EFFECTS OF EXPERIMENTAL *Fasciola gigantica* INFECTION ON SEMEN CHARACTERISTICS, TESTOSTERONE PROFILES AND SOME HAEMATOLOGICAL PARAMETERS IN YANKASA RAMS

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DECLARATION

I declare that the work in this thesis titled **''Effects of Experimental *Fasciola gigantica* Infection on Semen Characteristics, Testosterone Profiles and Haematological Parameters in Yankasa Rams,''** hasbeen performed by me in the Departmentof Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria, under the supervision of Dr. J. S. Rwuaan, Dr. I. U. Ate and Dr. N. P. Chiezey.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been previously presented for another degree or diploma at any institution.

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CERTIFICATION

This thesis titled **''EFFECTS OF EXPERIMENTAL *Fasciola gigantica* INFECTION ON SEMEN CHARACTERISTICS, TESTOSTERONE PROFILES AND SOME HAEMATOLOGICAL PARAMETERS IN YANKASA RAMS''** byDaudaILIYASU meets the regulations governing the award of Master of Science (M.Sc.) of Ahmadu Bello University Zaria, and is approved for the contribution of knowledge and development of sciences.

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DEDICATION

This thesis is dedicated to humanity, development of science, and my lovely family.

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ABSTRACT

This study was designed to determine the effects of experimental Fasciola infection on semen characteristics, testosterone profiles and haematological parameters of Yankasa rams. Twelve apparently healthy Yankasa rams aged 18-24 months were randomly divided into two groups of seven and five as infected and control groups (A and B) respectively. The rams were kept under intensive management and their base-line data on vital parameters, testosterone profiles, haematological parameters and semen characteristics were taken bi-weekly, for four weeks prior to experimental infection. Metacercariae were isolatedby crushing naturally infected*Lymnaeanatalensis*. Group A rams were orally inoculated with 800 metacercariae. From 2 to 12 weeks of infection, semen samples were collected once a week between 08:00 and 10:00 hours in both groups and evaluated for colour, volume, motility, pH, morphology, concentration and sperm viability. Five ml of blood was collected bi-weekly from the jugular vein, from week 2 to week 12 of infection for evaluation of haematological parameters as well as determination of testosterone levels. There was progressive decrease in PCV, haemoglobin concentration and total protein. Testosterone concentration as determined by competitive Enzyme Linked Immunosorbent Assay (cELISA) was higher by 8:00am in both groups and gradually became lower by 9:00am. The live weight, rectal temperature and scrotal circumference were measured twice a week. The live weight in Group A started decreasing at week 2 Post Infection (PI), but the weight in Group B rams progressively increased. The temperature was significantly ($p<0.05$) higher in Group A at week 9 PI, (40.1 ± 0.1^0C) compared to Group B, (38.7 ± 0.1^0C). The scrotal circumference of group A rams was significantly lower 1(9.3±0.7 cm) at week 11 PI, than those of group B rams (25.8±0.6 cm) at the same week. At the end of the experiment 2 rams from both groups were sacrificed, and their testes harvested for the determination of the gonadal and epididymal sperm reserves. The gonadal sperm reserves wassignificantly ($p>0.05$) lowerin group A(136.0 ± 70.0 $x10^6$/gm) compared to group B rams (377.0 ± 0.0 $x10^6$/gm). The epididymal sperm reserveswas significantly ($p>0.05$) lower in group A ($55.4\pm43x10^6$/gm) compared to group B rams (284.7 ± 0.0 $x10^6$/gm). From this study, it was concluded *Fasciolagigantica* infection caused loss of body condition, testicular damage and have deleterious effects on semen characteristics, haematological parameters and testosterone concentration in Yankasa rams. Further work is required to investigate if the low sperm output caused by infection is reversible by use of chemotherapy. The use of diagnostic aids like ultra sound should be employed to determine the extent of damage in the liver and the testes in Yankasa rams.

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ABBREVIATIONS

AI- Artificial Insemination

ADG- Average daily gain

BW- Body weight

BT- Bent tail

cm - Centimetre

CP- Crude protein

CT- Coiled tail

DH- Detached head

^{0}C- Degree celsius

E&N- Eosin and Nigrosin

EPG-Eggs per gram

ELISA- Enzyme-linked immunosorbent assay

EDTA-Ethylenediaminetetraacetic acid

ET- Ejaculation time

F-*Fasciola*

FAO- Food and Agriculture Organisation

FSH- Follicle stimulating hormone

FT- Flat tail

g- gramme

GDP- Gross domestic product

GH- Growth Hormone

GnRH- Gonadotropin Releasing Hormone

HPT- hypothalamic-pituitary-testicular

H&E stain- Hematoxylin and eosin stain

hrs- Hours

INF- Infection

Kg- kilogramme

L- litre

LH- Luteinizing Hormone

ml- millilitre

mg- milligram

ng- Nanogram

PI- Post Infection

RBC- Red blood cell

SC- Scrotal Circumference

SEM- Standard error of mean

SHBP- Sex Hormone Binding Protein

TP- Total Protein

WAD- West African dwarf

WBC- White blood cell

WHO- World Health Organization

µml- Micro millilitre

CHAPTERONE

INTRODUCTION

1.1 Background of the Study

Reproduction is one of the most important factors in livestock production (Radostits *et al*., 1995; Amann *et al*., 1998) and its success greatly depends on an interaction of factors that include genetic merit, physical environment, nutrition, diseases and management (Amann *et al*., 1998). In developing countries, nutrition and diseases are known to cause low productivity in livestock (Rasbech, 1984). Apart from infectious diseases like brucellosis, listeriosis and campylobacteriosis, some parasitic diseases like helminthosis particularly fasciolosis and haemonchosis are known to affect reproduction in animals (Phiri, *et al.*, 2007). The role of trypanosomiasis in alteration of reproductive function in cattle, sheep and goats, has been extensively reported and reviewed (Anosa and Isoun, 1983; Ogwu, 1984; Sekoni, 1991; Sekoni *et al.*, 2004). Fasciolosis is another parasitic disease that has received increased attention as a cause of reproductive dysfunction in animals (Ahmed *et al*., 2007).

In tropical countries, fasciolosis is considered the single most important helminth infection of livestock, with reported prevalence of 30-90% (Spithill *et al*., 1999; Phiri, *et al.*, 2005). The economic losses due to fasciolosis in many parts of Africa has been determined mainly from liver condemnations at slaughter (Mebrahtu and Beka, 2013). However, indirecteconomic losses may also result from decreased livestock productivity (Ahamed *et al*., 2006). Studies on female animals showed that fasciolosis caused delayed onset of puberty (Mebrahtu and Beka, 2013; Lopez-Diaz *et al*., 1998), reduced conception and pregnancy rates (Cawdery, *et al*., 1976; Dargie, 1987; Ahamed *et al*., 2006; Ahmed *et al*., 2007), and reduced multiple birth rates (Elrod *et al*., 1993). These investigators speculated that these effects may be a general consequence of reduced well-being and retarded growth due to the parasitic activity, which somehow disrupt hormonal balance (Fleming and Fettere, 1986).

The two most important species of liver fluke which are *Fasciola hepatica* found in temperate and in cooler areas of high altitude in the tropics and subtropics and *Fasciola gigantica*, which predominates in tropical areas. Both species coexist where ecology is conducive for the snail hosts (Yilma and Mesfin, 2000).

Fasciolosis occurs in two phases (Mebrahtu and Beka, 2013). The parenchymal phase during which there is traumatic hepatitis occurs during migration of young flukes through the liver parenchyma (Kassuku *et al*., 1986; Wamae, *et al*., 2007).The biliary phase coincides with the residence of flukes in the bile ducts and is characterized by hyperplasia of the bile duct epithelium that eventually leads to cholangitis and biliary obstruction (Kassuku *et al*., 1986; Wamae, *et al*., 2007).

Liver plays a vital role in testicular function, hence fasciolosis may have effects in male animals especially in fluke endemic areas like Nigeria (Ahamed, *et al*., 2006) where the increased use of irrigation for agriculture presents a conducive breeding ground for *Lymnea natalensis* the intermediate host of *Fasciola gigantica*(Max *et al*., 2006; Walker *et al*., 2008).

1.2 Statement of Research Problem

Fasciolosis is a major cause of morbidity and mortality in all classes of livestock in developed and developing countries (Tekelye *et al*., 1992a). Sheep have been reported to be more susceptible than cattle (Walker *et al*., 2008) especially under transhumance system of husbandry, where flock of sheep follow cattle and so migrate along with them during the dry season periods in search of food and water (Kumar *et al*., 2008). The unavailability of rich upland pasture during the prolonged dry season in the Northern parts of Nigeria often force herdsmen to graze their animals along river banks, most of which are infested with metacercariae from the snail intermediate hosts of *Fasciola*species (Yilma and Mesfin, 2000). Infections are also acquired in temporarily flooded areas as well as small rivers, streams, riverbanks and constructed dam sites (Kumar *et al*., 2008).

Although few published studies have investigated fasciolosis in male, mounting evidence suggest that liver flukes also have similar reproductive effects. A study on peripuberal rams suggested that fasciolosis impairs hepatic catabolism of androgen (Fleming and Fettere, 1986). Similar results were described in a study using a rat model in which liver fluke infection caused a significant decrease in hepatic P-450–dependent metabolism of progesterone and testosterone (Birosauveur *et al*., 1994). Other authors have reported infertility and sterility due to direct effects of fasciolosis on spermatogenesis and sperm survival due to pyrexia associated with the disease **(**Hoogenboezem and Swanepoel, 2000; Ahmad, *et al*., 2007;Marai *et al*., 2007**)**.

