end of testis.

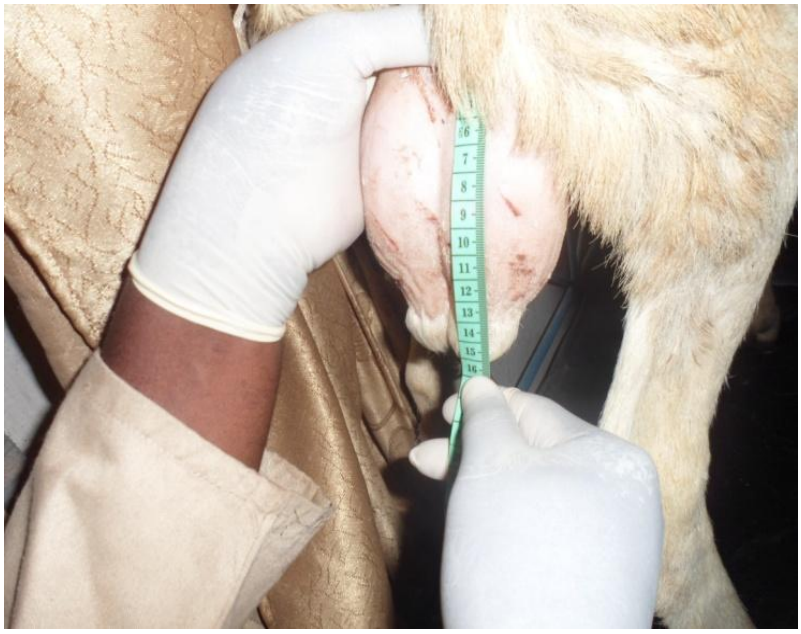


Plate VII Testicular length measurement using a flexible measuring tape.

Plate VIII: Shaving of the scrotum preparatory to ultrasound scanning.

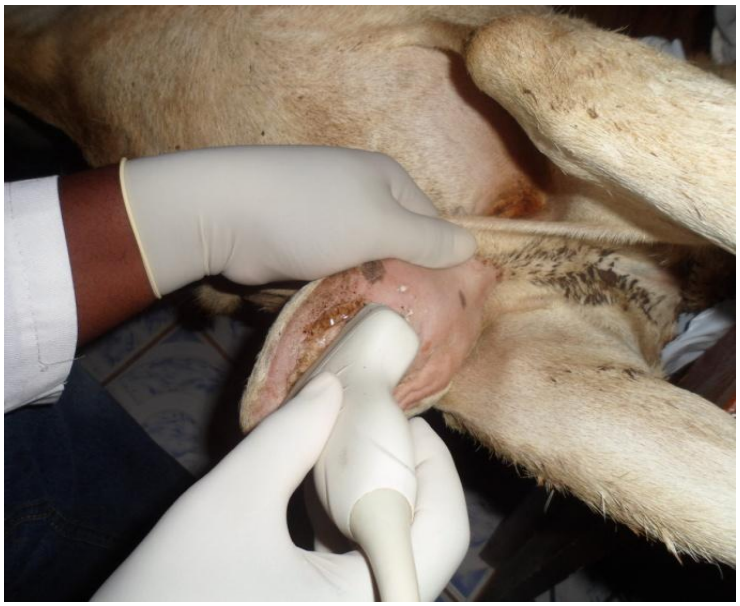


Plate IX: Measuring testicular length using a 5MHZ probe that was placed logitudinally

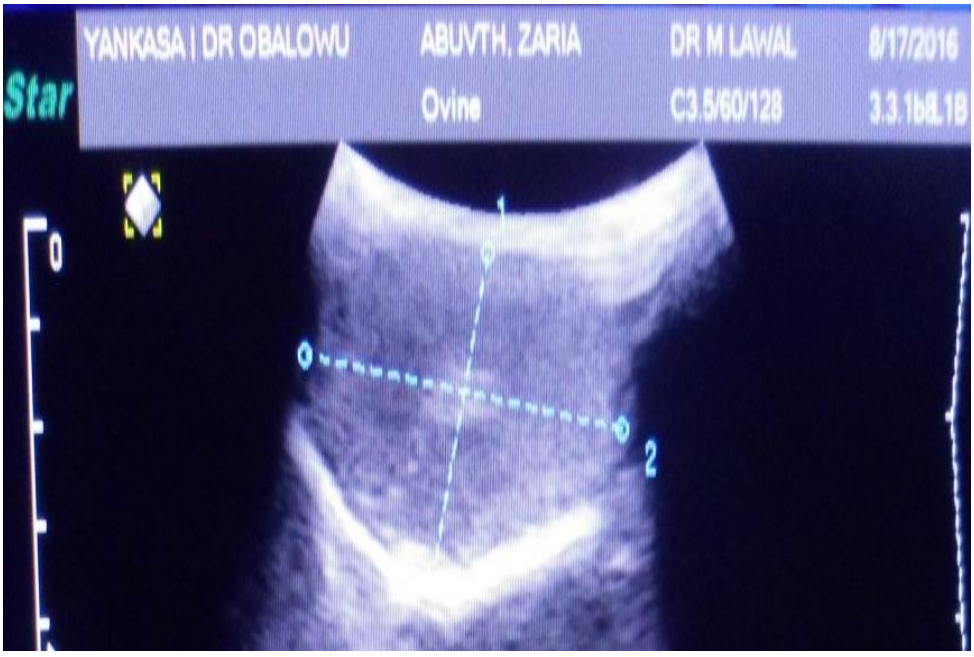


Plate X. Sonograph of a transverse view of the testes of a Yankasa ram

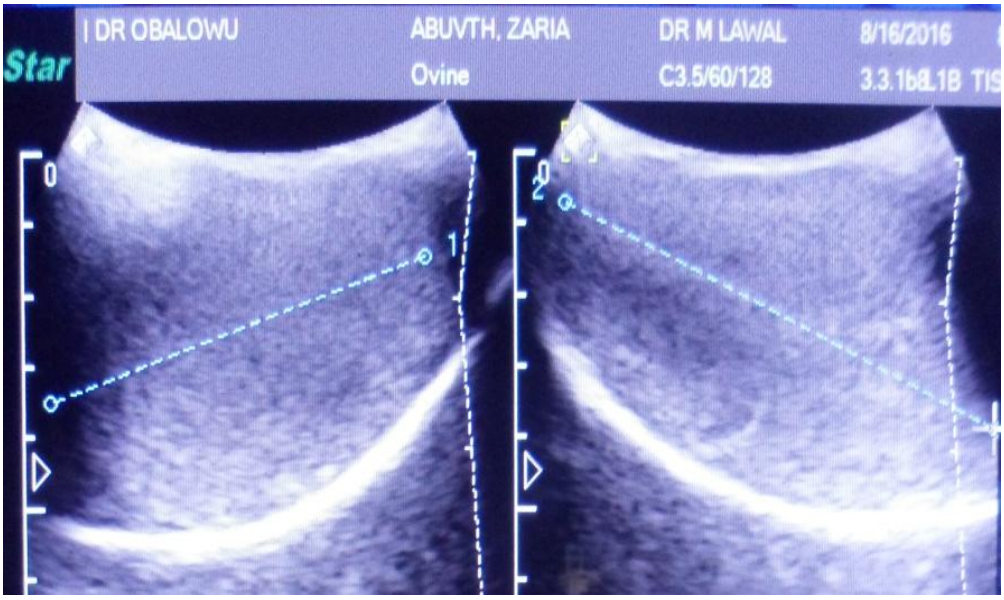


Plate XI. Sonograph of a longitudinal section of the testes of a Yankasa ram.

The 5 MHz linear array transducer connected to the B-mode Sonostar ultrasound scanner was used for all the procedures with a 4 cm standoff and gridline. The testicle was measured from the proximal pole to the gridline at the approximate midline of the testicle and then from this midline to the distal end, the summation of the 2 values yielded the testicular length. All the measurements were read to the nearest 0.1 cm as per the procedure described by Bailey *et al*., (1998).

The following parameters were measured after taking their echo texture and vascularity:

(i) Testicular length, width and volume.

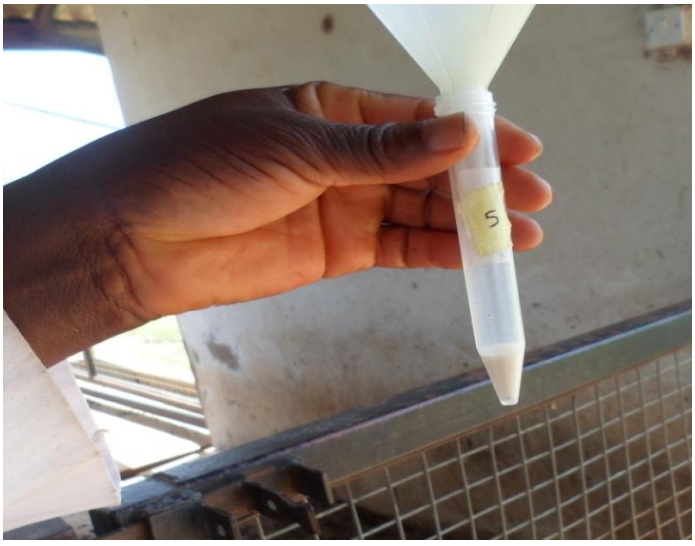
(ii) Epididymal head length, width and volume

(iii) Epididymal tail length, width and volume.

3.4 Semen Analysis

After breeding soundness examination, semen samples were collected from each animal using an electro-ejaculator (Plate XII) and labeled accordingly using a technique similar to that described by McGowan *et al., (1995).* The collected semen samples were immediately evaluated for colour, volume (Plate XIII), motility, pH (Plate XIV) as described by Zemjanis, (1970). Smear of each semen sample was prepared, air-dried, labeled and kept for further examination to determine sperm concentration using formaldehyde, sperm morphology using oil immersion and live/dead ratio using eosin negrosin stains.

Plate XII: The electro ejaculator was inserted *per rectum* to stimulate the release of semen in Yankasa rams.



PlateXIII: measuring the volume of the ejaculate from the Yankasa rams.

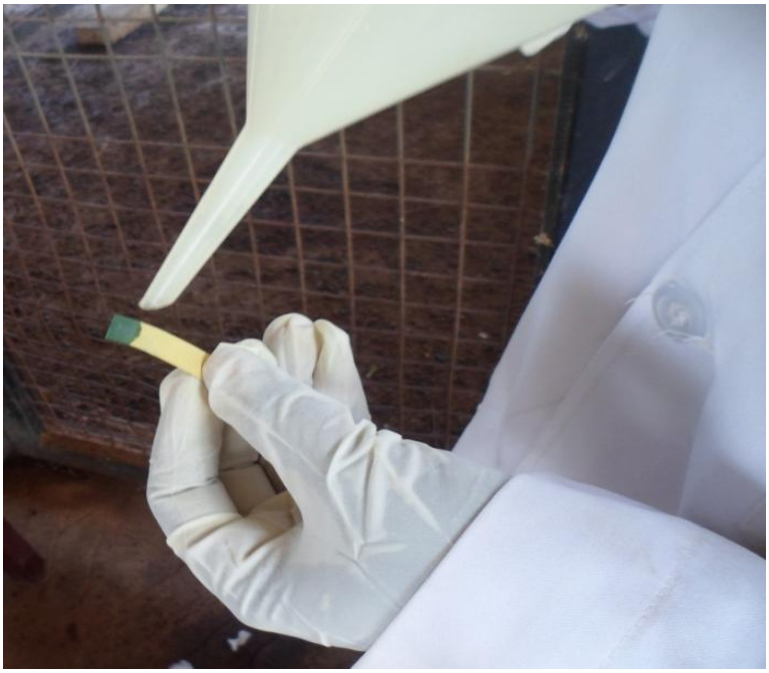


PLATE XIV. Measuring the pH of the semen collected from the Yankasa rams.

3.5 Hormonal Assay

3.5.1 Collection of blood.

Two mls of blood was collected every hour from each ram over a period of 6hrs (6:00 am to 11:00 am)twice on the days of semen collection. The blood samples were centrifuged using a table centrifuged at x3000g, the serum was harvested and stored at -20^0C until analysis.

3.5.2 Testosterone

The protocol provided by the Monobind Incoporateds Company on the use of testosterone assay kit was adapted.Ten microliter of the appropriate serum reference, control and serum samples were pipetted into the assigned microplate wells after which 50 µl of the working Testosterone Enzyme reagent was added to all the wells. The microplate was swirled gently for 30 seconds to mix. Fifty microlitre of the Testosterone Biotin reagent was then added and the microplate swirled gently for 30 seconds. The microplate was covered and incubated for 60 minutes at room temperature after which all the contents were discarded. The plate was washed three times by addition of 350 µl of wash buffer and decanted. 100 µl of working substrate solution was added to each well and incubated at room temperature for 15 minutes. Exactly 50 µl of stop solution was added to each well and gently mixed for 20 seconds. The absorbance in each well was read at 450nm using a reference wave length of 620nm within 30 minutes of adding stop solution (Monobind, unpublished).

3.5.3 Luteinizing Hormone(LH)

Adapting the protocol provided by the CALBIOTECH Company on LH assay kit, twenty fivemicrolitres of the LH standards, control and serum samples were pipetted into the selected wells. One hundred micrlitres of enzyme conjugate was then added to all the wells

after which the plate was covered and incubated for 60 minutes at room temperature. The content of the plate was decanted and washed three times with 300 µl of 1X wash buffer. Then blotted on absorbent paper towels. Exactly 100 µl of TMB substrate was added to all the wells and incubated for 15 minutes at room temperature. Fifty micro-litresof stop solution was added to all the wells and shaken gently for 30 seconds. The absorbance in each well was read on ELISA reader at 450nm within 15 minutes of adding the stop solution (Calbiotech, 2016b).

3.5.4 Follicle Stimulating Hormone (FSH)

Guidelines provided by the CALBIOTECH Company on FSH assay kit were adapted. Fifty micro-litres of the FSH standards, control and serum samples were pipetted into the selected wells, then added to 100 µl of enzyme conjugates to all the wells after which the plate was covered and incubated for 60 minutes at room temperature. The content of the plate was decanted and washed three times with 300 µl of 1X wash buffer. Then blotted on absorbent paper towels. Afterwards, one hundred microliters of TMB substrate was added to all the wells and incubated for 15 minutes at room temperature. Fifty microlitres of stop solution was added to all the wells and shaken gently for 30 seconds. The absorbance in each well was read on ELISA reader at 450nm within 15 minutes of adding the stop solution (Calbiotech, 2016a).

3.6 Statistical Analysis

The mean values (± SE) of the testicular and epididymal parameterstaken were calculated. In order to see the relationship between the ultrasound and caliper values, the correlation coefficients between these values were computed. The correlation coefficients between the

testes and epididymal parameters in relation to semen quality and hormonal profile were also evaluated using SPSS version 20.0`

CHAPTER FOUR

RESULT

4.1. Testicular Biometry Using Vernier Caliper

Data recorded for testicular biometry using vernier caliper were presented in the appendix 1c, 1d and 1e. The mean left testicular length was 15.7 ± 0.55 cm while that of the right was 15.9 ± 0.61 cm. The mean left and right testicular circumference was 26.83 ± 0.51 cm. The mean left and right testicular volumes were 1482 ± 64.37 cm^3 and 1503 ± 74.27 cm^3 respectively (Figure 4.5; Appendix 7.4).

4.2.1. Testicular biometry of Yankasa rams using ultrasound

The vertical axis (length) and the horizontal axis (breadth) along with the depth (height) of the testes were measured using the ultrasound machine (Appendix 1a and 1b). The mean values of left and right testicular length were 130.4 ± 2.73 mm and 125.5 ±2.57 mm respectively. While the mean values of the left and right breadth were 42.15 ±2.53 mm and 44.85 ±2.14 mm respectively. The mean values of left and right testicular heights were 52.25 ± 2.10 mm and 53.02 ± 2.20 mm respectively, while the volumes were 280340 ± 12780 mm^3 and 296432 ± 17549 mm^3 (Figure 4.1; Appendix 7.1).

4.2.2. Testicular Doppler parameters of Yankasa rams

The Doppler parameters of both the left and right testicular arteries observed using ultrasound machine were as presented in the appendix 4a and 4b, while the mean and standard error of mean were as presented in the table 4.2. The mean peak systolic velocities for left and right testes were 9.80 ± 0.97 (cm/s) and 8.99 ± 1.06 (cm/s) respectively. The mean end diastolic velocity for left and right testes were 3.50 ± 0.04(cm/s) and 3.49 ± 0.69 (cm/s) respectively. The mean minimum velocities for the left and right testes were 4.03 ± 0.70(cm/s) and 3.43 ± 0.72 (cm/s) respectively. The mean resistance indexes for left and right testes were 0.63 ± 0.053 and 0.59 ± 0.06 respectively. The average pulsatility index of the left testis at mean time 5.83 ± 0.83s was 3.16 ± 0.72 while that of the right testes at mean time 6.10 ± 0.78s was 7.76 ± 5.18 (Figure 4.2; Appendix 7.2).