Acute and sub-acute disease is seen in sheep, and occasionally occurs under conditions of heavy challenge in young calves (Kumar *et al*., 2008). The chronic form of the disease is common in cattle but also important in sheep (Lopez-Diaz *et al*., 1998). Liver fibrosis is a common sequel to liver injury in fasciolosis and the liver is an important metabolic organ, synthesising many blood proteins, as well as metabolising cholesterol the precursor of testosterone and other steroid hormones (Kumar *et al*., 2008).Although in milder infections, clinical signs may or may not be readily observed, however, a decreased appetite and interference with post-absorptive metabolism of protein, carbohydrates and minerals, may have a significant effect on reproduction (Kumar *et al*., 2008). Interactions among these physiologic, nutritional, and other pathologies associated with fasciolosis may have consequences on reproduction (Suhardono, 2001).

Therefore, there is need to investigate fasciolosis as a syndrome that could affect reproduction not just as a disease confined to the liver. Hence this study was designed to evaluate the effects of experimental *F. gigantica* infection on semen characteristics, testosterone profiles and haematological parameters of Yankasa rams.

1.3 Justification

The Livestock industry contributes 9-10 % of the Gross Domestic Product (GDP) and is only 35.5 % of the protein intake of Nigerians (Ugwu, 2007). The FAO and WHO recommended 35 g of the estimated minimum protein requirement for an average Nigerian per day should be obtained from animal protein, but only 10 g is currently derived from animal resources (Ugwu, 2007). Therefore, there is enormous challenge to the Nigerian livestock farmers to increase the supply of animal protein. Sheep and goats are widely distributed in Nigeria in rural, urban and peri-urban areas representing about 63.7 % of total grazing domestic animals in Nigeria (Ahemen and Bitto, 2007). The Yankasa sheep is the most numerous and most widely distributed throughout the various ecological zones, particularly Guinea and Sudan Savannah vegetation belts of Nigeria. It is estimated to constitute 60 % of the total national flock (Afolayan, 1996). The advantage of small ruminants includes their small size, low capital requirement, smaller risk on investiment, reproductive efficiency and economic use of available land (Armour, 1975; Ahamed *et al*., 2006).

One of the factors meditating against the productivity and reproductive performance of sheep is helminthosis, particularly haemonchosis and fasciolosis (Armour, 1975; Troncy, 1989). The economic losses due to fasciolosis in Nigeria and other parts of the world are enormous (Ahamed *et al*., 2006).These losses are associated with mortality, morbidity, reduced growth rate, foetal loss, reduced conception rate and liver condemnation as well as increased susceptibility to secondary infections (Mamabolo, 1999). In Nigeria, a lot of information is available on the prevalence of fasciolosis based on abattoir studies (Ahamed *et al*., 2006). Prevalence rates of between 19.5 % and 41.7 % have been reported in ruminants from different parts of the country (Ahamed *et al*., 2006).

In Nigeria, effects of the infection on male reproduction has been little studied (Ahamed *et al*., 2006). Ahmed *et al*. (2007) reported poor sexual attraction in infected rams, as a result of anorexia and pyrexia associated with the infection.Loss of body condition is a primary factor for endocrine dysfunction with respect to reproduction, identification and elimination of the factors may reduce infertility in the rams, give more successful breeding and high lambing rates for the farmer (Wamae, *et al*., 2007).

1.4 Aim of the Study

The aim of the study was to evaluate the effect of *F.gigantica* infectinon reproduction and haematological parameters of Yankasa rams.

1.5 Objectives of the Study

1. To evaluate the effects of experimental *F. gigantica* infection on semen characteristics of Yankasa rams.

2. To determine the effects of experimental *F. gigantica* infection on haematological parameters and total protein levelsof Yankasa rams.

3. To evaluate testosterone profiles of Yankasa rams experimentally infected with *F. gigantica*.

1.6 Research Question

What is the effect of experimental *F. gigantica* infection on semen characteristics, testosterone profiles and haematological parameters in Yankasa rams?

CHAPTER TWO

LITERATURE REVIEW

2.1 The Male Reproductive System

The male reproductive system is composed of several distinct organs. These include

i) Two gonads, the testes which produce the male germ cells and male sex steroid hormones.

ii) A long complicated set of tubes and tubules (epididymis and duct deferens) which connect the testes with the pelvic urethra and so permit spermatozoa produced in the testes to be stored and conducted through the penis.

iii) The accessory sex glands (vesicular gland, prostate gland, ampulla and bulbourethral gland) which provide a fluid vehicle for nourishing and transporting spermatozoa through the tract, propelled by the reflex contraction of the smooth muscles of their wall during coitus.

iv) A copulatory organ, the penis by which semen is delivered into the vagina of the female(Barth *et al*., 2008).

2.2 Spermatogenesis

Spermatogenesis is a process in which spermatozoa are produce from male primordial germ cells by way of mitosis and meiosis (Barth *et al*., 2008). The initial cells in this pathway are called spermatogonia which differentiate into spermatocytes and spermatid following mitosis and meiosis respectively.

2.3 The Process of Spermatogenesis

Spermatogenesis can be divided into two distinct phases; the first is spermatocytogenesis, a series of divisions during which spermatogonia form spermatids. The second is spermiogenesis, a phase where spermatids undergo a metamorphosis, forming spermatozoa (Barth *et al.*, 2008). The entire process is completed in 46 to 49 days in rams (Harder *et al.*, 1995). As spermatogenesis proceeds, the developing gametes migrate from the basement membrane of the seminiferous tubules toward the lumen of the seminiferous tubules (Harder *et al*., 1995).

2.3.1 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 A_1 and A_2 spermatogonium, the A_2 spermatogonium will divide, forming a dormant (A_1) spermatogonium and an active (A_3) 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 secondaryspermatocytes; 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 (A_3) spermatogonium, in bulls and rams. Since A_1 spermatogonium divide by mitosis to from A_2 spermatogonium, the potential yield of spermatids is higher than is actually realized. Degeneration of spermatogonia during mitotic divisions accounts for this loss.

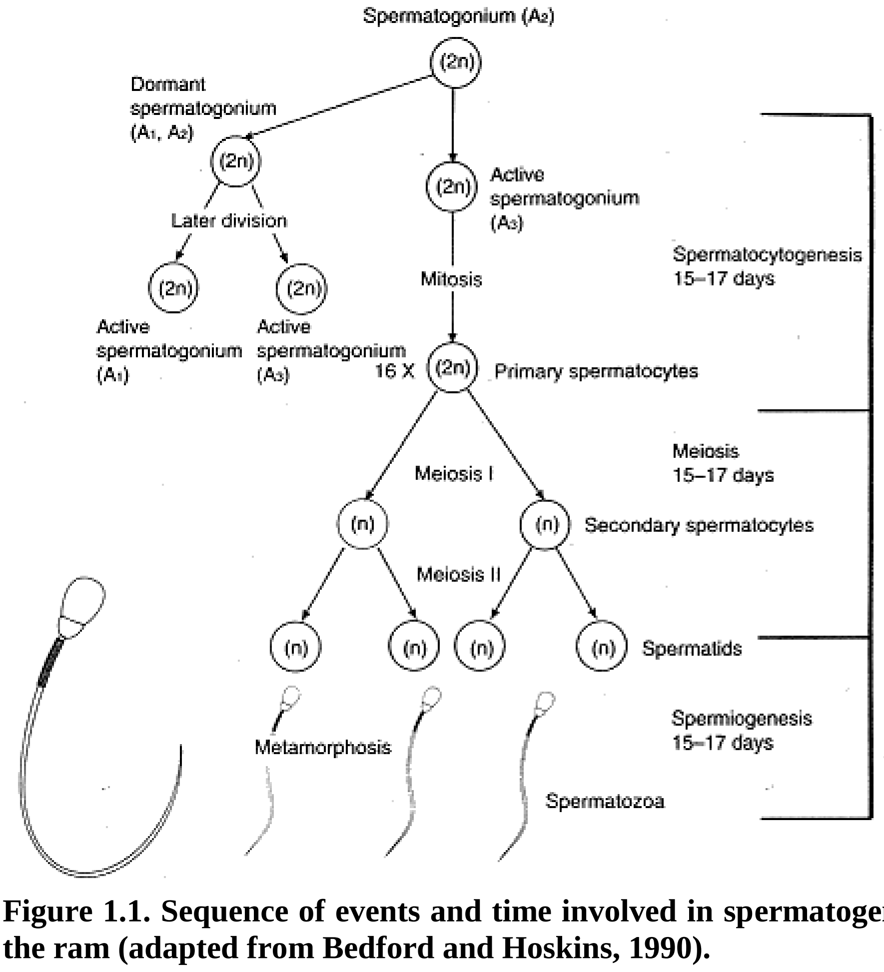


Figure 1.1. Sequence of events and time involved in spermatogenesis in the ram (adapted from Bedford and Hoskins, 1990).

Although A_0 spermatogonia (reserve stem cells) will occasionally divide, forming new A_0 and A_1 spermatogonia, formation of dormant spermatogonia from A_2 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).

2.3.2 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 spermatozoon, while the rest of the cell elongates, forming the tail (Rodriguez-Martinez, 2006). The acrosome, a cap around the head of the spermatozoon, will form from the Golgi apparatus of the spermatid (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 cytoplasmicdroplet will form on the neck of the spermatozoon. The mitochondria from the spermatid will form in a spiral around the upper one sixth of the tail, forming the mitochondrialsheath (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 rete testis (Bearden and Fuquay, 1997).

Transit of sperm through the efferent ductules and the epididymis 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), migration of cytoplasmic droplet from a proximal to distal mid-piece position (Rodriguez-Martinez, 2006). Sperm taken from the corpus and cauda epididymis already have the potential to fertilize (Harder *et al*., 1995; Bart *et al*., 2008).Sperm production and maturation are continuous processes, delicate in nature and stopped under certain limitation like thermal, nutritional and endocrine disorder. The sperm population in the epididymal reserves and in the ejaculate are heterogeneous in nature. Such heterogenecity can be reflected in differences in morphology even with the normal limits of accepted normality, viability and fertilizing capacity(Rodriguez-Martinez and Barth, 2007).

Although the mechanisms responsible for sperm maturation are not fully understood, it is generally believed that certain epididymal secretions, principally glycoprotein, coat the cell surfaces of immature sperm and thus cause them to undergo a functional change (Acott and Hoskins, 1989). The secretion of epididymal proteins is androgen-dependent, and any long-term disturbance in their synthesis or secretion can affect fertility (Voglmayr *et al*., 1985). In sheep, the entire cycle of spermatogenesis, from the first spermatogonial division to the release of sperm into the lumen of the seminiferous tubule, is completed in 49 days (Ortavant, 1990).

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; Bart *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 epididymis, 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).

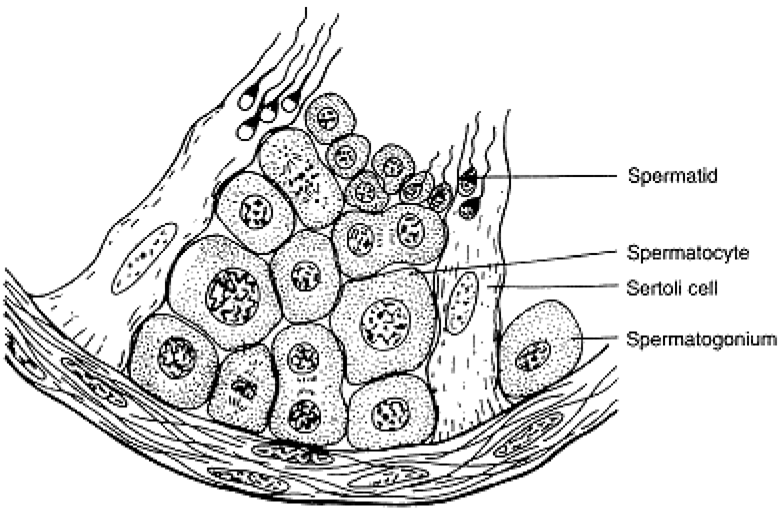


Figure 1.2. Small segment of an active seminiferous tubule showing the developmental stages that occur during spermatogenesis. The concentric layers of spermatogonia, spermatocytes, and spermatids progressing from the wall of the seminiferous tubules to the lumen. (Adapted from an original drawing by Brian Satchell, 1990).

2.4 Endocrine Control of Reproduction

The reproductive functions of animals are controlled by coordinated and efficient neuroendocrine system (Sekoni, 1991). The hormones involved in reproduction originate in 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).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 $PGF_2\alpha$ will stimulate the release of LH and testosterone (Wada*et al*., 2009). Therefore, $PGF_{2\alpha}$ 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 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 gonadotrophinare 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 were shown to partially maintain spermatogenesis provided the germinal epithelium had not completely atrophied (Wada*et al*., 2009; Gabor *et al*., 2011).

2.5 Diseases that Affect 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 (Anosa and Isoun, 1971; Sekoni *et al*., 1990). Severe epididymis degenerations and deterioration of semen characteristics and aspermatogenesis (Anosa and Isoun, 1984; Sekoni *et al*., 1990) and reduction in testosterone levels (Masake 1980, Wada*et al*., 2009).In female animals, ovaries of various species of animals were found to be affected, with a depletion in progesterone production in trypanosome-infected cows and ewes (Ogwu *et al*., 1984; Sekoni, 1991). Death has been reported in infected animals during pregnancy or after difficult parturition of weak or dead foetus (Ogwu *et al*., 1984; Bawa *et al*., 1991; Sekoni, 1990). In all cases, histology revealed degeneration of the pituitary gland and a consequent effect on gonadotropin and growth hormone production as well as detrimental effect on testicular function (Sekoni, 1991). 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).

2.6 Testosterone Production, Regulation and Function in Ruminants

Testosterone is a 19-carbon steroid hormone made from cholesterol. Testosterone circulates in blood bound to the sex hormone binding protein (SHBP), and in a smaller amount it is bound to other plasma proteins, for example albumin. SHBP is produced in the liver and plays an important role in regulating the amount of free testosterone in the body (Capell and Paulsen, 1972; Martins*et al*., 2006). Under normal conditions, 45 to 70 % of testosterone is bound to SHBP, 30 to 55 % to albumin (but this relationship is very weak) and approximately 2% circulates in the unbound form. The combination of free testosterone and albumin-bound testosterone is called a biologically free or bio available fraction (Martins*et al*., 2006).

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 convert 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 is no 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's sent back into the bloodstream, most of the testosterone binds to SHBG and albumin, becoming biologically inert leaving a small percentage that remains free and unbound that 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). Decreased levels of these hormones can also result in a reduced production of endogenous testosterone, because of the feedback mechanisms, which at high levels of testosterone provides for a reduction in the concentration of LH and also GnRH, and hence the amount of testosterone (Kletter *et al*., 1992).

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).The results of scientific research into testosterone show 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).

2.7 Metabolism of Testosterone Half-life in Blood

Testosterone is the most important androgenic-anabolic steroid (Van Eenoo and Delbeeke, 2006).About 4-10 mgof 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 sheep this oxidation mainly takes place in adipose tissue and in the testes. In ewe 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. There reductases and dehydrogenases catalyse the reactions of the D^4-double bond, the C3-carbonyl group and the C17-hydroxyl group. Finally the hydroxyl groups are connected to glucuronic acid or sulphate, followed by excretion via urine (Van Eenoo and Delbeeke, 2006).

2.8 Semen Evaluation

The American Society of Theriogenology has published guidelines for the examination of male animals for breeding soundness (Mickelsen *et al*., 1991). Evaluation of semen is one of the important part of successful reproduction.

2. 9 Semen Characteristics of Normal Ram

The characteristics of normal ovine semen are well documented by Zemjanis (1970), Roberts (1971), and Salisbury *et al*.(1976). 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 artificial vagina (Salisbury *et al*., 1976). Ejaculate volume may be influenced by duration of stimulation when semen collection is done by electro ejaculator 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 *Actinobacillusseminis*infection Zemjanis (1970).

2.9.1 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 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).

2.9.2 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 cause discolouration of the semen (Brito *et al*., 2002).

2.9.3 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. Wave motion depends on high sperm density and is therefore best in samples from bucks, bulls and rams which normally have high sperm concentrations; and decreases as sperm concentration decreases (Brito *et al*., 2002).

2.9.4 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). The percentage of abnormal spermatozoa and their types are recorded after observing 600 cells. 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 includes 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 epididymis 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 epididymis, the body of the epididymis, and into the tail of the epididymis, which serves primarily as a storage site until ejaculation. Failure of maturation and abnormal epididymal function may result to secondary defects (Acott and Hoskins, 1981).Secondary defects include tailless heads protoplasmic droplets on the mid piece and bent or broken tails or heads (Amann, 1998).

Tertiary abnormalities develop *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 artefact produced by smearing the semen (tertiary). High proportion of cells with distal droplet can be due to epididymal malfunction but may be caused by a lack of adequate seminal fluid which is one of the product of seminal vesicle that enhances cytoplasmic droplet release (Verstegen*et al*., 2002).

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).

Another classification system reported by Verstegen*et al*. (2002), labels defects as majoror minoraccording 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 (Amann, 1998; Sekoni *et al*., 1991).

2.9.5 Other cells in semen

It is documented that abnormal semen might have epithelial cells with few leukocytes, erythrocytes, but no bacteria or fungi except an infection is present. Cells from the germinal layers of the testis are an unusual finding in normal semen but their presence indicate severe testicular damage. Such cells would include spermatids, spermatocytes and large ciliated cells. These are classified as immature sperm cells (Amann, 1998; Sekoni *et al*., 1991).

2.10 Testicular Measurements

2.10.1 Scrotal circumference

Scrotal circumferencemeasurementis recommended in breeding soundness examination in rams for good semen motility.Some investigators have recommended that the best parameters for the evaluation of breeding soundness under field conditions should include spermatozoa morphology, semen motility and scrotal circumference (Okwun *et* *al*., 2006). A high correlation exists between testicular size and sperm output; consequently testicular size has influence on the number of sperm which can be harvested per unit of time ejaculated frequently (Foote *et al,* 1970).Bulls ejaculated 4x per week, about 55 million sperm can be collected per gram of testes per week. Thus, from a bull with paired testes weighing 500 g about 27.5 billion sperm per week could be expected. Each 100 g increase in size should result in 5.5 billion more sperm per week, a situation of particular value in artificial breeding (Hafez and Hafez, 2000; Ogunlade *et al*., 2006).

2.11 Testicular Pathology

This has significant negative impact on a livestock fertility causing disruption in steroid hormone production, spermatogenesis and libido (Voglmayr *et al*., 1985). Testicular pathology depends on severity,level of degeneration and trauma (Hafez and Hafez, 2000). Diagnosis of the different testicular disorders is based on palpation, ultrasonography examination, semen evaluation and in some cases, biopsy and histology (Hafez and Hafez, 2000).