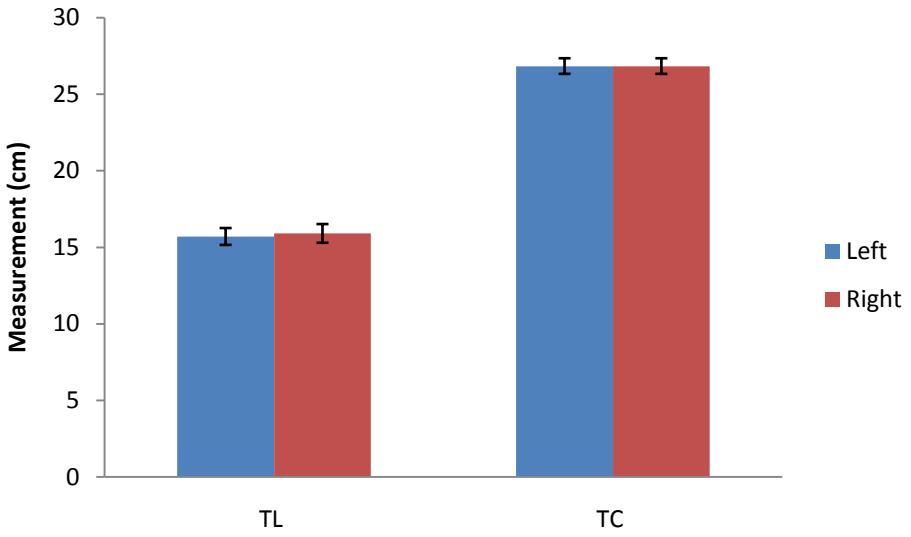


Figure 4.1 Mean ± SEM of testicular biometry using vernier caliper. TL (testicular length), TC (testicular circumference).

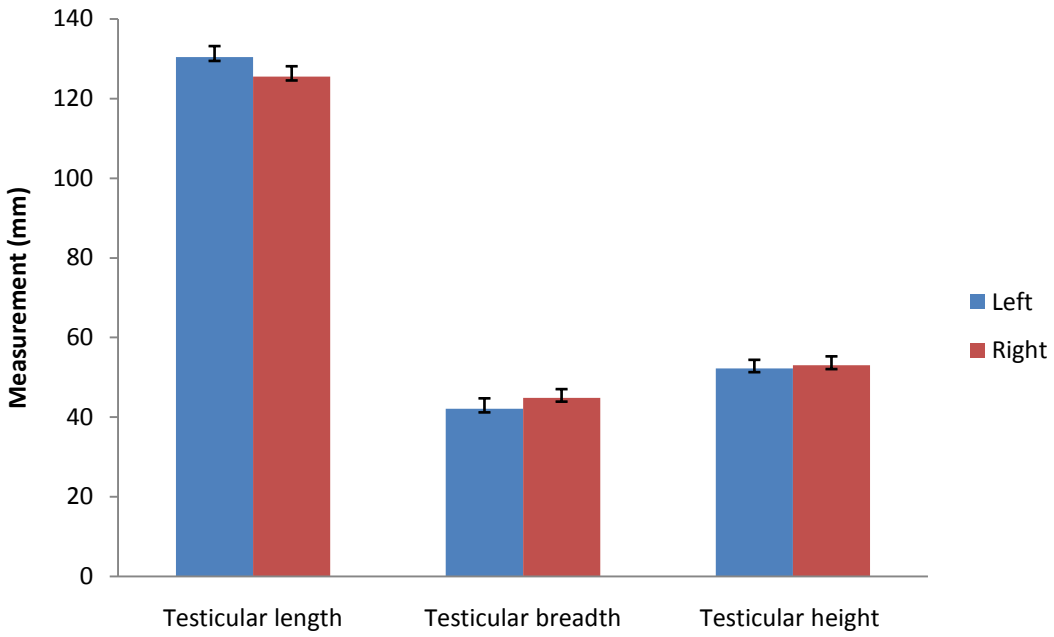


Figure 4.2 Mean ± SEM of testicular biometry of Yankasa ram using ultrasound

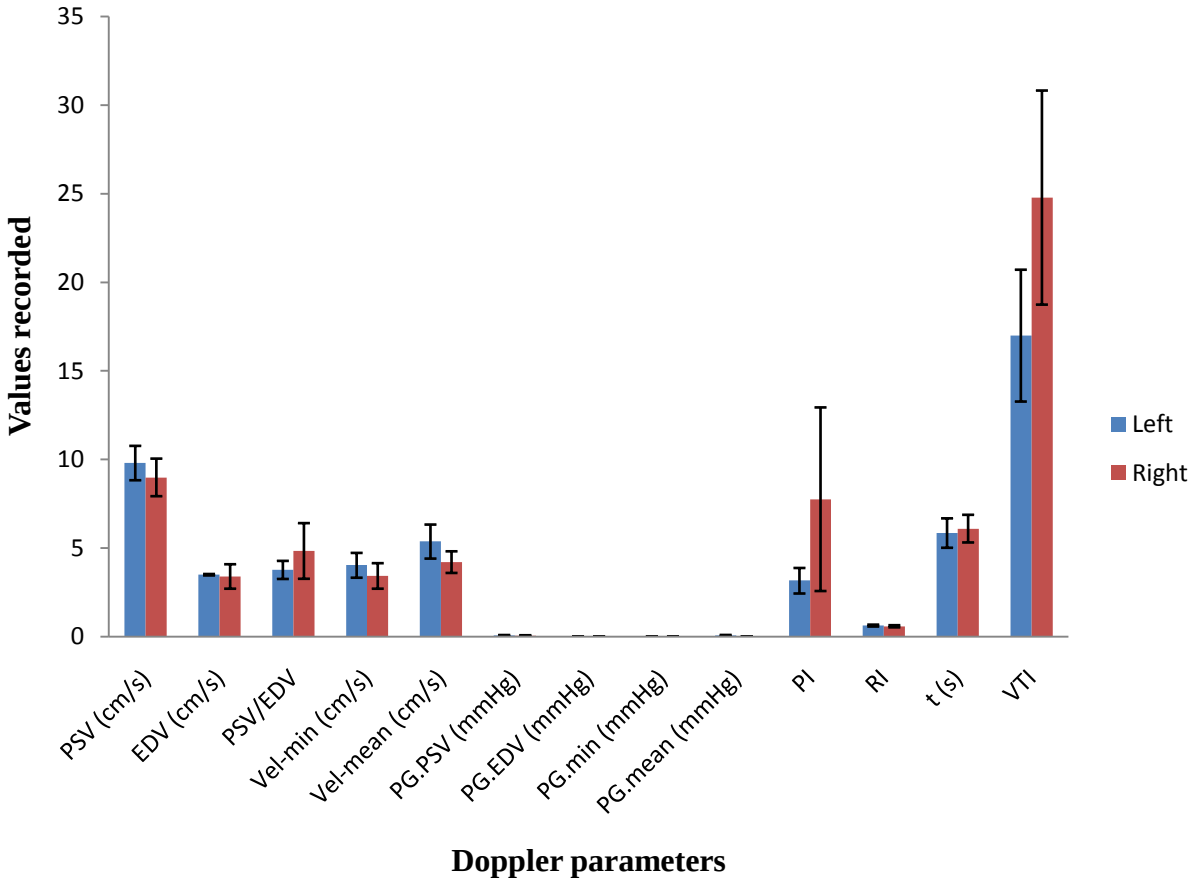


Figure 4.3 Mean ± SEM of testicular Doppler parameters of Yankasa rams using ultrasound. PSV (peak systolic velocity), EDV (end diastolic velocity), PSV/EDV (ratio of peak systolic and end diastolic velocity), VELmin (velocity minimum), VELmean (velocity mean), PGPSV (pressure gradient for peak systolic velocity), PGEDV (pressure gradient for end diastolic velocity), PGmin (pressure gradient minimum), PGmean (pressure gradient mean), PI (pulsatility index), RI (resistivity index), Time (time), VTI (velocity time integra).

4.2.3. Epididymal biometry of Yankasa ram

The parameters observed for the head and tail of the epididymides were presented in the appendix 3a, 3b and 3c. The mean value for the left's long axis of the epididymal head was 38.67 ± 1.47 mm while for the right was 35.15 ± 1.63 mm (Figure 4.3 and 4.4; Appendix 7.3). The mean value for the left's long axis of the epididymal tail was 27.64 ± 1.53 mm while for the right was 25.63 ± 1.91 mm. The mean value for the left's epididymal head volume was 6.48 ± 0.62 cm^3 while that of the right was 4.49 ± 0.42 cm^3. The mean value for the left's epididymal tail volume was 2.83 ± 0.30 cm^3 while that of the right was 3.01 ± 0.50 cm^3.

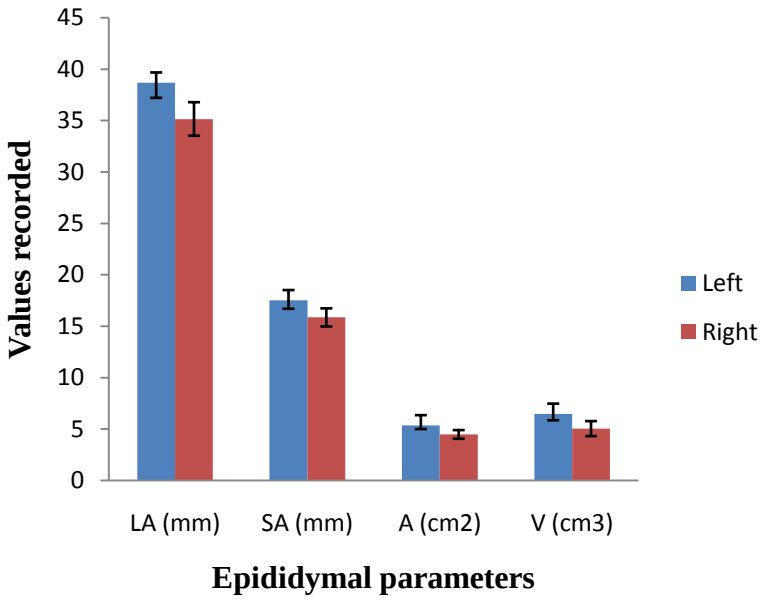


Figure 4.4 Mean ± SEM of the epididymal head biometry of Yankasa ram using ultrasound. LA (long axis), SA (short axis), A (area) and V (volume).

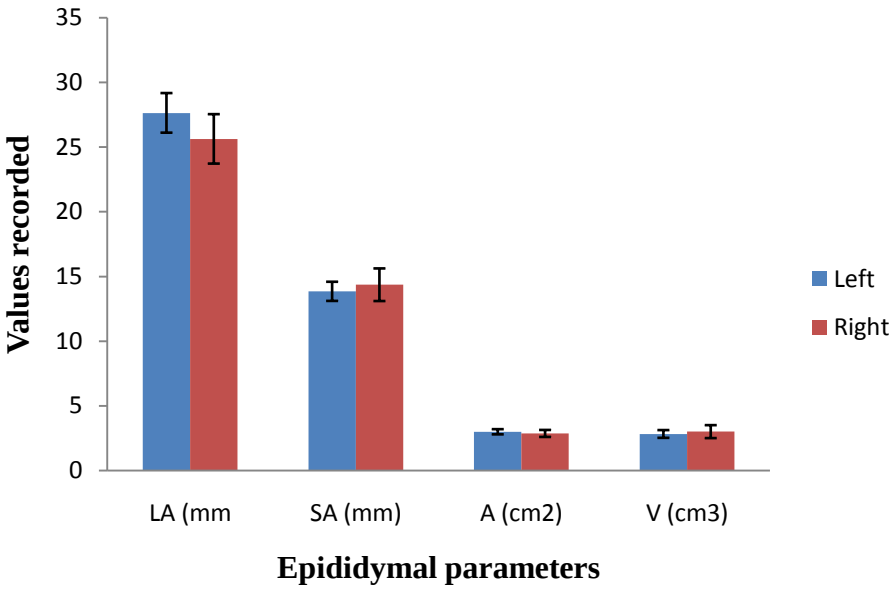


Figure 4.5 Mean ± SEM of the epididymal tail biometry of Yankasa ram using ultrasound. LA (long axis), SA (short axis), A (area) and V (volume).

4.3 The Echo texture of the Testes and Epididymides of Yankasa Ram

4.3.1 The testicular echo texture

The testicular parenchyma appeared as an isoechoic structure (midgray echo texture) with uniform echogenicity which was surrounded by a hyper-echoic structure (*Tunica albugina* and *Tunica vaginalis*) (Plate XV). The mediastinum testis was seen as a point of hyper echoic area in the center of testicular parenchyma when scanned in the transverse plane (Plate XVI). The Doppler parameters of the testicular arteries were shown in Plate XIX and XX.

4.3.2 The epididymidal echo texture

The echogenicity of the epididymides was seen similar to the echogenicity of the testes but with some hypo echoic area of echogenicity (Plate XVII and XVIII) in the epididymal head.

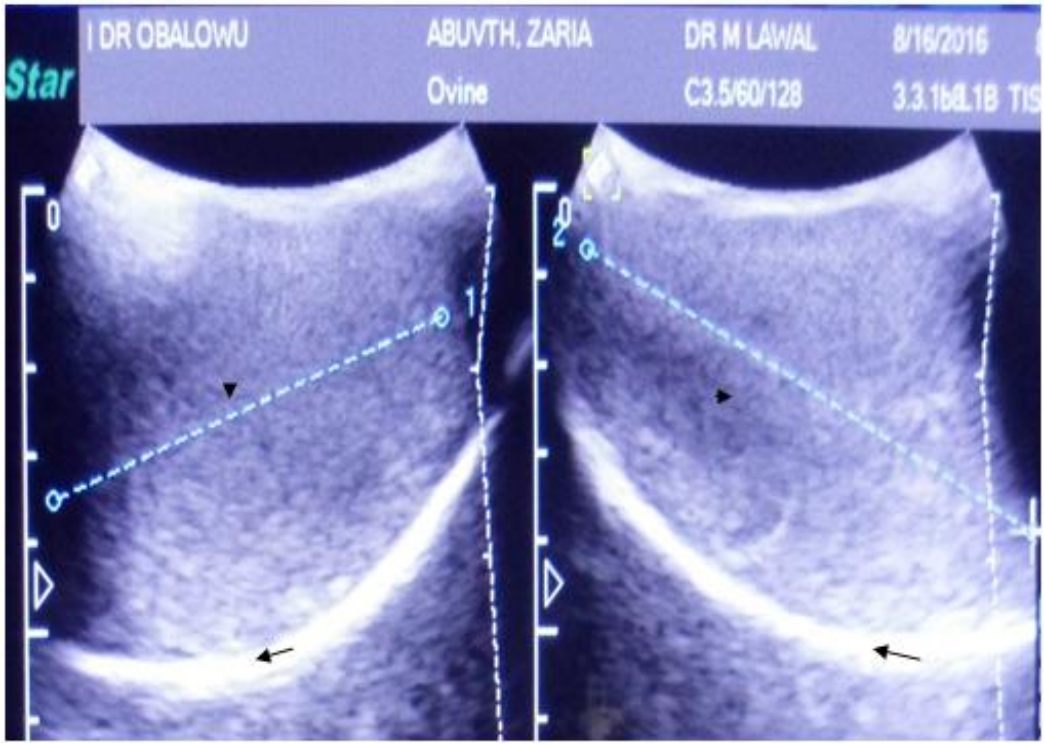


PLATE XV. Longitudinal scan of the Yankasa ram testis showing the sonograph with anhyper echoic band (arrows) representing the *Tunica albugina* and *Tunica vaginalis* and isoechoic testicular parenchyma (arrow heads)