Testiculardegenerationis a major cause of infertility in ruminants (Okwun *etal*., 2006). It is an acquired condition initiated by a variety of inciting causes varying only in degree of severity (Hafez and Hafez, 2000). Testicular degeneration may have multiple causes like thermal injury, vascular pathologies, trauma, systemic or local infections, malnutrition, dietary deficiencies, toxins, x-ray exposure, medications, testicular neoplasia, age-related degeneration or idiopathic (Ogunlade *et al*., 2006). These various factors may elicit the same temporary or permanent response in the testis, degenerating germ cells become more common, multinucleate giant germ cells form by coalescence of spermatocytes or spermatids, the ratio of germ cells to Sertoli cells is reduced, and spermatozoa production is adversely affected (Johnson *et al*., 1994). Testicular degeneration may be unilateral or bilateral and can be transient or permanent (Robaire and Viger, 1995). Diagnosis of testicular degeneration is based on physical and ultrasonography examination of the testicles, semen and hormone analysis (Robaire and Viger, 1995). In testicular degeneration the typical changes in semen include low sperm concentration and a high percentage of morphological defects, specifically a high number of immature germ cells, head defects and mid piece defects (Johnson *et al*., 1994).

Histologically there may be vacuolization of the seminiferous epithelium and loss of normal cellular arrangements in the seminiferous tubules (Robaire and Viger, 1995). Owing to a special survival mechanism, the testis has an amazing ability to completely recover spermatogenic capability sometime after the insult (Johnson *et al*., 1994). Elevated testicular temperature and endocrine disruption are probably the most common causes of mild testicular degeneration resulting in the production of abnormal sperm in the absence of any other clinical signs (Brito, 2002). Causes of increased testicular temperature include high ambient temperature, fever, intensive exercise, scrotal edema or dermatitis, hydrocele, tunic adhesions, and hernias. Treatment of testicular degeneration is usually unrewarding (Hafez and Hafez, 2000). Inciting causes that contribute to the condition should be diagnosed and treated appropriately (Robaire and Viger, 1995).

2.12 Factors that Affect Ram Fertility

Sheep are important source of meat in the tropics (Bielli, *et al*., 2001). Growth performance and body weight (BW) changes are crucial to attaining the required market BW with good carcass composition (Bielli, *et al*., 1999). Similarly, growth in testicular size is an important indicator of the breed potential of male animals there BW is closely associated with testicular mass (Nsoso *et al*., 2004). The pattern of the growth and sperm production capacity in rams and other domestic male animals, are influenced by factors such as breed, age, nutrition, season and health status (Rekwot *et al*., 1987b; Bielli, *et al*., 1999). Sperm production in male is highly sensitive to fluctuation in the environment particularly hormones, temperature and dietary deficiencies (Bearden and Fuquay, 1997; Jibril *et al*., 2010).

2.12.1 Nutrition

Nutrition is a major environmental cue that influences growth and sperm production in domestic animals (Salisbury *et al*., 1978). In a tropical environment, where the influence of photoperiod is minimal, nutrition appears to be the major modulator of sexual activity in small ruminants (Bielli, 2001). Generally, ruminant animals require nutrients for maintenance of their body metabolism, followed by growth, production and reproduction. Supplying nutrients as required is thus indispensable for optimum livestock performance. Improved quality of feed, protein content results in improved body weight, testicular size, semen characteristic and subsequently fertility (Abi-Saab *et al*., 1997). Adequate protein intake with good management had been reported to accelerate attainment of puberty (Rekwot *et al*., 1987a). Increasing the energy intake has also been reported to influence testicular growth and sperm production (Murray *et* *al*., 1990). Increased feeding level lead to heavier testicular weight (Bielli, 1999), indicating a link between better nutrition and higher sperm-producing testicular parenchyma of the animals. Higher quality semen is usually collected in the rainy rather than in the dry season in the tropics, directly indicating the positive influence of nutrition, as rainy season feeds have more nutrients than dry season feeds (Jibril *et al*., 2010). Bielli*et al*.(2001) demonstrated that improved protein supply in the form of rumen un-degradable protein increased average daily gain (ADG) of the body, scrotal circumference, testicular volume and spermatogenesis. It has also been demonstrated that feeding with sown pasture and grain increases the number of elongated spermatid per Sertoli cell (Bielli*et al*., 2001). Similarly, improved nutrition maintains and increases the secretion of gonadotropins and thereby enhances the proportions of morphologically normal spermatozoa (Johnson*et al*., 1994). Nsahlai *et al*. (2000) demonstrated that protein under-nutrition severely affects sperm motility and morphology. It is therefore essential to supply the required nutrient for the optimum testicular function, spermatogenesis and normal sperm maturation in the epididymis.

In rams, testis size which is highly correlated with spermatogenic activity, responds to nutrition, and sperm production can be increased by given high-protein supplement for about 8 weeks before mating starts (Abi-saab *et al*., 1997). Fatty acids and minerals are necessary for the production of the fluid medium produced by the cellular environment of the testes, which is necessary for sperm transport. Reports also indicate that vitamin A is important for the survival of sperms in the male genital tract and the sperm integrity (Abi-saab *et al*., 1997). Alabi (2005) reported an increase in abnormal sperm content after short-term deficiency of vitamin A in bulls and rams. Vitamin A is transported through epididymal segments and increases sperm maturation and survival rates (Al- Haboby*et al*., 1999). Reduction in vitamin A concentration at the level of the epithelium of the caput epididymis lowers the synthesis of certain proteins (transferrin and clusterin) required for sperm maturation. The increased sperm abnormalities particularly head abnormalities may be due to the deterioration of sperm DNA transcription. Amann *et al*. (1998) reported that epididymal secretions play a vital role in protecting the sperm plasma membrane, DNA and chromatin and increase the efficiency of energy utilization. Fasciolosis is known to compromise metabolic activities and glycolysis, which has direct effects on energy supply for optimum reproductive performance (Kumar *et al*., 2008).

Improved nutrition has been reported to have effect only on ejaculation latency and the percentage of sexually active males while severe and prolonged specific deficiencies, such as vitamin A, can cause spermatogenic failure Zarazaga *et al*., (2009). Deficiencies of trace minerals or excesses of substances such as cadmium also can reduce or block sperm output (Zarazaga *et al*., 2009).

2.12.2 Age

Difference in physiological status due to age influences body size and testicular growth in domestic animals (Karagiannidis *et al*., 2000). Under normal conditions animals grow fast when young, but grow slowly when they reach maturity. Testicular development is also rapid at an early age, which is followed by a period of slow growth in goat kids (Nsoso*et al*., 2004). Brito *et al*. (2002) demonstrated that scrotal circumference increases rapidly in young bulls, but only gradually in mature bulls, and even decreases in old bulls. In bucks the increase in scrotal circumference is curvilinearly related to age (Bongso*et al*., 1982). Sekoni *et al*. (2008) reported that testosterone concentration, scrotal circumference, semen volume, sperm concentration and sperm output of Bunaji and N'Dama bulls increased significantly with age. However, comparing the growth of male animals in terms of live-weight and testicular mass, growth of the latter is sluggish especially at higher ages (Brito *et al*., 2002). Similarly, Belibasaki and Kouimitzis (2000), reported that growth in testicular weight as a percentage of live-weight was higher before 12 months of age, but it decreased by 24 months, in Tokara Japanese goat. Age also influences sperm morphology in domestic male animals. Early studies have indicated that sperm head abnormality was higher at younger age but gradually decreased after sexual maturity and became higher again at old age (Dowsett and Knott, 1996; Amann *et al*., 2000). This is apparently due to immature spermatogenesis at younger age, a slight propensity to testicular degeneration with age and slight disturbances in epididymal function at older ages (Dowsett and knott, 1996). Rekwot *et al*. (1988) reported that live-weight, scrotal size and sperm production increased significantly with age. All this suggests that domestic male animals should be used for breeding or semen collection in an age range within which growth performance and spermiogram attributes are optimal.

2.12.3 Season

It is well documented that season has influence on reproductive performance of domestic animals. Seasonality influences are reflected by changes detectable in behaviour, testicular dimensions (mass and volume), gametogenesis and hormone secretion (Blench *et al*., 2006), which approximately follows the breeding activity of the ewes within a given breed (Delgadillo *et al*., 2004). However, these changes are more common in rams than in ewes, in contrast ewe has anoestrous period, but rams has uninterrupted spermatogenesis in life (Blench *et al*., 2006).

Rekwot *et al*. (1987b) observed improved semen volume, sperm concentration and percentage of live sperm in bulls during rainy as compared to dry season. Weight and scrotal circumference measurements taken on some rams during the non-breeding season showed a one-third decrease in their testicular weight and size (Delgadillo *et al*., 2004). Season also has strong influence on reproductive physiology of bucks, independent of feeding level as there were seasonal variations in body weight, testicular weight, testosterone secretion, sexual behaviours and semen characteristics despite the difference and constant level of feeding. It has been demonstrated that breeding season especially summer is characterised by high testicular weight and high testosterone production in bucks (Karagiannidis *et al*., 2000). Seasonal variation in semen quality has been reported (Salisbury, 1978; Ortavant *et al*, 1990).

However, where climates are so hot and humid that housing and shade do not prevent increase in body temperature, the number of sperm ejaculated and their motility often decline, while the percentage of abnormal sperm increases (Oyedipe *et al*., 1984). These effects may be most pronounced a few weeks after hot weather because of the time required to produce and transport sperm to the point of ejaculation. Controlled warm environment with temperature up to 38 to 40 ^{0}C resulted in decreased sperm output and high percentage of abnormal sperm (Ogunlade *et al*., 2006). Recovery usually followed return of the animals to an environment of thermal neutrality (Oyedipe *et al*., 1984). Extremely low temperatures can cause damage if testes become frozen. Light appears to have minimal effects on semen quality in bulls (Oyedipe *et al*., 1984). Fertility differences with season continue when bulls are housed in air-conditioned barns (Salisbury *et al*., 1978).