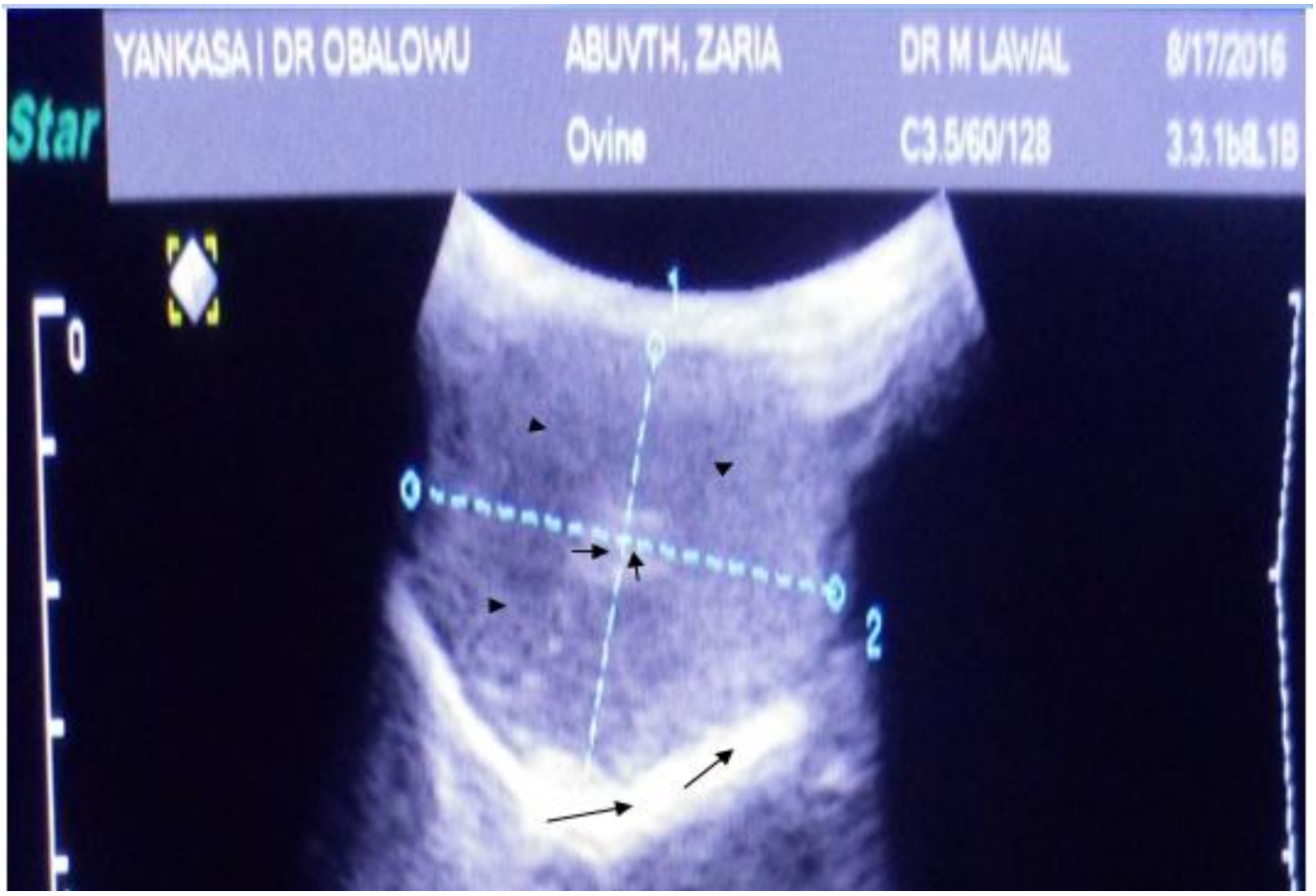


PLATE XVI. Transverse scan of the Yankasa ram testis showing the sonograph with homogenously isoechoic testicular parenchyma (arrowheads) and a hyper echoic circular area in the middle representing mediastinum testis (short arrows) with a hyper echoic band (long arrows) representing the *Tunica albugina* and *Tunica vaginalis.*

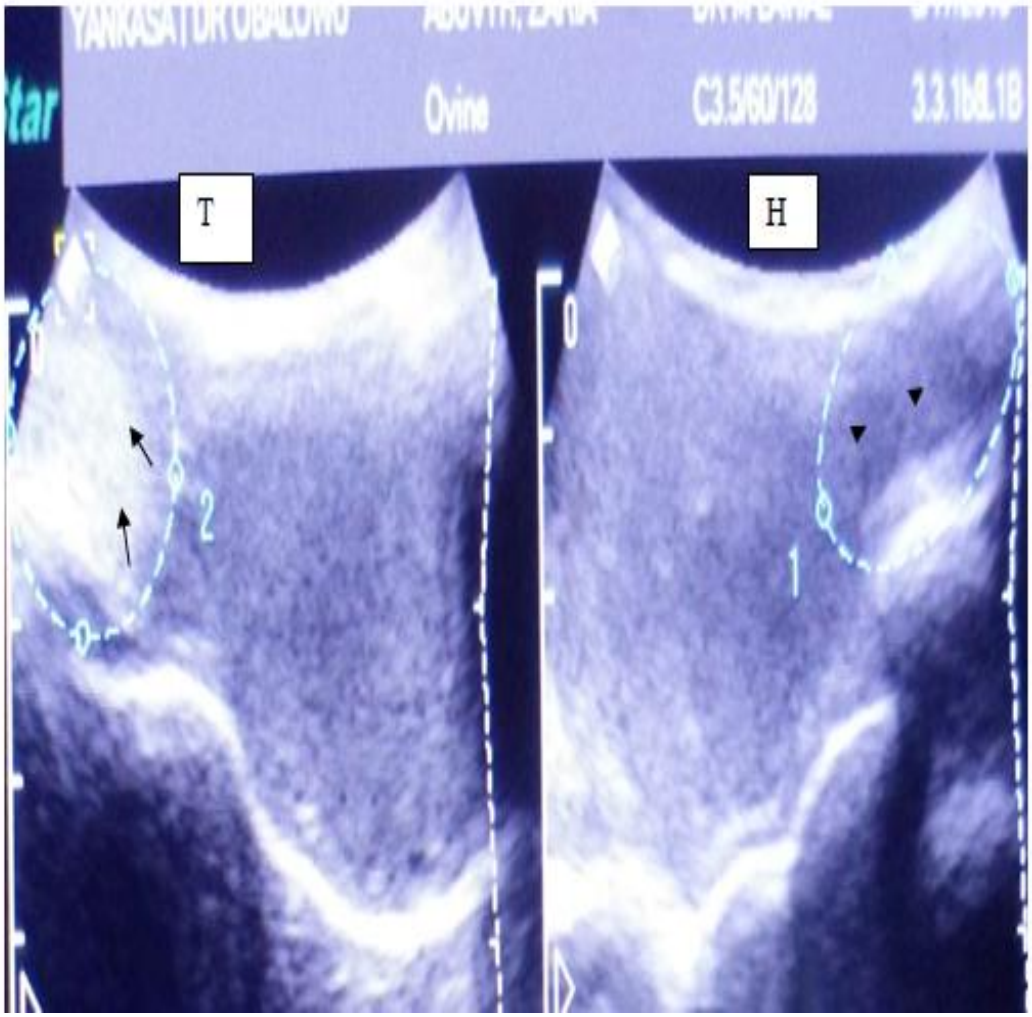


PLATE XVII: Echo texture of the right head (H) and tail (T) of the epididymides. Inscribed circle indicates measurement of the head (arrowheads) and the tail (arrows) epididymal parameters

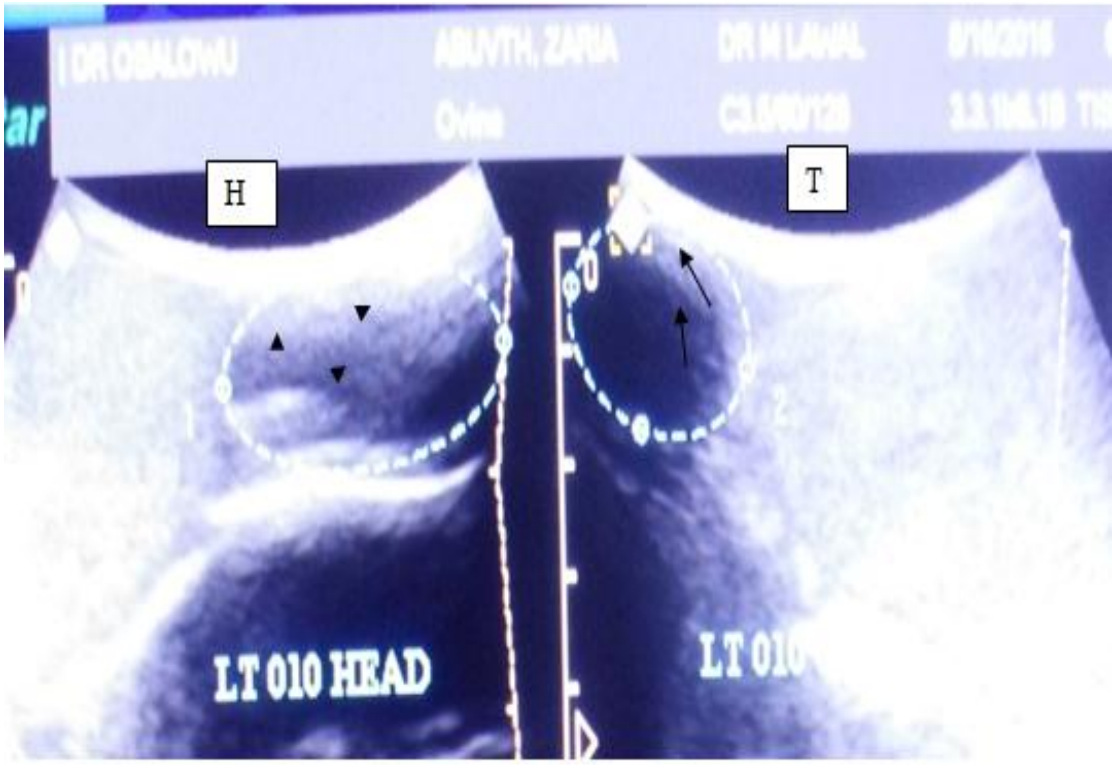


PLATE XVIII: Echo texture of the left head (H) and tail (T) of the epididymides. Inscribed circle indicate measurement of the head (arrowheads) and the tail (arrows) epididymal parameters.

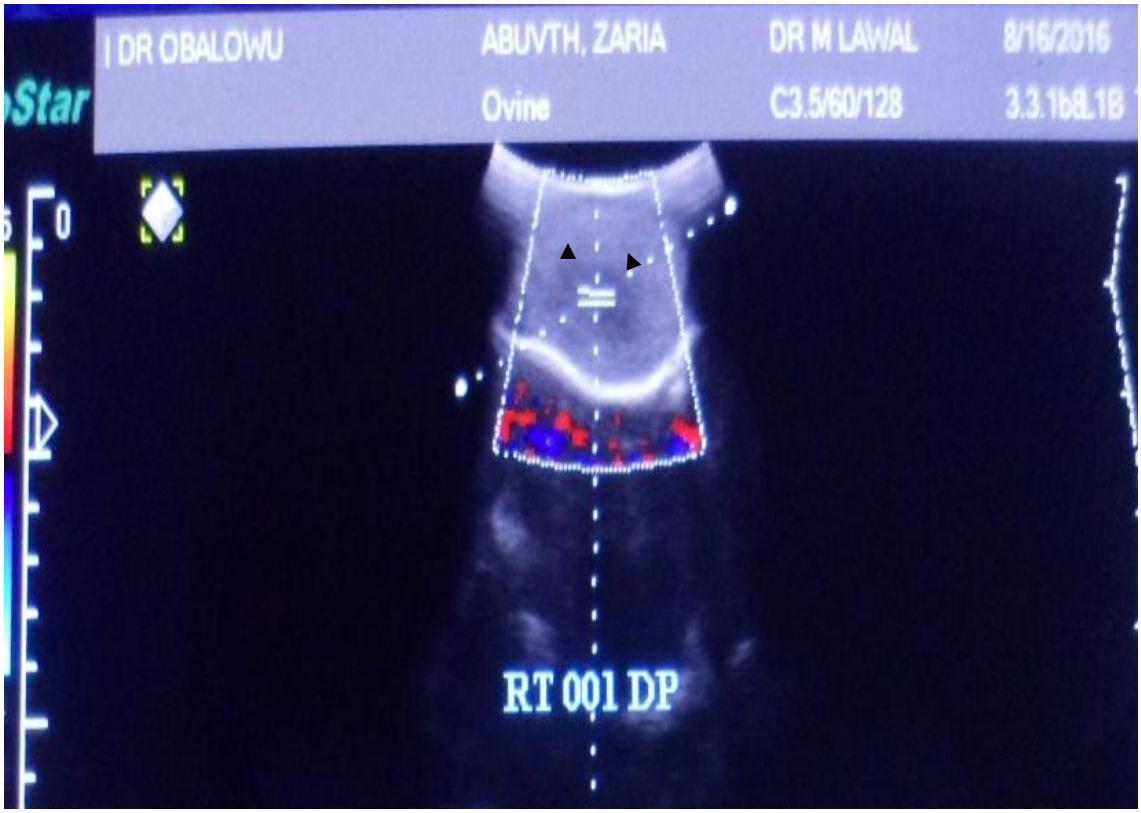


PLATE XIX: Testicular Doppler parameters of the right testis (arrowheads).

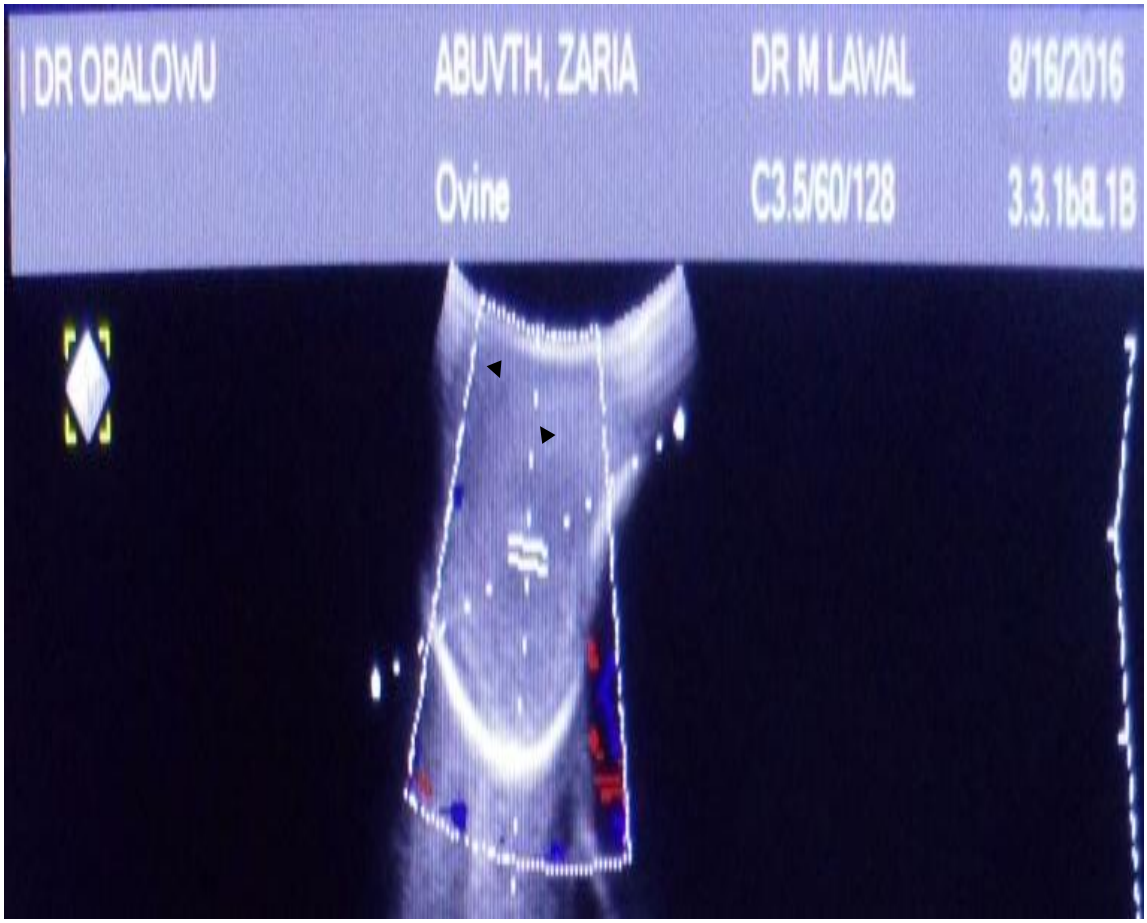


PLATE XX: Testicular Doppler parameters of the left testis (arrowheads).

4.4 The Testicular and Epididymal Biometry Compared to Semen Parameters

4.4.1 The relationship between testicular biometry and semen parameters

The sperm concentration, reaction time and normal sperm cells correlated positively with the testicular volume using vernier caliper or ultrasound, though the correlation was weak and not significant. The relationship between other semen parameters with the testicular volume did not follow a definite pattern (Table 4.1). Semen concentration and reaction time, the highest correlated with the left measurement testicular volume using vernear caliperat (r=0.225) and (r=0.346) respectively. While with the normal sperm cell count correlated with the right volumeat (r = 0.247) using vernier caliper.

The sperm motility correlated negatively with the left and right verniercaliper measurements of the testicular volumes and positively with the left and right ultrasound measurements of the testicular volumes (Table 4.1).

Table 4.1 Relationship between testicular vernier caliperic measurement, sonographic biometry and semen parameters (n=15)

		V	M	pH	C	RT	L	D	NC	FH	FT	CT	BT
LVC Volume	R	-0.085	-0.152	0.111	0.225	0.346	0.054	-0.054	0.170	0.236	0.005	-0.165	-0.360
RVC Volume	R	0.026	-0.260	0.103	0.216	0.215	-0.039	0.039	0.247	0.012	0.024	-0.224	-0.312
LUS Volume	R	-0.115	0.090	0.180	0.126	0.210	0.188	-0.188	0.166	-0.132	-0.106	-0.025	-0.128
RUS Volume	R	-0.028	0.328	0.231	0.222	0.096	0.274	-0.274	0.139	0.149	-0.124	0.114	-0.362

* Correlation is significant at the 0.05 level (2-tailed). V (Volume), M (Motility), pH, C (Concentration), RT (Reaction time), L (Live sperm), D (Dead sperm), NC (Normal cells), FH (Free Head), FT (Free Tail), CT (Coiled Tail) and BT (Bent Tail). LVC Volume (left vernier caliperic volume), RVC Volume (right vernier caliperic volume), LUS Volume (left ultrasonographic volume), RUS Volume (right ultrsonographic volume), andR(Pearson correlation coefficient).

4.4.2 The relationship between the vernier caliperic measurement of the testes and semen parameters

All the semen parameters did not have a specific pattern of correlation with the vernier caliperic measurement of the testicular length, circumference, volume and the weight (Table 4.2). Some parameters were positively correlated while others were negatively correlated. The sperm concentration was positively correlated with all the testicular parameters except with the right and left testicular circumference in which the correlation was negative. Reaction time and normal sperm count also positively correlated with all the testicular parameters except with the right and left testicular length in which was negatively correlated (Table 4.2).

Bent tail correlated negatively and significantly with the right and left testicular circumference (r=-0.516). The right and left testicular length correlate positively and significantly with the free tail (r=0.525) and (r=0.497) respectively. With both correlating negatively with the normal sperm cells (r=-0.270) and (r=-0.122).

Table 4.2 Relationship between the vernier caliperic measurement of the testes and semen parameters (n =15)

		V	M	pH	C	RT	L	D	NC	FH	FT	CT	BT
RTL	R	0.355	-0.296	-0.294	0.433	-0.034	-0.269	0.269	-0.270	0.331	0.522*	-0.101	0.022
RTC	R	-0.436	0.076	0.415	-0.107	0.443	0.305	-0.305	0.476	0.034	-0.480	-0.131	-0.516*
RTV	R	-0.085	-0.152	0.111	0.225	0.346	0.054	-0.054	0.170	0.236	0.005	-0.165	-0.360
RT Weight	R	-0.085	-0.152	0.111	0.225	0.346	0.054	-0.054	0.170	0.236	0.005	-0.165	-0.360
LTL	R	0.454	-0.436	-0.269	0.418	-0.147	-0.360	0.360	-0.122	0.057	0.497	-0.184	0.019
LTC	R	-0.436	0.076	0.415	-0.107	0.443	0.305	-0.305	0.476	0.034	-0.480	-0.131	-0.516*
LTV	R	0.026	-0.260	0.103	0.216	0.215	-0.039	0.039	0.247	0.012	0.024	-0.224	-0.312
LTWeight	R	0.026	-0.260	0.103	0.216	0.215	-0.039	0.039	0.247	0.012	0.024	-0.224	-0.312

* Correlation is significant at the 0.05 level (2-tailed). V (Volume), M (Motility), pH(Level of Acidity or Alcalinity), C(Concentration), RT (Reaction time), L (Live sperm), D (Dead sperm), NC (Normal cells), FH (Free Head), FT (Free Tail), CT (Coiled Tail) and BT (Bent Tail). RTL (right testicular length), RTC (right testicular circumference), RTV (right testicular volume), RTWeight (right testicular weight), LTL (left testicular length), LTC (left testicular circumference), LTV (left testicular volume), LTWeight (left testicular weight) and R (Pearson correlation coefficient).

4.4.3 The relationship between testicular Doppler biometry and semen parameters

The relationship between the semen parameters and the left testicular Doppler parameters did not follow a specific pattern because some parameters were positively correlated while others were negatively correlated (Table 4.3a). The pH of the semen however, correlated significantly and positively with the left peak systolic velocity(LPSV) (r=0.593), left end diastolic velocity (LEDV) (r=0.612), left velocity minimum (LVel min) (r=0.538), left pressure gradient end diastolic velocity (LPGEDV) (r=0.531) and left pressure gradient mean velocity (LPGVmean) (r=0.585). Also life sperm cells correlated significantly and negatively with the left end diastolic velocity (LEDV) and left pressure gradient end diastolic velocity(LPGEDV) (r= -0.554 and r= -0.573 respectively) and positively correlated with the resistant index (r= 0.591). Dead sperm cells correlated significantly and positively with the LEDV and LPGEDV (r= 0.554 and r= 0.573 respectively) and negatively with the resistance index (r= -0.591). The left resisitivity index(LRI) significantly correlated with the life sperm cells and dead sperm cells (r=0.591) and (r= -0.591) respectively.

Similarly the relationship between the semen parameters and the right testicular Doppler parameters did not follow a specific pattern as some parameters are positively correlated while others are negatively correlated (Table 4.3b). The pH of the semen correlated significantly with the RVel mean (r=0.511) and weakly with the RPGmean (r=0.448). The reaction time correlated significantly with right end diastolic velocity(REDV) (r= -0.523) and weakly with the right pressure gradient end end diastolic velocity(RPGEDV) (r= -0.495) (Table 4.7b).

Table 4.3a Relationship between the left testicular Doppler biometry and semen parameters (n =15).

		V	M	pH	C	RT	L	D	NC	FH	FT	CT	BT
LPSV	R	-0.023	0.088	0.593*	-0.077	-0.162	-0.120	0.120	-0.195	0.161	-0.255	0.302	0.164
LEDV	R	0.120	-0.348	0.612*	-0.231	0.150	-0.554*	0.554*	0.244	-0.034	-0.275	-0.152	-0.135
LPSVEDV	R	0.160	0.348	-0.399	0.484	-0.292	0.420	-0.420	-0.396	-0.139	0.404	0.371	0.309
LVELmin	R	0.386	-0.234	0.538*	0.054	-0.015	-0.390	0.390	0.062	-0.099	-0.011	-0.082	-0.018
LVELmean	R	0.013	0.188	0.384	-0.012	-0.344	-0.114	0.114	-0.210	0.183	-0.218	0.279	0.219
LPGPSV	R	-0.255	0.166	0.125	-0.065	0.457	0.148	-0.148	-0.109	0.363	-0.208	0.455	-0.258
LPGEDV	R	0.222	-0.320	0.531*	-0.149	0.143	-0.573*	0.573*	0.228	-0.088	-0.165	-0.161	-0.123
LPGmin	R	0.488	-0.186	0.440	0.154	-0.026	-0.368	0.368	0.030	-0.134	0.116	-0.088	-0.006
LPGmean	R	-0.203	0.031	0.585*	-0.169	-0.281	0.015	-0.015	-0.030	-0.041	-0.399	-0.070	0.414
LPI	R	0.073	-0.090	0.041	0.207	0.081	0.103	-0.103	0.098	-0.170	0.185	-0.076	-0.178
LRI	R	-0.103	0.541*	-0.308	0.366	-0.244	0.591*	-0.591*	-0.439	0.051	0.240	0.414	0.325
LTime	R	0.141	-0.089	0.083	0.089	0.118	-0.166	0.166	-0.108	0.306	0.114	0.194	-0.288
LVTI	R	0.015	0.007	0.131	0.328	-0.130	0.130	-0.130	-0.264	0.565*	0.064	0.419	-0.311

*Correlation is significant at the 00.05 level (2-tailed). V (Volume), M (Motility), pH, C (Concentration), RT (Reaction time), L (Live sperm), D (Dead sperm), NC (Normal cells), FH (Free Head), FT (Free Tail), CT (Coiled Tail) and BT (Bent Tail). LPSV (left peak systolic volume), LEDV (left end diastolic volume), LPSV/EDV (ratio of peak systolic and end diastolic volume), LVELmin (left velocity minimum), LVELmean (left velocity mean), LPGPSV (left pressure gradient for peak systolic volume), LPGEDV (left pressure gradient for end diastolic volume), LPGmin (left pressure gradient minimum), LPGmean (left pressure gradient mean), LPI (left pulsatility index), LRI (left resistivity index), L Time (left time), LVTI (left velocity time integra) and R (Pearson correlation coefficient).

Table 4.3b Relationship between the right testicular doppler biometry and semen parameters (n =15).

		V	M	pH	C	RT	L	D	NC	FH	FT	CT	BT
RPSV	R	0.122	0.203	-0.130	0.273	-0.193	0.004	-0.004	-0.327	0.324	0.249	0.393	-0.088
REDV	R	-0.114	-0.117	0.183	0.142	-0.523*	-0.308	0.308	0.054	0.015	-0.085	0.121	-0.122
RPSVEDV	R	0.271	0.249	-0.116	0.239	0.380	0.322	-0.322	-0.187	0.434	0.351	-0.058	-0.167
RVELmin	R	-0.173	-0.038	-0.206	-0.070	-0.235	-0.221	0.221	0.300	-0.264	-0.151	-0.198	-0.116
RVELmean	R	0.292	0.014	0.511	-0.105	-0.150	-0.059	0.059	-0.378	0.125	-0.021	0.246	0.417
RPGPSV	R	-0.226	0.253	0.061	0.032	0.384	0.238	-0.238	-0.148	0.388	-0.123	0.464	-0.261
RPGEDV	R	-0.123	0.007	0.069	0.259	-0.495	-0.175	0.175	0.011	0.123	-0.008	0.182	-0.209
RPGmin	R	-0.210	0.107	0.071	-0.072	-0.216	-0.006	0.006	0.321	-0.289	-0.222	-0.306	-0.008
RPGmean	R	0.197	0.283	0.448	0.008	-0.332	0.083	-0.083	-0.380	0.011	-0.008	0.294	0.491
RPI	R	-0.236	0.091	-0.049	0.154	-0.156	0.112	-0.112	0.069	-0.082	0.100	-0.078	-0.058
RRI	R	0.149	0.332	0.010	0.136	0.201	0.349	-0.349	-0.276	0.164	0.117	0.197	0.150
RTime	R	0.094	-0.143	0.042	0.112	0.096	-0.228	0.228	-0.183	0.353	0.128	0.262	-0.231
RVTI	R	0.077	-0.087	0.091	-0.030	-0.386	-0.167	0.167	-0.158	0.073	-0.102	0.356	0.008

*Correlation is significant at the 00.05 level (2-tailed). V (Volume), M (Motility), pH, C (Concentration), RT (Reaction time), L (Live sperm), D (Dead sperm), NC (Normal cells), FH (Free Head), FT (Free Tail), CT (Coiled Tail) and BT (Bent Tail). RPSV (Right peak systolic volume), REDV (Right end diastolic velocity), RPSV/EDV (Ratio of peak systolic velocity and end diastolic velocity), RVELmin (Right velocity minimum), RVELmean (Right velocity mean), RPGPSV (Right pressure gradient peak systolic velocity), RPGEDV (Right pressure gradientend diastolic velocity), RPGVmin (Right pressure gradient velocity minimum), RPGmean (right pressure gradient velocity mean), RPI (Right pulsatility index), RRI (right resistivity index), R Time (reaction time), RVTI (right velocity time integra), R (Pearson correlation coefficient)

4.4.4 The relationship between the epididymal biometry and the semen parameters

The relationship between the semen parameters and the left epididymal head and tail parameters did not follow a specific pattern because some parameters were positively correlated while others are negatively correlated (Table 4.4a). The semen volume is significantly correlated with the left epididymal head's long axis (r=0.599), area (r=0.526), volume (r=0.514) and weakly correlated with the short axis (r=0.328). While the semen volume is negatively correlated with all the left epididymal tail parameters (long axis, short axis, area and the volume). Sperm motility correlated negatively with all the left epididymal head parameters except with the short axis and correlated positively with all the left epididymal tail parameters except with the long axis. Sperm concentration correlated positively with all the left epididymal head parameters and negatively with all the epididymal tail parameters. Normal sperm cells correlate negatively with all the left epididymal head and tail parameters except epididymal tail long axis (r=0.103). Normal cells of the sperm correlate significantly with the left epididymal head's short axis (r= -0.531) and weakly with the area (r= - 0.446) and volume (r= -0.494). Free tail correlates positively with all the left epididymal head parameters and negatively with all the left epididymal tail parameters. It however, correlated significantly with the left epididymal head's area (r=0.541) and volume (r=0.596).

Similarly the relationship between the semen parameters and the right epididymal head and tail parameters does not follow a specific pattern because some parameters are positively correlated while others are negatively correlated (Table 4.4b). Semen volume correlates negatively with all the right epididymal tail parameters. The sperm motility correlated negatively with all the right epididymal head parameters and positively with all the right epididymal tail parameters. Free tail

correlated negatively with all the right epididymal head and tail parameters except the epididymal head's short axis.

4.5 Testicular and Epididymal Biometry Compared to Reproductive Hormones

4.5.1 The relationship between testicular biometry and reproductive hormones

Testosterone, luteinizing hormone and follicle stimulating hormone correlated negatively (weak) with the testicular volume using either vernier caliper or ultrasound machine except follicle stimulating hormone which correlated positively with the right testicular volume using vernier caliper (Table 4.5).

Although none of the testicular parameters using vernier caliper (length, circumference, volume and weight) significantly correlated with the reproductive hormones (testosterone, luteinizing hormone and follicle stimulating hormone), testosterone and luteinizing hormone correlated negatively with the right and left testicular length, circumference, volume and weight (Table 4.6).

Table 4.4a Relationship between the left epididymal measurements and semen parameters (n = 15)

		V	M	pH	C	RT	L	D	NC	FH	FT	CT	BT
LEHLA	R	0.599*	-0.303	0.350	0.390	0.090	-0.287	0.287	-0.256	0.345	0.429	-0.029	-0.037
LEHSA	R	0.328	0.045	0.204	0.450	0.055	0.099	-0.099	-0.531*	0.358	0.464	0.402	0.123
LEHA	R	0.526*	-0.097	0.249	0.473	0.126	-0.041	0.041	-0.446	0.443	0.541*	0.178	0.017
LEHV	R	0.514*	-0.002	0.180	0.498	0.152	0.069	-0.069	-0.494	0.476	0.596*	0.211	0.020
LETLA	R	-0.041	-0.279	0.629*	-0.108	0.023	-0.257	0.257	0.103	-0.073	-0.240	-0.139	0.131
LETSA	R	-0.036	0.009	-0.145	-0.047	-0.083	0.127	-0.127	-0.108	-0.003	-0.013	0.241	-0.001
LETA	R	-0.140	0.029	0.230	-0.180	0.043	0.127	-0.127	-0.104	0.079	-0.168	0.164	0.096
LETV	R	-0.104	0.083	0.003	-0.169	-0.036	0.162	-0.162	-0.149	0.062	-0.109	0.259	0.078

*Correlation is significant at the 00.05 level (2-tailed). V (Volume), M (Motility), pH, C (Concentration), RT (Reaction time), L (Live sperm), D (Dead sperm), NC (Normal cells), FH (Free Head), FT (Free Tail), CT (Coiled Tail) and BT (Bent Tail). LEHLA (left epididymal head's long axis), LEHSA (left epididymal head's short axis), LEHA (left epididymal head's area), LEHV (left epididymal head's volume), LETLA (left epididymal tail's long axis), LETSA (left epididymal tail's short axis), LETA (left epididymal tail's area), LETV (left epididymal tail's volume) and R (Pearson correlation coefficient)

4.4b Relationship between the right epididymal measurements and semen parameters (n = 15)

		V	M	pH	C	RT	L	D	NC	FH	FT	CT	BT
REHLA	R	-0.132	-0.051	0.410	-0.146	0.112	-0.167	0.167	0.042	-0.278	-0.259	0.241	0.170
REHSA	R	0.138	-0.150	0.360	0.260	-0.005	0.060	-0.060	-0.151	0.122	0.029	0.207	0.020
REHA	R	-0.023	-0.128	0.459	0.050	0.075	-0.031	0.031	-0.058	-0.081	-0.163	0.267	0.107
REHV	R	0.021	-0.140	0.434	0.104	0.055	0.028	-0.028	-0.083	0.010	-0.118	0.238	0.071
RETLA	R	-0.187	0.150	0.132	-0.010	-0.131	-0.056	0.056	-0.358	0.091	-0.158	0.333	0.495
RETSA	R	-0.206	0.038	-0.083	0.146	0.222	0.249	-0.249	0.231	-0.031	-0.128	0.192	-0.449
RETA	R	-0.342	0.188	-0.019	0.056	0.124	0.205	-0.205	0.013	-0.042	-0.259	0.333	-0.022
RETV	R	-0.285	0.162	-0.117	0.158	0.148	0.279	-0.279	0.070	-0.001	-0.169	0.348	-0.239

*Correlation is significant at the 00.05 level (2-tailed). V (Volume), M (Motility), pH, C (Concentration), RT (Reaction time), L (Live sperm), D (Dead sperm), NC (Normal cells), FH (Free Head), FT (Free Tail), CT (Coiled Tail) and BT (Bent Tail). REHLA (right epididymal head's long axis), REHSA (right epidermal head's short axis), REHA (right epididymal head's area), REHV (right epididymal head's volume), RETLA (right epididymal tail's long axis), RETSA (right epididymal tail's short axis), RETA (right epididymal tail's area), RETV (right epididymal tail's volume) and R (Pearson correlation coefficient)

Table 4.5 Relationship between testicular vernier caliperic measurement, sonographic biometry and reproductive hormone profile (n=15)

		T	LH	FSH
LVCVolume (cm^3)	R	-0.370	-0.078	-0.021
RVCVolume (cm^3)	R	-0.226	-0.161	0.122
LUSVolume (cm^3)	R	-0.125	-0.048	-0.216
RUSVolume (cm^3)	R	-0.003	-0.223	-0.292

T (testosterone), LH (luteinizing hormone), FSH (follicle stimulating hormone), LVC Volume (left vernier caliperic volume), RVC Volume (right vernier caliperic volume), LUS Volume (left ultrasonographic volume), RUS Volume (right ultrsonographic volume) and R (Pearson correlation coefficient)

Table 4.6 Relationship between the vernier caliperic measurement and reproductive hormonal profile (n =15)

		T	LH	FSH
RTL	R	-0.407	-0.086	0.150
RTC	R	-0.111	-0.011	-0.157
RTV	R	-0.370	-0.078	-0.021
RTWeight	R	-0.370	-0.078	-0.021
LTL	R	-0.243	-0.192	0.333
LTC	R	-0.111	-0.011	-0.157
LTV	R	-0.226	-0.161	0.122
LTWeight	R	-0.226	-0.161	0.122

T (testosterone), LH (luteinizing hormone), FSH (follicle stimulating hormone), RTL (right testicular length), RTC (right testicular circumference), RTV (right testicular volume), RTWeight (right testicular weight), LTL (left testicular length), LTC (left testicular circumference), LTV (left testicular volume), LTWeight (left testicular weight) and R (Pearson correlation coefficient),

4.5.2 The relationship between testicular Doppler parameter and reproductive hormones

The correlation between the reproductive hormones (testosterone, luteinizing hormone and follicle stimulating) and the Doppler parameters did not follow a definite pattern because some parameters are positively correlated while others were negatively correlated (Table 4.7a and 4.7b). Luteinizing hormone was positive and significantly correlated with the left pulsatility index (r = 0.586) and positive highly significant with the right pulsatility index (r = 0.953). Follicle stimulating hormone was negatively and significantly correlated with the left resistivity index (r = -0.553) and weakly correlated with the right resistivity index (r = -0.168). Luteinizing hormone was positively and significantly correlated with the right Velmin (r = 0.581) and weakly with the left Velmin (r = -0.116). It also correlated positively and significantly with the right PGmin (r = 0.639) and weakly with the left PGmin (r = -0.14).