Semen quality is more affected in rams of a breed that are seasonal breeders (Salisbury *et al*., 1978). Semen quality is best during periods of decreasing daylight and harmful during high temperature. Rathore(1970)reported that exposure of unshed rams to a temperature of 32 ^{0}C for 1 week decreased sperm concentration and percentage of motile sperm, and increased the proportion of abnormal sperm. A group of shed rams was not affected by the heat treatment. These environmental changes are presumably associated with changes in day length (Rathore, 1970). Enough evidence was found of thermal effects on semen quality. However, elevated testicular temperature, such as produced by cryptorchidism, interferes with spermatogenesis(Salisbury *et al*., 1978).

2.12.4 Diseases

Apart from the general debilitating effects of disease on the health status of an animal, reproductive disease impairs reproductive activities and thus leads to reduced productivity (Rekwot *et al*., 1998). Infections which lead to epididymitis and orchitis have been found to cause testicular degeneration in bulls (Kumi-Diaka, 1981). In rams epididymitis produced by *Actinobacillusseminus* hinders testicular development and resulted in decreased semen quality and quantity (Smith *et al*., 1994). Infectious forms of infertility and abortion reported in Nigeria include brucellosis (Bawa*et al*., 1991; Ate*et al*., 2007), trypanosomiasis (Sekoni*et al*., 1990; Sekoni and Rekwot, 2003), listeriosis (Akpavie and Ikheloa, 1992), campylobacteriosis (Bawa*et al*., 1991). These diseases cause embryonic death leading to abortions and infertility in farm animals which is of economic importance. Sekoni *et al*. (2004) reported significant elevation in ejaculation time and poor semen characteristics of bulls infected with trypanosome. The semen quality deteriorates progressively with drastic decrease in semen volume, sperm concentration and there was increased abnormal spermatozoa.Chronic dermatophilosis is also associated with infertility and sterility in bulls (Sekoni, 1992).

Infection with *Dermatophilus congolense* severely affects the semen characteristics of bulls with decreased semen volume, increased percentage of dead spermatozoa, increased percentage of total sperm morphological abnormalities, decreased progressive sperm motility and loss of libido (Sekoni, 1992). Sekoni *et al*. (1992) also reported loss of libido, deterioration of semen quality and terminal sterility in a bulls due to bestnoitiosis. Chronic *T. congolense* infections in cattle may lead to loss of libido, interference with reproduction and delayed puberty in calves (Sekoni, 1992). Wada*et al*. (2009) reported low levels of plasma testosterone in goats infected with *T. congolense*. Ikede and Akpavie, (1982) reported that male California white rabbits infectedwith *T. brucei* developed increased scrotal diameters, scrotal inflammation, periorchitis, epididymitis, preputial haemorrhage severe testicular degeneration and aspermatogenesis.

Pathogenic effects have also been reported in small ruminants.Severe testicular epididymal degenerations and deteriorated semen characteristics were reported in bucks and rams infected by *T. vivax* (Anosa and Isoun, 1984; Sekoni, 1991). Sekoni,(1991) reported that bulls infected with *T. congolense* had deteriorated semen characteristics, highly elevated ejaculation time, with either lack or decreased ejaculate in volume, poor sperm motility, and highly sperm morphological abnormalities (Sekoni*et al*., 1990; Sekoni*et al*., 1991; Sekoni, 2004). Histological findings revealed severe testicular and epididymal degeneration, seminiferous tubules lost their architectural design, and became collapsed and degenerated. The interstitial cells were highly fibrosed andshowed no spermatogenesis (Sekoni, 1990). Therefore, from the histological evidence trypanosomiasis is capable of destroying the endocrine function of the testes, production of testosterone by the interstitial cells of Leydig cells and the exocrine function of spermatogonial and germinal epithelium. The histology revealed degeneration of pituitary gland; this can affect the production of gonadotropin and growth hormones, consequently having detrimental effect on testicular function (Sekoni, 1991).

Many studies have reported an effect of *Fasciola* infection on reproduction in livestock; delay in puberty in both sheep and cattle have been reported (Fleming and Fetterer, 1986;López-Díaz *et al.* 1998) This delay in puberty is thought to result from an alteration in the metabolic clearance rate of estrogen or testosterone induced in the liver from the trauma of the migrating flukes. The increased hormonal concentrations then suppress the release of GnRH as a result of a negative feed- back effect (Fleming and Fetterer, 1986).

In infected female animals, abortions during all stages of gestation, still births (Wamae *et al*., 2007) decrease in conception rate and fecundity (Yüksek*et al*., 2013),parasite-induced-anorexia that led to poor nutrition in mid and late pregnancy, had reduced birth weight, and prenatal invasion of the foetus by *Fasciola hepatica* (Yüksek*et al*., 2013) have also been reported The liver is often incapacitated in its metabolic functions thus adversely affecting the overall performance of the animals (Wamae *et al*., 2007).

2.13 Fasciolosis

Fasciolosis is an economically important parasitic disease, which is caused by trematode of the genus *Fasciola* that migrate in the hepatic parenchyma, establish and develop in the bile ducts (Troncy, 1989). The two most important species, *Fasciola hepatica* found in temperate and in cooler areas of high altitude in the tropics and subtropics and *Fasciola gigantica*, which predominates in tropical areas. Both species coexist where ecology is conducive for the snail hosts (Yilma and Mesfin, 2000). The parasite is pathogenic to all classes of livestock, and had a cosmopolitan distribution (Roseby, 1970; Dargie, 1987; Ahmed, 2007).

2.14 Significance of Fasciolosis in Nigeria

In Nigeria, livestock is the main source of meat in both urban and rural areas (RIM 1992). A significant proportion of these animals are reared under the semi-intensive management system with little supplementation, which results in low productivity and high pre-weaning mortality. Fasciolosis is of great economic significance worldwidein distribution and occur in some part of Nigeria (Ofukwu, 2000 and Okoli *et at*., 2000). With losses estimated to exceed 2000 million dollars yearly, affecting more than 600 million animals around the globe. The disease is characterized by anaemia due to severe liver damage caused by immature fluke tunnelling through the liver parenchyma with extensive tissue damage and haemorrhage that culminate in severe clinical disease (Biffa *et al.*, 2006).

Fasciolosis poses a threat to the socio-economic wellbeing of the farmers, as a result of several complication including weight loss, drop in milk production, submandibular edema, delayed onset of estrous cycle, poor reproductive performance, abortion, infertility and diarrhoea which have been reported (Radostits *et al.*, 2000). According to Biffa *et al*.(2006), fasciolosis is mainly observed in chronic form, either in young animals during the rainy season due to recently acquired infections or in the dry season in older animals which are in poor condition and may not be able to withstand the effect of relatively small number of flukes.Irrigation activities during the dry season have been reported byOkaiyeto *et al*.(2012)to be one of the common sources of infection to animals grazing in areas infested with metacercariae.Nigeria is one of the tropical country that has conducive environment for survival of*Fasciolagigantica* known as causative agentsof fasciolosis in the tropics (Okaiyeto *et al*., 2012)

2.15 Morphology and Life Cycle of *Fasciolagigantica*

Fasciola gigantica-is leaf shaped, greyish-brown in colour, and is 3.5-7.5 cm in length. The shoulder and tail are parallel and are 0.65-1.2 cm in width. Adult *Fasciola* lives in the bile ducts and gall bladder of the liver and young fluke lives in the liver tissues (Yilma and Mesfin, 2000). The eggs of *Fasciola* enter the duodenum with the bile and leave the host in the faeces. The rate of development and the hatching of *F. gigantica* eggs depends on the environmental temperature, oxygen levels and humidity (Max*et al*., 2006; Walker *et al*., 2008). At a temperature of 20-26 ^{0}C eggs hatch in about 10-12 days producing the first larval stage, the miracidium. At temperatures over40 ^{0}C, eggs will die and in darkness, eggs develop well, though miracidia are not hatched out (Kassuku*et al*., 1986).

A miracidium is about 0.15 mm in length, its head is covered with gland tissues for penetrating into the intermediate host snail and with cilia surrounding it is equipped for movement. In the outside environment, miracidia survive for 2-3 hours. In the event that miracidia do not penetrate into the intermediate host snail *Lymnea* species, they die (Yilma and Mesfin, 2000). Following penetration, it casts off its ciliate covering and develops into the sporocysts, then rediae and cercariae. Development from miracidium to cercariae is 4-7 weeks (Kassuku*et al*., 1986). Cercariae leave the snail and within a few minutes to two hours the cercariae settle on blades of grass, water plants, rice stalks etc. just below water-level. Later, after casting off their tails, they secrete a covering from the cystogenous glands forming cysts at the surface of the water which sink to the bottom (Kassuku*et al*., 1986; Wamae*et al*., 2007). The encysted cercariae are calledmetacercariae which are now infective. Cattle and sheep became infected by ingesting grass, water plant, rice stalk, and rice straw with metacercariae or swallow them in drinking water. In some cases, infection can occur from mother to the offspring via the placenta (Kassuku *et al*., 1986; Wamae *et al*., 2007).

2.16 Pathogenesis and Clinical Signs of Fasciolosis in Ruminants

Pathogenesis depends primarily on the two different stages of development of the parasite in the liver of the host, the juvenile larvae and adult parasite, and the level of parasitaemia. Acute and sub-acute disease is seen in sheep, and occasionally occurs under conditions of heavy challenge in young calves. The chronic form of the disease is by far the most important in sheep, and specifically in cattle (Lopez-Diaz*et al*., 1998).