Table 4.7 Relationship between the vernier caliperic measurement and reproductive hormonal profile (n =15)

		T	LH	FSH
RTL	R	-0.407	-0.086	0.150
RTC	R	-0.111	-0.011	-0.157
RTV	R	-0.370	-0.078	-0.021
RTWeight	R	-0.370	-0.078	-0.021
LTL	R	-0.243	-0.192	0.333
LTC	R	-0.111	-0.011	-0.157
LTV	R	-0.226	-0.161	0.122
LTWeight	R	-0.226	-0.161	0.122

T (testosterone), LH (luteinizing hormone), FSH (follicle stimulating hormone), RTL (right testicular length), RTC (right testicular circumference), RTV (right testicular volume), RTWeight (right testicular weight), LTL (left testicular length), LTC (left testicular circumference), LTV (left testicular volume), LTWeight (left testicular weight) and R (Pearson correlation coefficient),

Table 4.8 Relationship between testicular Doplpler parameters and Reproductive hormonal profiles (n =15).

		T	LH	FSH
RPSV	R	-0.120	0.265	-0.033
REDV	R	-0.286	0.430	0.153
RPSVEDV	R	0.044	-0.154	-0.159
RVELmin	R	-0.105	0.581*	0.153
RVELmean	R	-0.166	-0.420	0.113
RPGPSV	R	-0.148	0.271	-0.115
RPGEDV	R	-0.263	0.467	0.054
RPGmin	R	-0.216	0.639*	0.029
RPGmean	R	-0.191	-0.049	-0.056
RPI	R	-0.214	0.953**	-0.063
RRI	R	-0.071	-0.158	-0.168
RTime	R	-0.437	0.214	0.210
RVTI	R	-0.060	-0.268	0.209

*Correlation is significant at the 00.05 level (2-tailed). **Correlation is significant at the 00.01 level (2-tailed), T (Testosterone), LH (Luteinizing hormone), FSH (Follicle stimulating hormone), RPSV (Right peak systolic volume), REDV (Right end diastolic velocity), RPSV/EDV (Ratio of peak systolic and end diastolic velocity), RVELmin (Right velocity minimum), RVELmean (right velocity mean), RPGPSV (right pressure gradient for peak systolic velocity), RPGEDV (Right pressure gradient for end diastolic velocity), RPGVmin (right pressure gradient velocity minimum), RPGVmean (right pressure gradient velocity mean), RPI (right pulsatility index), RRI (right resistivity index), R Time (right time), RVTI (right velocity time integra) and R (Pearson correlation coefficient),

4.5.3 The relationship between the epididymal parameters and reproductive hormones

Although there were varied degree of correlation between the epididymal parameters and the reproductive hormones, none of the correlation was significant (Table 4.8a and 4.8b). Luteinizing hormone showed a negative weak correlation with all the epididymal parameters except with left epididymal head's short axis.

Table 4.9a Relationship between the left epididymal measurements and reproductive hormonal profile (n = 15)

		T	LH	FSH
LEHLA	R	-0.374	-0.373	0.329
LEHSA	R	-0.431	0.039	-0.003
LEHA	R	-0.387	-0.188	0.131
LEHV	R	-0.320	-0.141	0.037
LETLA	R	-0.287	-0.280	0.126
LETSA	R	0.114	-0.112	0.091
LETA	R	0.006	-0.305	-0.094
LETV	R	0.132	-0.239	-0.048

LEHLA (left epididymal head's long axis), LEHSA (left epidiymal head's short axis), LEHA (left epididymal head's area), LEHV (left epididymal head's volume), LETLA (left epididymal tail's long axis), LETSA (left epididymal tail's short axis), LETA (left epididymal tail's area), LETV (left epididymal tail's volume), R (Pearson correlation coefficient),

Table 4.9b Relationship between the right epididymal measurements and reproductive hormonal profile (n = 15)

		T	LH	FSH
REHLA	R	-0.095	-0.052	0.030
REHSA	R	-0.312	-0.244	0.229
REHA	R	-0.229	-0.193	0.165
REHV	R	-0.247	-0.225	0.201
RETLA	R	-0.439	-0.290	-0.314
RETSA	R	0.255	-0.097	-0.002
RETA	R	0.068	-0.292	-0.269
RETV	R	0.140	-0.213	-0.180

*Correlation is significant at the 0.05 level (2-tailed). REHLA (right epididymal head's long axis), REHSA (right epidiymal head's short axis), REHA (right epididymal head's area), REHV (right epididymal head's volume), RETLA (right epididymal tail's long axis), RETSA (right epididymal tail's short axis), RETA (right epididymal tail's area), RETV (right epididymal tail's volume) and R (Pearson correlation coefficient)

4.6 The Relationship between the Demographic Data and Semen Characteristics

Reaction time significantly correlated with the body weight (r=0.601), body length (r=0.592) and height (r=0.609) while the pH was significantly correlated with the height (r=0.531) (Table 4.9).

4.7 The relationship between body weight, demographic data and testicular volume

Body weight was significantly correlated with the body length (r=0.88), girth (r=0.777), height (r=0.618), left vernier caliperic volume measurement (0.655), right vernier caliperic volume measurement (r=0.622), left ultrasonograhic volume measurement (r=0.754) and right ultrasonographic volume measurement (r=0.693) (Table 4.10).

4.8 The relationship between demographic data and hormone profile

None of the demographic parameters is significantly correlated with the reproductive hormones profiles (Table 4.11). Luteinizing hormone correlated negatively with all the demographic parameters; age (r= -0.105), body weight (r= -0.071), body length (r= -0.124), girth (r= -0.001) and height (r= -0.256).

Table 4.10The relationship between the demographic data and semen characteristics (n = 15)

		V	M	Ph	C	RT	L	D	NC	FH	FT	CT	BT
AGE	R	-0.044	-0.074	0.201	0.180	0.430	0.001	-0.001	-0.178	0.271	0.157	0.110	-0.009
Body Weight	R	-0.248	-0.005	0.383	-0.132	0.601*	0.085	-0.085	0.287	0.112	-0.312	-0.023	-0.397
Body Length	R	-0.222	-0.048	0.197	-0.156	0.592*	0.071	-0.071	0.294	-0.035	-0.211	-0.100	-0.282
Girth	R	-0.332	0.134	-0.011	0.018	0.421	0.331	-0.331	0.260	0.043	-0.157	0.017	-0.426
Height	R	0.151	-0.111	0.531*	-0.243	0.609*	-0.043	0.043	0.362	0.082	-0.218	-0.448	-0.312

*Correlation is significant at the 00.05 level (2-tailed). V (Volume), M (Motility), pH, C (Concentration), RT (reaction time), L (Live sperm), D (Dead sperm), NC (Normal cells), FH (Free Head), FT (Free Tail), CT (Coiled Tail) and BT (Bent Tail).

Table 4.11The relationship between the body weight, demographic data, vernier caliperic and sonographic volume (n =15).

		AGE	Body Weight	Body Length	Girth	Height	LVCVolume	RVCVolume	LUSVolume
Body Weight	R	0.432	-	-	-	-	-	-	-
Body Length	R	0.523*	0.880**	-	-	-	-	-	-
Girth	R	0.319	0.777**	0.813**	-	-	-	-	-
Height	R	0.200	0.618*	0.394	0.109	-	-	-	-
LVC Volume	R	0.628*	0.655*	0.583*	0.542*	0.444	-	-	-
RVC Volume	R	0.550*	0.622*	0.596*	0.572*	0.378	0.949**	-	-
LUS Volume	R	0.280	0.754**	0.697**	0.793**	0.295	0.681**	0.717**	-
RUS Volume	R	0.025	0.693**	0.489	0.602*	0.349	0.453	0.481	0.616*

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). LVCVolume (left vernier caliperic measurement), RVCVolume (right vernier caliperic measurement), LUSVolume (left ultrasonograhpic volume), RUSVolume (right ultrasonogrphic measurement), R (spearman's rank correlation), PV (P-value)

Table 4.12The relationship between the demographic parameters and the hormone profile. (n = 15)

		T	LH	FSH
AGE (months)	R	-0.323	-0.105	-0.175
Body Weight (Kg)	R	-0.127	-0.071	-0.106
Body Length (cm)	R	0.144	-0.124	-0.133
Girth (cm)	R	0.203	-0.001	-0.312
Height (cm)	R	-0.149	-0.256	0.130

T (testosterone), LH (luteinizing hormone), FSH (follicle stimulating hormone), R (spearman's rank correlation), and PV (P-value)

CHAPTER FIVE

DISCUSSION

This study shows that the left testicular length measurement using ultrasound exceeds that of the right testes but the left testicular volume was lower than the right testis. The observed mean values of the left and right testicular width or breadth using ultrasound (4.22 ±0.25 cm and 4.49 ±0.21 cm respectively) are similar to the testicular diameter of right and left testes in a fertile bull (4.29 ± 0.25 and 4.27 ± 0.24 cm respectively) reported by Ali *et al.,* (2011). The observed mean values of left and right testicular length in the current study agrees with the findings of Abdullahi *et al.,* (2012). Who used flexible tape to measure the testicular length in an in vitro study which excludes the scrotum However, the volume of the testes they observed by using Archimedes principles of water displacement in a measuring cylinder (130.50 ± 13.43 ml) was greater than the testicular volume obtained using ultrasound. This is due to the accuracy of the ultrasound method as explained by Harriet *et al.,*(2002). Thoughseveral researchers have tried to address the issue of accuracy and precision of ultrasound measurements of testicular volume (Rivkees *et al.,* 1987; Behre *et al.,* 1989; Costabile *et al.,* 1992; Lenz *et al.,* 1993) by introducing different formulas such as (a) length (L) × width (W) × height (H) × 0.52; (b) L × W2 × 0.52 and (c) L × W × H × 0.71. Harriet *et al.,* (2002) reported the formula of Lambert, *L* x *W* x*H* x 0.71 to be the most accurate formula for determining testicular volume though. However, Sakamoto *et al.,* (2007), Hsieh *et al.,* (2009) and Timothy *et al.,* (2013) reported that there was no statistically significant difference in precision among the three ultrasound formulas stated above.

The values for Doppler biometry obtained in the current studies were similar to the Doppler parameters of the supratesticular artery in humans reported by Middleton *et al*., (1989). The authors reported that the peak systolic velocities ranged from 7.5 to 27.7 cm/secs (mean, 14.0

cm/sec), end-diastolic velocities range from 0 to 4.7cm/secs (mean, 1.9cm/secs), resistivity index ranged from 0.63 to 1.0 (mean, 0.8) and pulsatility index ranged from 1.3 to 5.9 (mean, 3.0). Though there are limited information available on Doppler evaluation of testicular parenchyma,current studies observed that,the pressure and velocity of blood in the right testes of Yankasa rams was higher than in the left testes because the resistance to blood flow was higher in the left testes. This could aid in diagnosis of scrotal condition that alters blood flow as similarly reported by Middleton *et al*., (1989).

The epididymal length, width and volume observed by Suchana, (2013) in her study of the epididymal biometry and testicular circumference of the small Bangladeshi indigenous breed of ram was smaller than values obstained for Yankasa rams in this study. The differences were due to the breeds of ram used. In support of this, Suchana (2013) reported that such difference was due to the variation in age, breed, bodyweight, season and nutrition. The testicular volume had been reported by England, (1991) to be positively correlated to the epididymal volume in Dogs.

The mean left and right testicular length using vernier caliper was greater than the values obtained with ultrasound machine. This finding was also higher than the value recorded by Abdullahi *et al.,* (2012) in their*invitro* study of Yankasa rams testicular biometry using thread and tape (12.25 ± 0.35cm). The observed values however agrees with the report by Wahid and Yunus, (1994) who recorded testicular length to be within the range of 12.53 ± 0.05 - 15.65 ± 0.11, and slightly in agreement with Akpa, (2006) who reported the Yankasa testicular length to be within the range of 11.0 ± 0.16 – 14.2 ± 0.19 using flexible tape. Higher testicular length reported in this study using vernear caliper (VC) was due the fact that the scrotum is included in VC measrements.

The mean left and right testicular circumferencerecorded in this study using flexible tape compares favourably with the results obtained by several authours; Wahid and Yunus, (1994), Jibril *et al.,* (2011), and Abdullahi *et al.,* (2012).

The observed mean left and right testicular volumes using Bailey's *et al*,(1998)formula in the current studies were higher than the values obtained by Abdullahi *et al.,* (2012) in Yankasa rams. The difference wasdue to differences in the methods used in determining the volume. Abdullahi *et al.,* (2012) used Archimedes principle of water displacement in a measuring cylinder and this is more accurate than the vernier caliperic method whose value incorporates the scrotum.

Similar to the findings in this study, Andrade *et al.* (2014) reported that testicular measurement obtained with calipers was higher than the ultrasound measurements. This was similarly supported by a finding in a study that determined the relationship between *in vivo* measurements of bovine testes obtained with ultrasound and vernear caliper, in which Bailey *et al*., (1998) observed that the width measured with ultrasound showed a higher correlation with the real width than that obtained with the vernear caliper because only the testicular parenchyma was measured using ultrasound, while vernearcaliper measurements also included the body of epididymides, scrotum and testicular tunics. However, regarding the measurement of testicular volume, according to Bailey *et al*., (1998), the volume obtained with vernear calipers had higher correlation with the real volume when compared to volume measurements obtained with ultrasound because of difficulties in assessing the testicular length by ultrasound in older animals as the transducer is sometimes smaller than the testis.

It was observed in the current studies that the echo texture of the testicular parenchyma and epididymides were uniform (isoechoic) surrounded by an hyper echoic structure (*Tunica*

albuginea and *Tunica vaginalis*) in a longitudinal plain scan, however in transverse plain, the mediastinum testis was seen as a circular region of hyper echoic appearance in the center of the testicular parenchyma. This is similar to the findings of Ali *et al.,* (2011) who reported that the testicular parenchyma of fertile bulls to be uniformly homogeneous with moderate echogenicity. Likewise, Andrade *et al.,* (2014), reported that the testicular parenchyma appeared homogeneous, ranging from low to moderate echogenicity regardless of the testis (right or left) and the scan plane used. As similarly found in this study, Andrade *et al.* (2014) reported the mediastinum testis was seen to be hyper echoic line of variable thickness in the center of the testicular parenchyma when scanned in the frontal plane and as a point in the center of hyper echogenic parenchyma when scanned in the transverse plane. The scrotal septum was seen as a hyper echoic line between the right and left testes (Andrade *et al.,* 2014), but since individual testis was scanned separately, scrotal septum was not visualised in this study. Suchana, (2013) also supports our observation by describing the normal testis to appear as an oval structure with a fairly homogenous medium-gray to dark-gray internal echo pattern, the borders of which were well circumscribed and distinguishable from the surrounding structures.

It was observed that the echogenicity of the epididymides is isoechoic with uniform appearance. an area of hypo echoic structure in the epididymal head. Ali *et al.,* (2011) similarly reported the epididymal head to be homogeneous and less echogenic than the testicular parenchyma of a fertile bull. England, (1991) reported that the tail of the epididymides in dogs was easily identified and appeared as a circular moderately hypo echoic structure with respect to testicular parenchyma in vivo, but of similar echo texture in vitro. In support of our findings, Suchana, (2013) and Andrade *et al.* (2014) reported the epididymal tail to possess reduced echogenicity when compared to testicular parenchyma in prepubertal animals.

It was observed currently, and similar to the report by Akpa, *et al*., (2012) thatsperm concentration, reaction time and normal sperm cells correlated positively with the testicular volume using vernier caliper or ultrasound, though the correlation was weak and not significant. However, The positive and weak relationship between the semen pH and testicular measurement disagrees with the report by Akpa, *et al*., (2012).

Similar to the finding in this study in which concentration of spermatozoa was positively correlated to the testicular volume measured with verniercaliper or ultrasound, England, (1991) reported the correlation between the total testicular volume and total sperm output (r= 0.29) and in the bull, measurements of testicular size have been used for the prediction of sperm output (Ott 1986**).** Similarly measurements of testicular size in the dog have been shown to be highly correlated with measures of sperm production (Mialot *et al.,*1985).Testicular weight and sperm concentration were positively correlated (Vidament *et al.,* 2007; Akpa *et al*., 2012). Increase in mean testicular weight could be due to increase in seminiferous tubules size and proliferation of germ cells characterizing higher spermatogenic activity (Melo *et al*., 2010).

All the semen parameters measured in this study did not have a specific pattern of correlation with the vernier caliperic measurement of the testicular length, circumference, volume and the weight. This disagrees with Wahid and Yunus, (1994) who reported a positive and significant correlation between testicular length with semen volume, sperm motility and concentration. The sperm concentration was however, positively correlated with all the testicular parameters except with the right and left testicular circumference which were negatively correlated.

The result obtained from this study suggests that the testicular length can affect livability of

sperm cells and not sperm production because it positively correlated with the semen volume (r= 0.355 and r= 0.454) as supported by Wahid and Yunus, (1994) who reported a positive and significant correlation between testicular length and semen volume.

Testicular size was ultimately correlated with capacity of sperm production; number of sperm ejaculated and testicular circumference had a direct correlation with spermatozoa output as observed by some researchers (Osinowo, 1979; Akpa *et al*., 2012). This disagrees with what was observed in this study in which both the right and left testicular circumferences were negatively correlated with the semen volume (r= -0.436). Since the right and left testicular circumferences also correlated positively with the life and normal sperm cells (r=0.305 and r=0.476 respectively) this suggests that increase in testicular circumference does not lead to increased sperm production but is necessary for the healthiness or survival of spermatozoa. It was observed that testicular volume was weakly correlated with the sperm concentration (r= 0.225 and r= 0.222 for vernier caliper and ultrasound measurement respectively). This is not in support of Akpa *et al.,* (2012) who reported that testicular size is strongly correlated with sperm count.

The results of the pH obtained in this study suggest that the pH of the semen determines the velocity and pressure of blood flow in the left and right testicular parenchyma.

The number of life and dead sperm cells in an ejaculate can be predicted from End Diastolic Velocity, Pressure Gradient of the End Diastolic Velocity and resistance index, because the higher the number of life sperm cells the higher the resistance index, and lower value of end diastolic velocity and Pressure Gradient of the End Diastolic Velocity of the left testes. The reverse is the case for dead sperm cells. As supported by Batissaco *et al.,* (2013),who showed that the increase in sperm defect can be related to increase in testicular blood flow. High

circulatory resistance in the veins results in a relatively low diastolic flow and a high value of RI (Batissaco *et al.,* 2013), this has been reported to lead to increase in number of abnormal sperm cells in humans (Pinggera *et al*., 2008), which is similar to our observation in this study in which the left and right testicular resistance index was negatively but not significantly correlated with the normal cells (r=-0.439 and r=-0.276 respectively) and positively correlated with the abnormal sperm cells (free head, free tail, coiled tail and bent tail). Since it also correlated negatively and significantly with the dead cells (r=-0.591 and r=-0.34 respectively) it means that increase resistivity does not lead to death of spermatozoa but only leads to their abnormal morphology, unlike what is reported by Batissaco *et al.,* (2013), who stated that vascular changes in the testis may lead to damage in sperm production as it is reflected in sperm motility and morphology (Batissaco *et al.,* 2013)

This study also showed that motility of the spermatozoa reduces the flow of blood in the testicle. This observation, however, disagrees with Batissaco *et al.,* (2013) who observed no correlation between motility and hemodynamic characteristics.

The semen volume significantly correlated with the left epididymal head's long axis (r=0.599), area (r=0.526), volume (r=0.514) and weakly correlated with the short axis (r=0.328) while the semen volume was negatively correlated with all the left epididymal tail parameters (long axis, short axis, area and the volume). This indicates that the head of the epididymides could be used to predict the volume of the ejaculate, this is further supported by the fact that sperm concentration correlated positively with all the left epididymal head parameters and negatively with all the epididymal tail parameters. However, this result disagrees with Suchana, (2013) who reported the epididymal volume to be negatively correlated with the semen volume (r=-0.237),

but partly in support of England, (1991) who reported the correlation between cross-sectional area of the epididymal tail and the total sperm output (r=0.05).

Normal sperm cells correlated negatively with all the left epididymal head and tail parameters except epididymal tail long axis (r=0.103). Normal cells of the sperm correlated significantly with the left epididymal head's short axis (r= -0.531) and weakly with the area (r= - 0.446) and volume (r= -0.494). This shows that large size of the epididymides has detrimental effects on the spermatozoa. This is further supported by the fact that left epididymal head area and volume correlated positively and significantly with the abnormal cells and negatively with the normal cells (r= -0.446 and r= -0.494).

Similar to the findings in this study, Suchana, (2013) reported a positive correlation between epididymal volume and sperm morphology. There was a significant correlation (r=0.56) between the total testicular volume and the cross-sectional epididymal area (England, 1991).

Although none of the testicular parameters using vernier caliper (length, circumference, volume and weight) significantly correlated with the reproductive hormones (testosterone, luteinizing hormone and follicle stimulating hormone), testosterone and luteinizing hormone correlated negatively with the right and left testicular length, circumference, volume and weight (table 4.10).

In this study it was observed that testosterone and luteinizing hormone correlated negatively with the right and left testicular length, circumference, volume and weight, this is not in support of Yarney and Sanfordi, (1990) who reported that testicular size in rams at 6-7 month of age was related positively (r=0.58-0.65) to mean testosterone concentration while testicular size and spermatogenic function in yearling of Suffolk rams related to serum LH and (or) testosterone

concentrations in the neonatal period (50 days) and at puberty (150 days). They however observed that the yearling's scrotal circumference was related (r= 0.58) to FSH concentration. Yarney and Sanfordi, (1990) partly agrees with our observation in this study by reporting that testicular size measurements taken in the rams' first breeding season, testicular size and daily sperm output assessments made following the spring, were negatively correlated to most characteristics of LH release and to mean FSH concentration between 90 and 130 days of age. Yarney and Sanfordi, (1990) also observed that the magnitude or the frequency of LH pulses was correlated negatively with the testosterone concentration (r= -0.69), testicular size (r= -0.53) and sperm number in the ejaculate (r= -0.53). Wahid and Yunus, (1994) reported variable relationship between the testicular measurement and libido in the temperate breeds of ram and also showed that as the testicular length influenced the quality of the semen, testicular circumference influenced libido of rams (Wahid and Yunus, 1994). Kuijper *et al.*, (2008) also reported that testicular volume is related to various reproductive endocrine parameters which were not in support of what is observed in these our studies.

There is limited literature published on the relationship between testicular Doppler parameter and reproductive hormones. Luteinizing hormone was positively and significantly correlated with the left pulsatility index (r=0.586) and positive highly significant with the right pulsatility index (r=0.953). This may be due to the pulsatile nature of the LH. Hence this study shows that quantity of LH in the system could be predicted from the measure of pulsatility index of the testicular artery. Follicle stimulating hormone was negatively and significantly correlated with the left resistivity index (r= -0.553) and weakly correlated with the right resistivity index (r= -0.168). This suggests that FSH enhances the flow of blood in the testicular arteries. Luteinizing hormone was positively and significantly correlated with the right Velmin (r=0.581) and weak

with the left Velmin (r= -0.116). Since it also correlates positively and significantly with the right PGmin (r=0.639) and weakly with the left PGmin (r= -0.14) which suggest that LH could affect the rate of blood flowing through the testicular arteries.

There is no significant relationship between the epididymal parameters and reproductive hormones, though there is varied degree of correlation between the epididymal parameters and the reproductive hormones. This shows that none of the reproductive hormone studied had effects on the epididymides.

Most of the semen characteristics were not significantly correlated with the demographic data which disagrees with significant correlations reported by Akpa, *et al*., (2012). It was found that semen volume correlates negatively with the body weight and body length (r=-0.25 and r=-0.22); semen pH was positively correlated with the body weight and body length (r=0.38 and r=0.2); sperm concentration was negatively correlated with the body weight and body length (r=-0.13 and r=-0.16); and sperm motility was negatively correlated with the body weight and body length (r=-0.005 and r=-0.048). This result totally disagrees with (Akpa, *et al*., (2012), who reported that semen volume was positively and significantly correlated with body weight and body length (r=0.37-0.48; P<0.01); semen pH was negatively and significantly correlated with body weight and body length (P=0.01-0.05; r=-0.29 to-0.38); sperm concentrations were positively and significantly correlated with body weight and body length (P<0.01-0.05; r=0.27-0.35); Live/dead ratio was positively and significantly correlated with body length ((P<0.01-0.05; r=0.27-0.30); and sperm motility with positive, but less significant correlations with body weight and body length in Yankasa rams (P>0.05; r= 0.16-0.25).

In this study, it was observed that reaction time significantly correlated with the body weight (r=0.601), body length (r=0.592) and height (r=0.609) while the pH was significantly correlated with the height (r=0.531). This shows that body weight, body length and height can be used to predict the libido and sexual stimulation in rams.

Similar to this finding Koyuncu *et al.*, (2005), also reported statistically significant ($p<0.001$) positive correlations between age, body weight, testicular diameter, testicular length, scrotal length, scrotal circumference and volume in growing Kivircik male lambs. This is further supported by Akpa *et al.*, (2006), who reported highly significant ($P<0.01$) positive correlations ($r = 0.898 – 0.0969$) among age and the following demographic parameters; Body weight (BW), Body length (BL), Height at withers (HW) Heart girth (HG), Scrotal circumference (SC) and Scrotal length (SL). Testicular size varies with age, breed and time of the year (NseAbasi, 2015). Salheb *et al.* (2001) observed that parental size, age and body weight affected testicular growth however, there was no significant difference between measurement of the left and right testes (NseAbasi, 2015).

None of the demographic parameters significantly correlated with the reproductive hormones profile. Luteinizing hormone correlated negatively with all the demographic parameters; age (r= -0.105), body weight (r= -0.071), body length (r= -0.124), girth (r= -0.001) and height (r= -0.256). This disagrees with Yarney and Sanfordi, (1990) who reported that daily sperm output in urine (DSO) and testicular size in rams at 6-7 month of age were related positively (r= 0.58-0.65) to mean testosterone concentration. Likewise the testicular size and spermatogenic function in yearling Suffolk rams were related to serum LH and (or) testosterone concentrations in the neonatal period (50 days) and at puberty (150 days) while scrotal circumference was related (r=0.58) to FSH concentration (Yarney and Sanfordi, 1990). The disagreement may be due to

differences in the age of the animal used in the study. Yarney and Sanfordi, (1990) worked on lambs while our study was on the young Yankasa rams.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has established the following about the evaluation of sonographic parameters of the testes in relation to the spermogram and hormonal profile (testosterone, follicle stimulating hormone and luteinizing hormone) of clinically healthy Yankasa rams.

The mean values of ultrasound measurement of the left and right testicular length were 130.4 ± 2.73 mm and 125.5 ±2.57 mm respectively; the mean values of the ultrasound measurement of the left and right testicular breadth were 42.15 ±2.53 mm and 44.85 ±2.14 mm respectively; the mean values of the ultrasound measurement of the left and right height were 52.25 ± 2.10 mm and 53.02 ± 2.20 mm respectively. While the volumes were 280340 ± 12780 mm^3 and 296432 ± 17549 mm^3 as observed in Yankasa rams.

From the normal Doppler evaluation of testicular arteries, the velocity, pressure and resistance to blood flow in the left testes of Yankasa rams is were higher than in the right testes (figure 4.2).

i. The mean value for the left epididymal head volume using ultrasound was 6.48 ± 0.62 cm^3 while that of the right was 4.49 ± 0.42 cm^3 and the mean value for the left's epididymal tail volume was 2.83 ± 0.30 cm^3 while that of the right was 3.01 ± 0.50 cm^3.

ii. The mean left testicular length (15.7 ± 0.55 cm) and that of the right (15.9 ± 0.61 cm) using vernier caliper was greater than value obtained with ultrasound machine (13.04 ± 0.27 cm and 12.55 ± 0.2.7 cm respectively). Likewise the mean left and right testicular volume using vernier caliper was 1482 ± 64.37 cm^3 and 1503 ± 74.27 cm^3 respectively which was also higher than the ultrasound measurement.

iii. The testicular parenchyma of Yankasa rams was an isoechoic structure (midgray echo texture) with uniform echogenicity which was surrounded by a hyper-echoic structure (*Tunica albugina* and *Tunica vaginalis*). The mediastinum testis was seen as a point of hyper echoic area in the center of testicular parenchyma when scanned in the transverse plane. The echogenicity of the epididymides was similar to the echogenicity of the testes but with some hypo echoic area of echogenicity in the epididymal head.

iv. The sperm concentration, reaction time and normal sperm cells correlated positively and insignificantly with the testicular volume using vernier caliper or ultrasound, while the sperm motility correlates negatively with the left and right vernier caliperic measurement of the testicular volume and positively with the left and right ultrasound measurement of the testicular volume.

v. The relationship between the vernier caliperic measurement of the testes and semen parameters showed that testicular length affected the livability of sperm cells and not sperm production and increase in testicular circumference did not lead to increased sperm production but was necessary for the health or survival of spermatozoa.

vi. The relationship between testicular Doppler biometry and semen parameters showed that the pH of the semen determines the velocity and pressure of blood flow in the testicular parenchyma; the number of life and dead sperm cells in an ejaculate can be predicted from End Diastolic Velocity, Pressure Gradient of the End Diastolic Velocity and resistance index; increase resistivity to blood flow did not lead to death of spermatozoa but to their abnormal morphology, and motility of the spermatozoa reduced the flow of blood in the testicular artery.

vii. The relationship between the epididymal biometry and the semen parameters showed that the head of the epididymides could be used to predict the volume of sperm production but large size of the epididymides has detrimental effects on the spermatozoa.

viii. The relationship between testicular biometry and reproductive hormones showed that testosterone and luteinizing hormone correlate negatively and insignificantly with the right and left testicular length, circumference, volume and weight.

ix. The relationship between testicular Doppler parameter and reproductive hormones showed that quantity of LH in the system could be predicted from the measure of pulsatility index of the testicular artery, LH could affect the rate of blood flowing through the testicular arteries while FSH enhances the flow of blood in the testicular arteries.

x. The relationship between the epididymal parameters and reproductive hormones showed that none of the reproductive hormone studied had effects on the epididymides of Yankasa rams.

xi. The relationship between the demographic data and semen characteristics showed that body weight, body length and height can predict the libido and sexual stimulation in rams.

xii. The relationship between body weight, demographic data and testicular volume revealed that body weight was significantly correlated with the body length (r=0.88), girth (r=0.777), height (r=0.618), left vernier caliperic volume measurement (0.655), right vernier caliperic volume measurement (r=0.622), left ultrasonograhic volume measurement (r=0.754) and right ultrasonographic volume measurement (r=0.693)

xiii. The relationship between demographic data and hormone profile revealed that none of the demographic parameters significantly correlated with the reproductive hormone profiles studied in Yankasa rams.

In conclusion, evaluation of echo texture and biometry of the Yankasa rams testes and epididymides using ultrasound is essential for maximum and rational utilization of the breeding stock and can be used to predict future semen parameters.

6.2 Recommendations

i. It is recommended that routine ultrasound examination should be employed alongside other diagnostic procedures in breeding soundness examination of Yankasa Ram

ii. We also recommend further studies be carried out on the use of ultrasound to measure testicular and epididymal parameters *in vitro* after castrating the animals to eliminate error of few millimeters due to scrotum and subcutaneous tissues that is unavoidable during *in vivo* scanning.

iii. We also recommend morestudies be carried out on Doppler evaluation of testicular arteries in predicting the sperm morphology and abnormalities

iv. Further study is required to explore the relationship between the epididymal biometry and the semen parameters in predicting future ram breeders.