The clinical signs of acute disease are characterised by sudden death, weakness, anaemia and dyspnoea (Kassuku *et al*., 1986; Wamae *et al*., 2007). Sub-acute and chronic fasciolosis is characterized by progressive loss of condition, anaemia, hypoalbuminaemia, emaciation, pallor of the mucous membranes, submandibular oedema and ascites. Anaemia is hypochromic and macrocytic and an accompanying eosinophilia is usually present. In milder infections clinical signs may or may not be readily observed, however, a decreased appetite and interference with post-absorptive metabolism of protein, carbohydrates and minerals, may have a significant effect on production (Kumar *et al*., 2008).

2.16.1 Sub-acute fasciolosis in ruminants

Sub-acute disease is usually seen during raining season and cool season, and 6–10 weeks after ingestion of smaller numbers (500-1500) of metacecariae (Kumar *et al*., 2008). At this stage some parasites may have reached the bile ducts whilst others may still be migrating through the parenchyma. Sub capsular haemorrhages may be present but usually these do not rupture.

2.16.2 Acute fasciolosis in ruminants

Acute disease is associated with mostly immature flukes, and usually seen in raining season and cool season, 2-6 weeks after ingestion of metacercariae in large numbers (> 2000) (Kumar *et al*., 2008). The immature flukes migrate through the liver parenchyma and create migratory tracts, which results into direct trauma, coagulative necrosis and release of toxic excretions (e.g catephsin) from the flukes (Yilma and Mesfin, 2000). Lesions may vary from mild (low infestations) to severe in heavy or repeat infestations

(Yilma and Mesfin, 2000). The liver may be enlarged and haemorrhagic with fibrinous to fibrous exudates on the capsular surface (usually the ventral lobes). The migratory tracts may be visible as dark acute haemorrhagic streaks to, more yellowish white streaks typical of post necrotic scarring and granulation (Kumar *et al*., 2008). Sometimes flukes may be seen in the migratory tunnels. If severe haemorrhages are present it may result in large subcapsular haemorrhages, which in turn may rupture with severe intra-abdominal haemorrhage and acute haemorrhagic anaemia as consequence (Yilma and Mesfin, 2000). In some heavy and repeat infestations acute lesion of multifocal pin-point serosal haemorrhages and fibrinous peritonitis, to more chronic fibrous peritonitis may be present (Kumar *et al*., 2008).

2.16.3 Chronic fasciolosis in ruminants

Chronic fasciolosis is associated with mature flukes, and seen mainly in late winter or early spring. It is usually 4-5 months after ingestion of moderate numbers (200-500) of metacercariae (Kumar*et al*., 2008). Mature flukes, which are present in the bile ducts, cause necrosis and ulceration of the epithelium giving rise to peribiliary inflammation and severe hyperplasia of the epithelial layer (Max *et al*., 2006; Walker *et al*., 2008) Mechanical irritation by their scales, and suckers, biliary retention and the production of toxic or irritant products by the flukes may contribute to lesions. The reduced growth rate in sheep challenged with *F. hepatica*, or highest dose of *F.gigantica*, which indicate a real reduction in energy expenditure attributable to disease and fasciolosis (Raadsma *et al*., 2007).

Anaemia and hypoalbuminaemia are the most important consequences contributing mostly to the pathogenesis (Yilma and Mesfin, 2000). More than 0.05 ml blood per fluke can be lost per day(Kumar *et al*., 2008). Plasma protein may be lost through the bile ducts into the intestine due to the increased permeability of the hyperplastic bile duct epithelium, and loss of plasma proteins through the fluke's digestive tract (Raadsma *et al*., 2007). Bile ductular distention in sheep, swine and horses may be more mechanical due to accumulation of parasites and bile, whereas in cattle the inflammatory lesion associated with erosions and granulation seems more prominent (Kumar*et al*., 2008). However, *F. gigantica* is slower to develop in ruminant hosts and takes greater than 14 weeks following infection to reach the bile ducts while *F. hepatica* parasites establish within the bile ducts after just 8–10 weeks of infection (Wiedosari and Copeman, 1990).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Animals

Twelve apparently healthy Yankasa rams aged 18-24 months were purchased from Makarfi market, Makarfi Local Government Area of Kaduna State, Nigeria. The rams were housed in the small ruminant pens of the Department of Veterinary Parasitology and Entomology. The pens were fumigated and disinfected before housing the rams. The rams were given adequate basal (Jibril, 2010) diet of hay (*Digitaria smutsii*), concentrate supplement(0.5 kg/ram/day) (ground corn 80.05%, groundnut cake 6.53 %, wheat bran 11.42%, table salt 0.50 %, bone meal 1.25 %, vitamin premix 0.25 %) , salt lick and water were provided *ad libitum*.

The rams were screened for the presence of parasitic infections, and respiratory disease. The physical examination involved checking the mucus membrane and hair coat for signs of anaemia and ecto parasites, respectively. The rams were acclimatized for two weeks during which samples of faeces and blood were collected for laboratory investigation. Rams that were infected with strongyloidiasis were treated with1% Ivermectin injectable(Ivermec®) at a dose rate of 0.2 mg/kg, administered subcutaneously. Thereafter all the experimental rams were routinely dewormed with albendazole bolus (Shanuzole®) at a dose rate of 7.5 mg/kg, administered per os.

The rams were randomly assigned into two groups; Group A (infected rams;n=7) and group B (non-infected/control; n=5), respectively. Rams in each group (A and B) were kept in separate pens and managed intensively. Prior to experimental infection, semen was collected using an electro-ejaculator from each ram once a week for 4 weeksby the method described by Belibasaki and Kouimitzis (2000), andevaluated for semen characteristics.

Blood samples were asepticallycollected via the jugular vein using 5 ml sterile 21 gauge hypodermic needles (Zarinject®)twice weekly for four weeks and used for evaluation of haematological indices as described by Dacie and Radish (1975). Serum was also harvested from the blood samples and used for the determination of testosterone levels. Live weights of the rams were also determined weekly by weighing balance up to the end of the experiment.

3.2 Collection of *Lymnaeanatalensis*and Harvesting of *Fasciolagigantica*Metacecariae

Infected snails *(Lymnaea natalensis)* were collected from streams located along area BZ, Ahmadu Bello University, Zaria, Staff Quarters, and put into rubber containers containing water and transported to the Helminthology laboratory of the Department of Veterinary Parasitology and Entomology. The snails were crushed in a petridish using forceps and a dissection pin. The shells were removed while the tissues were washed with water and sieved. The filtrate was left uncovered in a Petridish for 3 days, and the supernatant discarded according to the method described by Ajanusi (1987). The petri dishes were emptied of water and kept at 4 ^{0}C in a refrigerator. The attached metacercariae on the petri dish were scraped into 40 mls of distilled water in a cylinder and mixed.After through mixing, 0.1 ml was pipetted unto a ruled glass slide for counting of metacercariae and counting of viable cysts under x10 magnification of a light microscope. Each 0.1 ml of the mixture contained an average of 97 viable metacercariae.

3.3 Experimental Infection

Eight hundred (800) metacecariae of *F. gigantica* in 0.82 ml were drenched orally to (group A) rams**,** and flushed with 100 ml of portable water to each ram in group A as described by Ajanusi (1987). Rams in group B received 0.82 ml portable water orally. Thereafter, rams in both groups were monitored weekly for manifestation of clinical signs.

3.4 Sample Collection and Handling

3.4.1 Coprology

Faeceswerecollected once every week for 4 weeks pre-infection and 12 weeks PI, from all the experimental rams, for identification of fasciola eggs as described by (Yilma and Mesfin, 2000).

3.4.2 Blood collectionfor haematology,total protein and testosterone profiles

Haematological studies and preparation of sera for total protein determination, 5 ml of blood was collected aseptically via the jugular vein using 21 gauge hypodermic needles (Zarinject®)twice per week (Monday and Thursdays) throughout the 4 weeks pre-infection and 12 weeks period of infection.

To obtain sera for testosterone assay, 3 ml of blood was collected three times at 30 minutes interval between 8:00-900am every Monday and Thursdays during the period stated above. However, due to cost of analysis, only sera obtained randomly at weeks 3, 5, 7, and 9 of infection were assayed.

3.5 Haematological Evaluation

3.5.1 Determination of packed cell volume (PCV)

The Packed Cell Volume (PCV) was determined as descried by Benjamin (1978) using the microhaematocrit method.

3.5.2 White blood cell count

Total white blood cell (WBC) count was done using haemocytometer method as described by Schalm*et al*. (1975) and Dacie and Lewis (1975)..

Procedure:- A white blood cell diluting pipette fitted with rubber and plastic mouth piece was filled with the blood sample to the 0.5 mark, and the excess blood clinging to the exterior of the pipette was wiped off using a piece of cotton wool. The pipette was then dipped in a bottle containing the WBC diluting fluid (2 % glacial acetic coloured with 2 drops of gentian violet). The diluting fluid was drawn to the 101 mark on the pipette. The blood and the diluting fluid were then adequately mixed by horizontal agitation, thereby lysing the red blood cells. Six drops of the diluting fluid was removed by releasing the thumb intermittently. The haemocytometer was then filled by capillary action through contact with the pipette containing the fluid,and examined under x40 magnification ofa light microscope. The leukocytes were counted in the four large squares to obtain the total leukocytes count. The value obtained was multiplied by a factor of 50.