6.3 **Limitations**

i. Non-availability of software system to actually quantify the pixel intensity of the echo texture

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Appendices

Appendix 1a. Ultrasonographic measurement of the left testes

Tag No	Length (mm)	Breadth (mm)	Height (mm)	Volume (mm^3)
1	141	33.6	61.9	293257.44
2	149.6	36.5	52.6	287217.04
3	127	40.4	49.8	255513.84
4	132.3	39.1	51.8	267957.774
5	136.6	39.7	65	352496.3
6	114.8	33.6	51.4	198264.192
7	135.8	31.9	56.6	245192.332
8	115.6	33.4	54.2	209268.368
9	133.6	57	46.6	354868.32
10	140.6	35.3	55.5	275456.49
11	134	44.1	55.7	329153.58
12	129.8	48.3	55.6	348575.304
13	111	39.3	55.9	243852.57
14	129.4	62.5	36.4	294385
15	124.9	57.6	34.7	249640.128

Appendix 1b.Ultrasonographic measurement of the right testes

Tag No	Length (mm)	Breadth (mm)	Height (mm)	Volume (mm^3)
1	129	43.4	60	335916
2	118.4	39.6	46.4	217552.896
3	120.6	37.8	48.9	222919.452
4	136.4	42.9	60.9	356360.004
5	120.6	46.7	57.2	322151.544
6	101.5	39.6	49.2	197754.48
7	122.1	33.4	57.6	234900.864
8	122.1	36.6	58.5	261428.31
9	118	59.7	47.5	334618.5
10	140.2	47	64.4	424357.36
11	130.2	46.7	56.7	344755.278
12	139.3	45.3	59.2	373569.168
13	132.5	37.8	53.8	269457.3
14	122.5	61.4	43.1	324176.65
15	129.6	54.8	31.9	226556.352

Appendix 1c.Vernier caliperic measurement of the left testes

Tag No	TL (cm)	TC (cm)	TW (cm)	TW^2 (cm^2)	TLX TW^2 (cm^3)	Volume {0.5236 (TLX TW^2)} (cm^3)	Weight {0.5533 (TLX TW^2) }g
1	15	28	14	196	2940	1539.384	1626.702
2	19	26	13	169	3211	1681.2796	1776.6463
3	15.5	26	13	169	2619.5	1371.5702	1449.36935
4	16	25	12.5	156.25	2500	1309	1383.25
5	18	26	13	169	3042	1592.7912	1683.1386
6	12	24	12	144	1728	904.7808	956.1024
7	17	26	13	169	2873	1504.3028	1589.6309
8	18	26	13	169	3042	1592.7912	1683.1386
9	18	28	14	196	3528	1847.2608	1952.0424
10	13	29.5	14.75	217.5625	2828.3125	1480.904425	1564.905306
11	15	29	14.5	210.25	3153.75	1651.3035	1744.969875
12	14	31	15.5	240.25	3363.5	1761.1286	1861.02455
13	15	28	14	196	2940	1539.384	1626.702
14	17	25	12.5	156.25	2656.25	1390.8125	1469.703125
15	13	25	12.5	156.25	2031.25	1063.5625	1123.890625

Appendix 1d.Vernier caliperic measurement of the right testes

Tag No	TL (cm)	TC (cm)	TW (cm)	TW^2 (cm^2)	TLX TW^2 (cm^3)	Volume {0.5236 (TLX TW^2)} (cm^3)	Weight {0.5533 (TLX TW^2) }g
1	15	28	14	196	2940	1539.384	1626.702
2	18	26	13	169	3042	1592.7912	1683.1386
3	15	26	13	169	2535	1327.326	1402.6155
4	16	25	12.5	156.25	2500	1309	1383.25
5	19	26	13	169	3211	1681.2796	1776.6463
6	12	24	12	144	1728	904.7808	956.1024
7	19	26	13	169	3211	1681.2796	1776.6463
8	17	26	13	169	2873	1504.3028	1589.6309
9	20	28	14	196	3920	2052.512	2168.936
10	13.5	29.5	14.75	217.5625	2937.09375	1537.862288	1625.093972
11	14	29	14.5	210.25	2943.5	1541.2166	1628.63855
12	15	31	15.5	240.25	3603.75	1886.9235	1993.954875
13	15	28	14	196	2940	1539.384	1626.702
14	17	25	12.5	156.25	2656.25	1390.8125	1469.703125
15	13	25	12.5	156.25	2031.25	1063.5625	1123.890625

Appendix 1e. Summary of the vernier caliperic measurement

Tag No	Left				Right			
	TL (cm)	TC (cm)	TV (cm^3)	Weight (g)	TL (cm)	TC (cm)	TV (cm^3)	Weight (g)
1	15	28	1539.384	1626.702	15	28	1539.384	1626.702
2	19	26	1681.2796	1776.6463	18	26	1592.7912	1683.1386
3	15.5	26	1371.5702	1449.3694	15	26	1327.326	1402.6155
4	16	25	1309	1383.25	16	25	1309	1383.25
5	18	26	1592.7912	1683.1386	19	26	1681.2796	1776.6463
6	12	24	904.7808	956.1024	12	24	904.7808	956.1024
7	17	26	1504.3028	1589.6309	19	26	1681.2796	1776.6463
8	18	26	1592.7912	1683.1386	17	26	1504.3028	1589.6309
9	18	28	1847.2608	1952.0424	20	28	2052.512	2168.936
10	13	29.5	1480.904425	1564.9053	13.5	29.5	1537.862288	1625.093972
11	15	29	1651.3035	1744.9699	14	29	1541.2166	1628.63855
12	14	31	1761.1286	1861.0246	15	31	1886.9235	1993.954875
13	15	28	1539.384	1626.702	15	28	1539.384	1626.702
14	17	25	1390.8125	1469.7031	17	25	1390.8125	1469.703125
15	13	25	1063.5625	1123.8906	13	25	1063.5625	1123.890625

Appendix 1f.Summary of the ultrasonographic measurement

Tag No	Left				Right			
	L (mm)	B (mm)	H (mm)	V (mm^3)	L (mm)	B (mm)	H (mm)	V (mm^3)
1	141	33.6	61.9	293257.44	129	43.4	60	335916
2	149.6	36.5	52.6	287217.04	118.4	39.6	46.4	217552.896
3	127	40.4	49.8	255513.84	120.6	37.8	48.9	222919.452
4	132.3	39.1	51.8	267957.77	136.4	42.9	60.9	356360.004
5	136.6	39.7	65	352496.3	120.6	46.7	57.2	322151.544
6	114.8	33.6	51.4	198264.19	101.5	39.6	49.2	197754.48
7	135.8	31.9	56.6	245192.33	122.1	33.4	57.6	234900.864
8	115.6	33.4	54.2	209268.37	122.1	36.6	58.5	261428.31
9	133.6	57	46.6	354868.32	118	59.7	47.5	334618.5
10	140.6	35.3	55.5	275456.49	140.2	47	64.4	424357.36
11	134	44.1	55.7	329153.58	130.2	46.7	56.7	344755.278
12	129.8	48.3	55.6	348575.3	139.3	45.3	59.2	373569.168
13	111	39.3	55.9	243852.57	132.5	37.8	53.8	269457.3
14	129.4	62.5	36.4	294385	122.5	61.4	43.1	324176.65
15	124.9	57.6	34.7	249640.13	129.6	54.8	31.9	226556.352

Appendix 1g.Comparing testicular caliperic and sonographic measurements

	LEFT				RIGHT			
	Caliper		Ultrasound		Caliper		Ultrasound	
Tag No	TL (cm)	TV (cm^3)	TL (mm)	TV (mm^3)	TL (cm)	TV (cm^3)	TL (mm)	TV (mm^3)
1	15	1539.384	141	293257.44	15	1539.384	129	335916
2	19	1681.2796	149.6	287217.04	18	1592.7912	118.4	217552.896
3	15.5	1371.5702	127	255513.84	15	1327.326	120.6	222919.452
4	16	1309	132.3	267957.77	16	1309	136.4	356360.004
5	18	1592.7912	136.6	352496.3	19	1681.2796	120.6	322151.544
6	12	904.7808	114.8	198264.19	12	904.7808	101.5	197754.48
7	17	1504.3028	135.8	245192.33	19	1681.2796	122.1	234900.864
8	18	1592.7912	115.6	209268.37	17	1504.3028	122.1	261428.31
9	18	1847.2608	133.6	354868.32	20	2052.512	118	334618.5
10	13	1480.904425	140.6	275456.49	13.5	1537.862288	140.2	424357.36
11	15	1651.3035	134	329153.58	14	1541.2166	130.2	344755.278
12	14	1761.1286	129.8	348575.3	15	1886.9235	139.3	373569.168
13	15	1539.384	111	243852.57	15	1539.384	132.5	269457.3
14	17	1390.8125	129.4	294385	17	1390.8125	122.5	324176.65
15	13	1063.5625	124.9	249640.13	13	1063.5625	129.6	226556.352

Appendix 1h. Difference between testicular ultrasonographic and vernier caliperic volume measurement. (n=15)

Tag No	Left testicular ultrasound volume (cm^3)	Right testicular ultrasound volume (cm^3)	Left testicular vernier caliper volume (cm^3)	Right testicular vernier caliper volume (cm^3)
1	293.2574	335.916	1539.384	1539.384
2	287.217	217.5529	1681.28	1592.791
3	255.5138	222.9194	1371.57	1327.326
4	267.9578	356.36	1309	1309
5	352.4963	322.1516	1592.791	1681.28
6	198.2642	197.7545	904.7808	904.7808
7	245.1923	234.9009	1504.303	1681.28
8	209.2684	261.4283	1592.791	1504.303
9	354.8683	334.6185	1847.261	2052.512
10	275.4565	424.3574	1480.904	1537.862
11	329.1536	344.7553	1651.303	1541.217
12	348.5753	373.5692	1761.129	1886.923
13	243.8526	269.4573	1539.384	1539.384
14	294.385	324.1766	1390.813	1390.813
15	249.6401	226.5564	1063.563	1063.563
Mean ± SEM	280.3 ± 12.78	296.4 ± 17.55	1482 ± 64.37[a]	1503 ± 74.27[a]

(P<0.0001)

Appendix 2a.Demographic data

Tag No	Age (months)	Weight (Kg)	Body length (cm)	Girth (cm)	Height (cm)	Stature
1	18	33	88	75	77	Normal
2	30	30	89	84	69	Normal
3	18	22	80	82	65	Normal
4	18	22.5	86	75	73	Normal
5	18	30	81	90	73	Normal
6	18	18	78	70	66	Normal
7	18	29	87	76	74	Normal
8	30	25	85	76	75	Normal
9	30	37.4	106	104	66	Normal
10	18	32.9	86	97	66	Normal
11	24	53.3	105	101	77	Normal
12	30	54.4	110	107	81	Normal
13	18	25	81	78	74	Normal
14	12	31.9	95	97	72	Normal
15	18	22.9	89	87	67	Normal

Appendix 2b.Demographic data and testicular volume

Tag No	Age (months)	Weight (Kg)	Body length (cm)	Girth (cm)	Height (cm)	V.C volume (cm^3) Left	V.C volume (cm^3) Right	S.volume (mm^3) Left	S.volume (mm^3) Right
1	18	33	88	75	77	1539.384	1539.384	293257.44	335916
2	30	30	89	84	69	1681.2796	1592.7912	287217.04	217552.896
3	18	22	80	82	65	1371.5702	1327.326	255513.84	222919.452
4	18		86	75	73	1309	1309	267957.774	356360.004
5	18	30	81	90	73	1592.7912	1681.2796	352496.3	322151.544
6	18	18	78	70	66	904.7808	904.7808	198264.192	197754.48
7	18	29	87	76	74	1504.3028	1681.2796	245192.332	234900.864
8	30	25	85	76	75	1592.7912	1504.3028	209268.368	261428.31
9	30	37.4	106	104	66	1847.2608	2052.512	354868.32	334618.5
10	18	32.9	86	97	66	1480.904425	1537.862288	275456.49	424357.36
11	24	53.3	105	101	77	1651.3035	1541.2166	329153.58	344755.278
12	30	54.4	110	107	81	1761.1286	1886.9235	348575.304	373569.168
13	18	25	81	78	74	1539.384	1539.384	243852.57	269457.3
14	12	31.9	95	97	72	1390.8125	1390.8125	294385	324176.65
15	18	22.9	89	87	67	1063.5625	1063.5625	249640.128	226556.352

Appendix 3a.Sonographic measurement of epididymal head and tail

	Left								Right							
	Head				Tail				Head				Tail			
Tag No	LA (mm	SA (mm)	A (cm^2)	V (cm^3)	LA (mm	SA (mm)	A (cm^2)	V (cm^3)	LA (mm	SA (mm)	A (cm^2)	V (cm^3)	LA (mm	SA (mm)	A (cm^2)	V (cm^3)
1	41.9	21.8	7.17	10.415	32.5	11.9	3.03	2.398	36.9	14.7	4.25	4.125	32.8	9.9	2.5	1.625
2	36.9	17.7	5.12	6.02	42.7	10.6	3.55	2.505	37.1	15.9	4.63	4.897	35.7	11.4	3.21	2.446
3	35.5	14	3.89	3.621	21.6	12.1	2.06	1.662	26.8	16	3.37	3.594	18.5	13.5	1.97	1.771
4	43	13.9	4.7	4.365	31.1	11	2.7	1.986	37.2	14.3	4.17	3.963	24.7	9.2	1.78	1.087
5	30.7	19.3	4.64	5.964	26.7	12.1	2.19	2.034	39.1	20.1	6.16	8.264	19.9	14.4	2.3	2.248
6	42.7	17.5	5.88	6.878	30.7	12.7	3.07	2.607	32.5	18.3	4.67	5.685	25.3	23.2	4.62	7.155
7	39.1	20.1	6.16	8.264	19.9	14.4	2.3	2.248	42.6	15.7	5.27	5.521	28.5	16.1	2.59	2.785
8	33.2	20.4	5.33	7.219	27	13.5	2.86	2.567	50.1	22.1	8.72	12.866	28.5	17.8	3.99	4.748
9	47.7	19	7.11	9.008	27.6	19.1	4.14	5.266	32.5	17.4	4.45	5.199	21.8	15	2.57	2.465
10	39.5	19	5.88	7.432	25.6	19	3.83	4.846	33	16.3	4.22	4.592	26	20.9	4.27	5.966
11	43.4	20.6	7.02	9.66	26.7	16.7	3.49	3.887	34.3	17.6	4.74	5.572	21.1	11.1	1.84	1.363
12	47.8	16.8	6.31	7.06	24.1	16	3.02	3.216	38.1	17.4	5.21	6.053	35	15	4.13	4.142
13	34.3	16.9	4.54	5.106	30.2	15.6	3.7	3.848	31.3	13.1	3.29	2.803	21.1	21.6	3.58	5.156
14	29.8	9.7	2.28	1.476	18.1	10.2	1.44	0.98	23.7	9.2	1.71	1.048	9.5	7.6	1.31	0.83
15	34.6	16.1	4.36	4.67	30.11	12.9	3.66	2.463	32.1	9.8	2.48	1.624	36	8.7	2.45	1.417

Appendix 3b. Comparing demographic data and left epididymal parameters

Tag No	Age (months)	Weight (Kg)	Body length (cm)	Girth (cm)	Height (cm)	Head LA (mm	Head SA (mm)	Head A (cm^2)	Head V (cm^3)	Tail LA (mm	Tail SA (mm)	Tail A (cm^2)	Tail V (cm^3)
1	18	33	88	75	77	41.9	21.8	7.17	10.415	32.5	11.9	3.03	2.398
2	30	30	89	84	69	36.9	17.7	5.12	6.02	42.7	10.6	3.55	2.505
3	18	22	80	82	65	35.5	14	3.89	3.621	21.6	12.1	2.06	1.662
4	18		86	75	73	43	13.9	4.7	4.365	31.1	11	2.7	1.986
5	18	30	81	90	73	30.7	19.3	4.64	5.964	26.7	12.1	2.19	2.034
6	18	18	78	70	66	42.7	17.5	5.88	6.878	30.7	12.7	3.07	2.607
7	18	29	87	76	74	39.1	20.1	6.16	8.264	19.9	14.4	2.3	2.248
8	30	25	85	76	75	33.2	20.4	5.33	7.219	27	13.5	2.86	2.567
9	30	37.4	106	104	66	47.7	19	7.11	9.008	27.6	19.1	4.14	5.266
10	18	32.9	86	97	66	39.5	19	5.88	7.432	25.6	19	3.83	4.846
11	24	53.3	105	101	77	43.4	20.6	7.02	9.66	26.7	16.7	3.49	3.887
12	30	54.4	110	107	81	47.8	16.8	6.31	7.06	24.1	16	3.02	3.216
13	18	25	81	78	74	34.3	16.9	4.54	5.106	30.2	15.6	3.7	3.848
14	12	31.9	95	97	72	29.8	9.7	2.28	1.476	18.1	10.2	1.44	0.98
15	18	22.9	89	87	67	34.6	16.1	4.36	4.67	30.11	12.9	3.66	2.463