3.5.3 Determination of total protein (TP)

Total protein was determined according to the method described by Benjamin (1978)which is popularly known as Biuret reaction.Biuret reaction is based on the principles that protein forms coloured complex with copper ions (Cu^{2+}) in alkaline solution as a result of nitrogen reaction complex.

3.6 Testosterone Assay

Testosterone determination was by the cELISA technique as described below;

3.6.1 Reagent preparation for testosterone assay

Working enzymes reagent was constituted by combining testosterone enzyme reagent and steroid conjugate buffer. The working substrate solution was constituted by combining substrate A solution with substrate B solution and mixed thoroughly. Wash buffer was diluted with 100 ml of distilled water, as described by the manual. All the constituted reagents were used immediately.

3.6.2 Testosterone assay procedure

Before proceeding with the assay, all reagents were brought to room temperature 20-27 ^{0}C.The microplate well were formatted.Microplate well for each serum reference was assayed in duplicate. Serum sample (10 µl)were pipetted into the assigned wells, 50 µlof the working testosterone enzyme reagent was added to all wells and the microplate was swirled gently for 20-30 sec.Testosterone biotin reagent (50 µl) was added to all wells and the microplates were again swirled gently for 20-30 sec. The microplates was covered with parafilm paper and incubated for 60 minutes at room temperature. The contents of the microplates were discarded by decantation and the microplate were dried using absorbent paper. Wash buffer (350 µl) was aspirated into the wells of the microplate trice and discarded. Working substrate solution (100 µl) was added to all wells. The microplate was incubated for 15 min and 50 µl of stop solution was added to each well andwas gently mixed for 15-20 sec.The absorbance was read in each well at 450 nm using a reference wavelength of 620-630 nm to minimize well imperfections in the microplate reader(spectroMax plus®).

3.7 Measurement of Scrotal Circumference

The scrotal circumference was measured with a flexible tape at the broadest part of the scrotum. This was done by applying gentle pressure with hand above the head of the epididymides thereby forcing the testes into the scrotum. The rams were restrained in a standing position during measurements.

3.8Semen Collection

With rams adequately restrained in a standing position, semen was collected by two person; one person operating the electro-ejaculator while the second person collected the semen into a calibrated tube (plastic tube). Semen samples were collected with the aid of a battery-operated electro ejaculator (Bailey® MOD2, Western Instrument Company, Colorado USA) for small ruminants. The Bailey® is powered by 6 voltsbatteries and designed for one-hand operation. Erection and ejaculation were achieved using the method described by Belibasaki and Kouimtzis (2000).The probe was lubricated with petroleum jelly for easy insertion into the rectum and pushed forward slowly and a series of short electrical stimulation was done intermittently for approximately 2-5 seconds,until erection and ejaculation was achieved. The time taken to ejaculate were noted as described by Sekoni *et al*. (1991). Ejaculates were placed in a water bath at 37 ^{0}C before microscopic evaluations.

3.9 SemenEvaluation

3.9.1 Macroscopic examination

Semen samples were evaluated according to the method described by Zemjanis (1970). The gross semen characteristics examined included volume, colour, pH, and presence or absence of foreign bodiesas described by Maina *et al*. (2006). The volume was read from the graduated collecting tubes, colour was coded as 1, 2 and 3 corresponding to creamy, milky and watery colours, respectively.A drop of semen was placed on the pH paper and read immediately. pH colour change were matched with standard chart (universal pH 1-14®) reflecting the pH value.

3.9.2 Microscopic examination

Microscopic examination for gross sperm motility was determined as described by Kridli *et al*.(2002) and Oyeyemi *et al*.(2009). A drop of raw, undiluted semen on a prewarmed slide was covered by a cover slip and viewed under a field microscope at x40 magnification.

Sperm concentration was determined byusing the haemocytometer method as described by Bearden and Fuquay (1992). Semen sample was sucked into thered blood cell diluting pipette up to 0.1 mark and the volume made up to 101 mark with 10% formal saline and mix thoroughly. The mixture was dropped and allowed to spread under the cover slip placed tightly on the haemocytometer after discarding few drops. The cells were allowed to settle before counting under x40 magnification. Sperm cells were counted diagonally from top left to right bottom in 5 small squares of the improved Neubauer haemocytometer. Sperm output of individual ram was calculated as described by Verstegen *et al*. (2002).

Live-dead ratio of the sperm cells was determined as described by Esteso *et al*. (2006). A thin smear of the semen sample was made on clean grease-free glass slides and stained with Eosin-Nigrosin stain.The dead sperm cells were identified as thosethat absorbedthe stain. At least 400 sperm cells were counted using light microscope at x40 magnification. Sperm abnormalities were determined as described by Esteso *et al*.(2006). A thin smear of the semen sample was made on a clean grease-free glass slide and fixed with buffered formal saline. The preparation was then examined and abnormal sperm cells were counted in a regular sequence using light microscope at x100 magnification with oil immersion. A total of 400 well-spaced spermatozoa were carefully examined in each preparation and the percentage of head,mid piece and tail sperm abnormalitieswere determined as described by Melrose and Laing(1970).

3.10 Measurement of Gonadal and Epididymal Sperm Reserves

Two rams were selected randomly from each of groups A and B were sacrificed with their testes removed intact after being dissected free from extraneous tissues. The tunica albuginea was removed using scalpel blade and the testicular parenchyma was weighed and measured using digital weight balance (essae®) and tape respectively.

The left and right epididymides wereseparated into the caput, corpus and cauda portions based on gross anatomy. They were then measured, and weighed using digital weighing balance, after which they were placed in normal saline for subsequent estimation of epididymal sperm reserves.

3.11 Determination of Gonadal and Epididymal Sperm Reserves

Gonadal and epididymal sperm reserves were determined as described by Coulter *et al*.(1987). The testicular parenchyma was sliced and homogenized with a high-speed blender for two minutes with 50 ml of 0.9 % NaCl solution containing antibiotics (sodium penicillin G, 100IU/ml and streptomycin sulphate 1 mg/ml) to prevent bacterial growth.

For determining the epididymal sperm reserves, the caput, corpus and cauda epididymides were isolated, and minced with a pair of scissors separately in 20 ml of 0.9 % NaCl solution. All tissues were homogenised for 2-6 hour after being minced. Testicular homogenates and epididymal sample were refrigerated overnight. After 24 hours, the samples was filtered through gauze with the filtrate volume measured. One millilitre of the gonadal and epididymal filtrates was diluted separately with 2 ml of normal saline solution. Sperm concentration of the testicular and epididymal sample were determined using haemocytometer and light microscope as described by Kwari and Waziri 2001).

3.12 Evaluation of Gross and Histological Lesions

Two rams were randomly selected and sacrificed from each group for post-mortem examination.All the major organs (liver, kidney, intestine, gall bladder, bile duct, spleen, heart and testes)from the two sacrificed rams were examined for gross lesions.The liver and the bile duct were incised.The liver, kidney, testis, spleen and heart were examinedgrossly, and sections and fixed in 10 % formalin solution and later transferred to buffered formalin after 24 hours. These were later processed into slides first by initially embedding them in paraffin wax before being sectioned with microtome. The slides were stained with haematoxylin and eosin (H&E) as described by Luna (1960) and examined under the light microscope at x100 magnification.

3.13 DataAnalysis

Data on haematological parameters, total protein, semen characteristics and testosterone profiles were expressed as mean(±SEM).These variables were compared between the experimentallyinfected (group A) and control (group B) rams using t-test. Levels of $p<0.05$ were considered significant.

CHAPTER FOUR

RESULTS

4.1 Baseline Pre-infection Data

The pre-infection data areaverage bi-weekly valuesrectal temperature, body weight and scrotal circumference as presented in (AppendixI). Rectal temperatures of rams in both groups (A and B) were within the normal range established for sheep. The haematological parameters (packed cell volume, haemoglobin concentration, total protein, red blood cells count, differential blood count) and testosterone concentrations evaluated were all within normal range as presented in (Appendix IV and V) respectively.

TheMean (±SEM) ejaculationtimes of the rams in both groups (A and B) prior to experimental infection 198.0±29.3 and178.0±12.4 seconds, respectively. Semen characteristics (semen volume, sperm motility, pH, sperm concentration, sperm viability and sperm morphology) were within the normal range (Appendix VII). Mean live-weight (Mean ±SEM)16.0±1.0 and 17.6±0.9 and scrotal circumference (Mean ±SEM)were19.4±1.1 and 21.0±1.6 for group A and group B rams, respectively.

4.2 Clinical Signs of Yankasa Rams Experimentally Infected with*F. gigantica*

4.2.1 Clinical signs

Manifestation ofclinical signs was gradually observed from two weeks post infection in group A. These signs observed from week 4-7 PI were distended abdomen, anorexia, rough hair coat, diarrhoea, bilateral muco purulent ocular and nasaldischarges. However, rams in group B showed no clinical signs or variation from the normal pre-infection ranges in rectal temperature, body weight and scrotal circumference as observed in group A (Appendix I).

4.2.2 Rectal temperature

Marginal increases in rectal temperatureswere observed for rams group Afrom week 1 PI (Figure 4.1). This steadily increased to 39.6 ± 0.1 ^{0}C by week 6 PI (Figure 4.1). Rectal temperature was significantly higher ($p<0.05$) in group A than B by week 3 PI (Figure 4.1). The maximum rectal temperature (Mean ±SEM) observed for group A was 40.1 ± 0.1 ^{0}C at week 9 PI. Thereafter, temperatures began to decline and was 39.0 ±0.1^{0}C by week 11 in the infected group(Figure 4.1).