Appendix 3c.Comparing demographic data and right epididymal parameters

Tag No	Age (months)	Weight (Kg)	Body length (cm)	Girth (cm)	Height (cm)	Head LA (mm	Head SA (mm)	Head A (cm^2)	Head V (cm^3)	Tail LA (mm	Tail SA (mm)	Tail A (cm^2)	Tail V (cm^3)
1	18	33	88	75	77	36.9	14.7	4.25	4.125	32.8	9.9	2.5	1.625
2	30	30	89	84	69	37.1	15.9	4.63	4.897	35.7	11.4	3.21	2.446
3	18	22	80	82	65	26.8	16	3.37	3.594	18.5	13.5	1.97	1.771
4	18		86	75	73	37.2	14.3	4.17	3.963	24.7	9.2	1.78	1.087
5	18	30	81	90	73	39.1	20.1	6.16	8.264	19.9	14.4	2.3	2.248
6	18	18	78	70	66	32.5	18.3	4.67	5.685	25.3	23.2	4.62	7.155
7	18	29	87	76	74	42.6	15.7	5.27	5.521	28.5	16.1	2.59	2.785
8	30	25	85	76	75	50.1	22.1	8.72	12.866	28.5	17.8	3.99	4.748
9	30	37.4	106	104	66	32.5	17.4	4.45	5.199	21.8	15	2.57	2.465
10	18	32.9	86	97	66	33	16.3	4.22	4.592	26	20.9	4.27	5.966
11	24	53.3	105	101	77	34.3	17.6	4.74	5.572	21.1	11.1	1.84	1.363
12	30	54.4	110	107	81	38.1	17.4	5.21	6.053	35	15	4.13	4.142
13	18	25	81	78	74	31.3	13.1	3.29	2.803	21.1	21.6	3.58	5.156
14	12	31.9	95	97	72	23.7	9.2	1.71	1.048	9.5	7.6	1.31	0.83
15	18	22.9	89	87	67	32.1	9.8	2.48	1.624	36	8.7	2.45	1.417

Appendix 4a.Left testicular Doppler evaluations

Tag No	PSV (cm/s)	EDV (cm/s)	PSV /EDV	Vel-min (cm/s)	Vel-mean (cm/s)	PG.PSV (mmHg)	PG.EDV (mmHg)	PG.min (mmHg)	PG.mean (mmHg)	PI	RI	t (s)	VTI
1	12.9	5.4	2.4	5.4	10.9	0.0662	0.0115	0.0115	0.506	0.69	0.58	1.297	10.59
2	11.5	3.2	3.58	3.2	6.4	0.0532	0.0041	0.0041	0.2206	1.29	0.72	4.313	19.79
3	7.5	2.1	3.5	2.1	1.1	0.0225	0.0018	0.0018	0.0056	8.47	0.71	7.969	5.53
4	13.9	8.3	1.68	8.3	11.8	0.0777	0.0276	0.0276	0.058	0.48	0.4	7.969	7.32
5	10.5	1.3	7.8	8.6	2.1	0.0437	0.0007	0.0294	0.0095	9.17	0.87	7.969	35.78
6	13.9	2.7	5.2	2.7	8	0.0777	0.0029	0.0029	0.036	2.08	0.81	7.969	15.2
7	8.6	6.7	1.28	6.7	3.4	0.0294	0.018	0.018	0.0139	4.48	0.22	7.969	19.36
8	9.4	2.1	4.37	1.6	6	0.0352	0.0018	0.001	0.0163	1.3	0.77	7.969	26.59
9	2.2	0.3	8.5	0.4	1.1	0.002	0	0.0001	0.0006	2.43	0.88	0.406	0.87
10	14.2	2.9	4.82	2.7	11.5	0.0808	0.0035	0.0029	0.0578	1	0.79	7.969	54.24
11	10.2	5.6	1.81	5.6	2.8	0.415	0.0127	0.0127	0.0133	5.7	0.45	7.969	26.45
12	12.6	7.5	1.6	7.5	5	0.0635	0.0225	0.0225	0.0204	4.06	0.4	7.969	16.53
13	9.1	1.9	4.86	1.9	5.8	0.0332	0.0014	0.0014	0.0181	1.91	0.79	7.969	11.34
14	2.4	1.3	1.8	1.3	1.8	0.0023	0.0007	0.0007	0.0013	0.59	0.44	1	1.66
15	8	2.7	3.33	2.4	2.8	0.0259	0.0023	0.0023	0.0066	3.71	0.6	1	3.6

Appendix 4b.Right testicular Doppler evaluations

Tag No	PSV (cm/s)	EDV (cm/s)	PSV /EDV	Vel-min (cm/s)	Vel-mean (cm/s)	PG.PSV (mmHg)	PG.EDV (mmHg)	PG.min (mmHg)	PG.mean (mmHg)	PI	RI	t (s)	VTI
1	3.7	2.9	4.64	2.4	8	0.0748	0.0035	0.023	0.0362	2	0.78	1.297	11.2
2	7.5	3.2	2.33	2.9	4	0.0225	0.0041	0.0035	0.0077	1.15	0.57	6.594	12.84
3	13.1	7.8	1.69	9.9	0.3	0.069	0.0242	0.0394	0.0129	79.75	0.41	7.969	6.08
4	12.9	7	1.85	7	4.5	0.0662	0.0194	0.0194	0.0233	4.44	0.48	7.969	34.23
5	9.5	2.1	4.37	1.6	5.1	0.0352	0.0018	0.001	0.0128	2.14	0.77	7.969	32.84
6	13.7	2.9	4.64	2.7	8.5	0.0748	0.0035	0.0029	0.0345	1.3	0.78	7.969	62.09
7	7.8	4.6	1.71	4.3	4.9	0.0242	0.0083	0.0074	0.0104	0.71	0.415	7.969	41.79
8	12.9	0.5	24	0.3	6.3	0.0662	0.0001	0	0.0222	2	0.96	7.969	16.53
9	1.8	1.1	1.75	1.1	0.3	0.0014	0.0004	0.0004	0.0005	9.84	0.43	1.906	0.03
10	13.9	8.3	1.68	2.9	4.3	0.0777	0.0276	0.0035	0.017	3.93	0.4	7.969	80.08
11	9.1	1.6	5.67	1.6	4.7	0.332	0.001	0.001	0.0133	2.3	0.82	7.969	7.961
12	5.1	3.6	1.44	1.1	3.8	0.0105	0.0051	0.0005	0.0061	1.65	0.31	7.969	31.03
13	10.5	0.8	13	8	2	0.0437	0.0003	0.0259	0.0095	1.39	0.92	7.969	31.28
14	2.9	2	1.27	1.6	2.1	0.0025	0.0016	0.001	0.0018	0.44	0.21	1	1.99
15	10.5	4	2.6	4	4.3	0.0437	0.0065	0.0065	0.0138	3.37	0.62	1	1.72

Appendix 5a. Semen analysis 1

Tag No	Volume (ml)	Colour	Motility (%)	PH	Concentration (x106)	Reaction time (s)	Life (%)	Dead (%)	Normal count (%)	Free head (%)	Free tail (%)	Coiled tail (%)	Bent tail (%)	Midpiece droplet (%)
1	0.6	creamy	95	7	284	29	95	5	85	2	2	7	4	0
2	0.7	creamy	55	9	106	50	65	35	76	9	2	7	6	0
3	0.7	creamy	85	7	24	39	85	15	89	6	1	1	3	0
4	0.8	creamy	95	7	274	15	95	5	93	1	0	0	6	0
5	0.8	creamy	95	7	236	26	95	5	90	5	0	0	5	0
6	0.3	milky	75	9	99	30	75	25	79	7	0	8	6	0
7	0.3	creamy	80	9	123	31	95	5	88	4	3	1	4	0
8	0.5	creamy	95	7	261	95	95	5	75	12	9	1	4	0
9	0.6	milky	70	9	139	48	80	20	89	3	0	3	5	0
10	0.2	milky	35	7	106	35	90	10	93	2	1	0	4	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0.3	creamy	80	9	193	29	80	20	93	3	0	2	2	0
13	0.2	Watery	45	9	62	19	45	55	98	1	0	0	1	0
14	0.3	creamy	35	7	59	16	30	70	24	34	38	0	4	0
15	0.7	creamy	95	7	256	33	95	5	88	2	2	4	4	0

Appendix 5b.Semen analysis 2

Tag No	Volume (ml)	Colour	Motility (%)	PH	Concentration (x106)	Reaction time (s)	Life (%)	Dead (%)	Normal count (%)	Free head (%)	Free tail (%)	Coiled tail (%)	Bent tail (%)	Midpiece droplet (%)
1	0.4	Milky	95	9	132	9	95	5	84	4	0	2	10	0
2	0.1	Milky	65	7	140	15	65	35	72	11	2	6	9	0
3	0.2	Creamy	90	7	202	13	90	10	83	6	4	3	4	0
4	1	creamy	95	7	256	9	60	40	74	7	6	6	7	0
5	1	creamy	95	7	296	9	95	5	73	6	7	5	7	0
6	0.5	milky	95	7	70	9	85	15	67	4	3	9	15	0
7	0.9	milky	20	7	150	9	40	60	88	5	4	1	2	0
8	1	creamy	95	7	298	35	95	5	70	14	9	4	3	0
9	0.6	creamy	90	6	268	16	90	10	77	3	6	6	8	0
10	0.2	milky	95	7	241	1	95	5	79	11	2	8	0	0
11	0.2	milky	95	7	162	50	95	5	79	11	2	8	0	0
12	0.3	milky	80	9	59	49	85	15	94	4	0	0	2	0
13	0.2	watery	95	6	88	35	95	5	94	4	0	1	1	0
14	0.4	creamy	90	6	89	25	90	10	83	8	3	2	4	0
15	0.5	creamy	90	7	172	23	90	10	88	3	3	2	4	0

Appendix 6a.Concentration of Testosterone (mIU/ml) in the Yankasa rams from 6:00 am to 11:00 am

Animal I.D	6:00	7:00	8:00	9:00	10:00	11:00
1	1.055	1.172	1.743	1.903	1.92	1.739
2	0.319	0.391	0.434	0.338	0.199	0.223
3	1.563	1.668	1.683	0.48	0.784	1.252
4	0.475	1.518	1.366	2.75	0.634	0.812
5	1.699	1.587	1.567	1.443	0.422	0.553
6	1.927	1.904	2.057	1.971	2.094	2.143
7	1.352	1.22	1.334	1.56	0.918	0.463
8	0.763	0.888	0.944	1.15	1.006	1.131
9	1.825	1.567	1.681	1.628	1.505	1.627
10	0.937	1.003	0.313	0.378	0.518	0.867
11	0.473	0.602	0.292	0.309	0.246	0.355
12	0.294	0.462	0.605	0.76	0.288	0.347
13	1.252	1.262	1.23	1.215	0.576	0.345
14	1.12	1.239	1.285	1.201	1.15	1.23
15	1.5	1.3	1.5	0.9	1.2	1.64

Appendix 6b.Concentration of FSH (mIU/ml) in the Yankasa rams from 6:00 am to 11:00 am

Animal I.D	6:00	7:00	8:00	9:00	10:00	11:00
1	1.243	1.07	1.34	1.206	1.279	1.206
2	1.144	1.221	1.43	1.082	1.245	1.134
3	1.388	1.137	1.149	1.218	1.318	1.238
4	1.471	1.168	1.233	1.211	1.214	1.257
5	1.144	1.139	1.173	1.228	1.301	1.422
6	1.146	1.434	1.173	1.146	1.223	1.313
7	4.948	1.129	4.609	1.173	1.185	1.247
8	1.084	1.115	1.173	1.216	1.11	1.262
9	1.274	1.105	1.272	1.072	1.132	1.093
10	1.103	1.141	1.257	1.07	1.214	1.105
11	1.214	1.223	1.279	1.228	1.209	1.067
12	1.206	1.115	1.264	1.132	1.262	1.132
13	1.199	1.146	1.221	1.33	1.199	1.177
14	1.17	1.11	1.122	1.146	1.173	1.091
15	1.2	1.13	1.24	1.17	1.32	1.17

Appendix 6c.Concentration of LH (mIU/ml) in the Yankasa rams from 6:00 am to 11:00 am

Animal I.D	6:00	7:00	8:00	9:00	10:00	11:00
1	0.72	0.694	0.786	0.999	0.841	0.788
2	0.678	0.69	0.657	0.655	0.841	0.754
3	2.74	5.078	5.988	5.976	6.054	0.663
4	0.925	0.762	0.801	0.826	0.846	0.797
5	0.655	0.647	0.9	0.707	0.701	0.694
6	0.804	0.786	0.801	0.756	0.819	0.773
7	0.756	0.735	0.726	0.69	0.713	0.669
8	0.728	0.703	0.726	0.649	0.701	0.637
9	0.663	0.694	0.659	0.735	0.715	0.795
10	0.688	0.667	0.713	0.686	0.709	0.819
11	1.725	1.686	1.717	1.823	1.795	1.789
12	0.884	0.95	0.861	0.911	0.969	1.192
13	0.699	0.692	0.705	0.732	0.804	0.715
14	0.701	0.709	0.775	0.684	0.705	0.855
15	0.874	0.985	0.961	0.921	0.969	1.292

Appendix 7a Mean ± SEM (range) of testicular biometry of Yankasa ram using ultrasound (n=15)

	Length (mm)	Breadth (mm)	Height (mm)	Volume (mm^3)
Left	130.4 ± 2.73	42.15 ± 2.53	52.25 ± 2.10	280340 ± 12780
	(111-149.6)	(31.9-62.5)	(34.6-65)	(198264-354868)
Right	125.5 ± 2.57	44.85 ± 2.14	53.02 ± 2.20	296432 ± 17549
	(101.5-140.2)	(33.4-61.4)	(31.9-64.4)	(197754-424357)

Appendix 7b Mean ± SEM (range) of testicular Doppler parameters of Yankasa rams using ultrasound (n=15)

	PSV (cm/s)	EDV (cm/s)	PSV/EDV	Vel-min (cm/s)	Vel-mean (cm/s)	PG.PSV (mmHg)	PG.EDV (mmHg)	PG.min (mmHg)	PG.mean (mmHg)	PI	RI	t (s)	VTI
Left	9.80 ± 0.97 (2.2-14.2)	3.5 ± 0.04 (0.3-8.3)	3.77 ± 0.51 (1.28-8.5)	4.03 ± 0.70 (0.4-8.6)	5.37 ± 0.96 (1.1-11.8)	0.069 ± 0.026 (0.002-0.42)	0.0074 ± 0.0023 (0.0-0.028)	0.0093 ± 0.0027 (0.0001-0.0294)	0.066 ± 0.034 (0.0006-0.51)	3.16 ± 0.72 (0.48-9.17)	0.63 ± 0.053 (0.22-0.88)	5.85 ± 0.83 (0.41-7.91)	16.99 ± 3.72 (0.87-54.24)
Right	8.99 ± 1.06 (1.8-13.9)	3.4 ± 0.69 (0.5-8.3)	4.84 ± 1.57 (1.27-24)	3.43 ± 0.72 (0.3-9.9)	4.21 ± 0.61 (0.3-8.5)	0.063 ± 0.020 (0.0014-0.332)	0.0072 ± 0.0023 (0.0001-0.028)	0.009 ± 0.0031 (0.0-0.039)	0.015 ± 0.0027 (0.0005-0.036)	7.76 ± 5.18 (0.44-7.975)	0.59 ± 0.06 (0.21-0.96)	6.10 ± 0.78 (1.0-7.97)	24.78 ± 6.04 (0.03-80.08)

PSV (peak systolic volume), EDV (end diastolic volume), PSV/EDV (ratio of peak systolic and end diastolic volume), VELmin (velocity minimum), VELmean (velocity mean), PGPSV (pressure gradient for peak systolic volume), GEDV (pressure gradient for end diastolic volume), PGmin (pressure gradient minimum), PGmean (pressure gradient mean), PI (pulsatility index), RI (resistivity index), Time (time), VTI (velocity time integra).

Appendix 7c Mean ± SEM (range) of the epididymal biometry of Yankasa ram using ultrasound (n=15)

	Head				Tail			
	LA (mm	SA (mm)	A (cm^2)	V (cm^3)	LA (mm	SA (mm)	A (cm^2)	V (cm^3)
Left	38.67 ± 1.47	17.52 ± 0.82	5.36 ± 0.35	6.48 ± 0.62	27.64 ± 1.53	13.85 ± 0.74	3.00 ± 0.2	2.83 ± 0.30
	(29.8-47.8)	(9.7-21.8)	(2.28-7.17)	(1.48-10.42)	(18.1-42.7)	(10.2-19.1)	(1.44-4.14)	(0.98-5.27)
Right	35.15 ± 1.63	15.86 ± 0.88	4.49 ± 0.42	5.05 ± 0.73	25.63 ± 1.91	14.36 ± 1.26	2.87 ± 0.27	3.01 ± 0.50
	(23.7-50.1)	(9.2-22.1)	(1.71-8.72)	(1.05- 12.87)	(9.5-36)	(7.6-23.2)	(1.31-4.62)	(0.83-7.16)

Appendix 7d Mean ± SEM (range) of testicular biometry using vernier caliper (n=15)

	TL (cm)	TC (cm)	TV (cm^3)
Left	15.7 ± 0.55	26.83 ± 0.51	1482 ± 64.37
	(12-19)	(24-31)	(904.8-1847)
Right	15.9 ± 0.61	26.83 ± 0.51	1503 ± 74.27
	(12-20)	(24-31)	(904.8-2053)

TL (testicular length), TC (testicular circumference), TV (testicular volume)