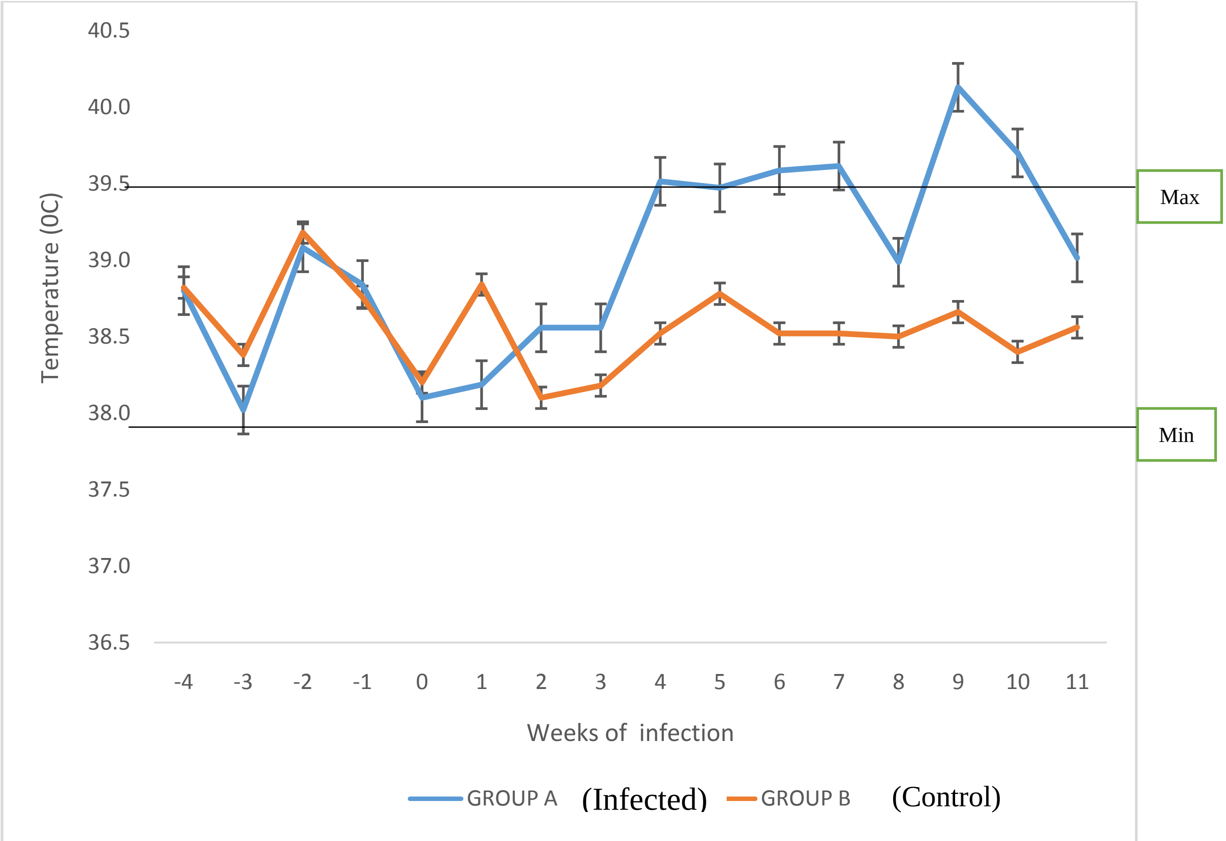


Figure 4.3: Rectal temperatures of Yankasa rams prior to and post experimental infection with *F. gigantica*

4.2.3 Live-weight

There was marginal decrease inlive-weight (Mean±SEM) of rams in group A from 22.3±0.7 kg to 21.0±0.6 kgby week 2 Post Infection (PI). Live-weight progressively decreased to 17.7 ± 0.9 kg at week 11 PI. Significantly higher differences ($p<0.05$) in live-weight were observed for rams group B than A from week 3 onward (Figure 4.2). Although live weight continued to decrease in group A rams, the reverse was observed in group B rams which reached a maximum weight of 26.0 ±0.7 at week 11(Figure 4.2).

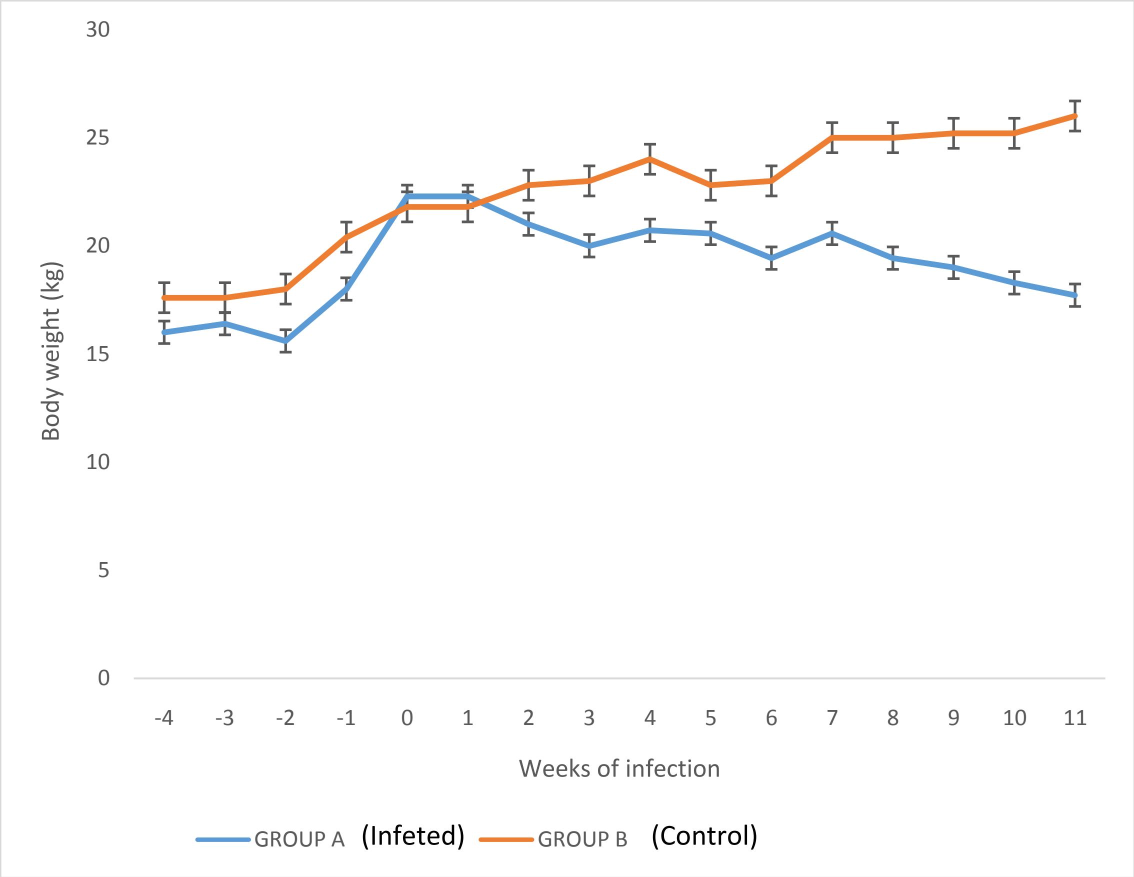


Figure 4.4:Live-weight of Yankasa rams prior to and post experimental infection with *F. gigantica*.

4.2.4 Scrotal circumference

The mean values of scrotal circumference is presented in Table 4.1 *Fasciolagigantica* infection significantly ($p<0.05$) decreased scrotal circumference (SC) from 22.3±0.6-19.3±0.7cm from week 5 to week 11 PI in group A. On the other hand, gradual and progressive increases in scrotal circumference of rams were observed in group B.

Table 4.1: Scrotal circumference of Yankasa rams prior and post experimental infection with *Fasciolagigantica*

Weeks	Group A	Group B
-1	19.4± 1.1	21.0± 1.6
-2	18.8 ± 1.3	20.8± 1.3
-3	19.4 ± 1.3	22.6 ± 1.8
-4	21.0 ± 1.0	23.2 ± 1.6
0	24.0 ± 0.5	23.6± 0.2
1	24.0 ± 0.5	23.6 ± 0.2
2	24.0 ± 0.7	23.6 ± 0.2
3	23.4 ± 0.6	24.0 ± 0.3
4	22.9± 0.7	24.6± 0.2
5	22.3 ± 0.6[a]	24.6 ± 0.2[b]
6	22.0 ± 0.7[a]	24.8 ± 0.4[b]
7	20.9 ± 0.8[a]	25.20 ± 0.4[b]
8	21.3 ± 0.6[a]	25.2 ± 0.4[b]
9	20.0 ± 0.7[a]	25.6 ± 0.5[b]
10	19.9 ± 0.8[a]	25.6 ± 0.5[b]
11	19.3 ± 0.7[a]	25.8 ± 0.6[b]

[a,b]Means in the same row with different superscriptsare statistically ($p<0.05$) different.

4.2.5 Ejaculation time

Data on ejaculation time for rams in the infected (Group A) and control (Group B) groups are given in (Figure 4.3).Generally, ejaculation time gradually decreased in both groups from the pre-infection phase to week 4 of the experiment (Figure 4.3).However, asignificant ($p>0.05$) increase was observed mean in ejaculation time for rams in group A from week 8 PI (184.1±23 seconds) compared to rams in group B (35.4±3.2 seconds).This increase continued for rams in group A and reached a high mean value (Mean ± SEM) of 232.1±3.6 seconds by week 12 PI (Figure 4.3).

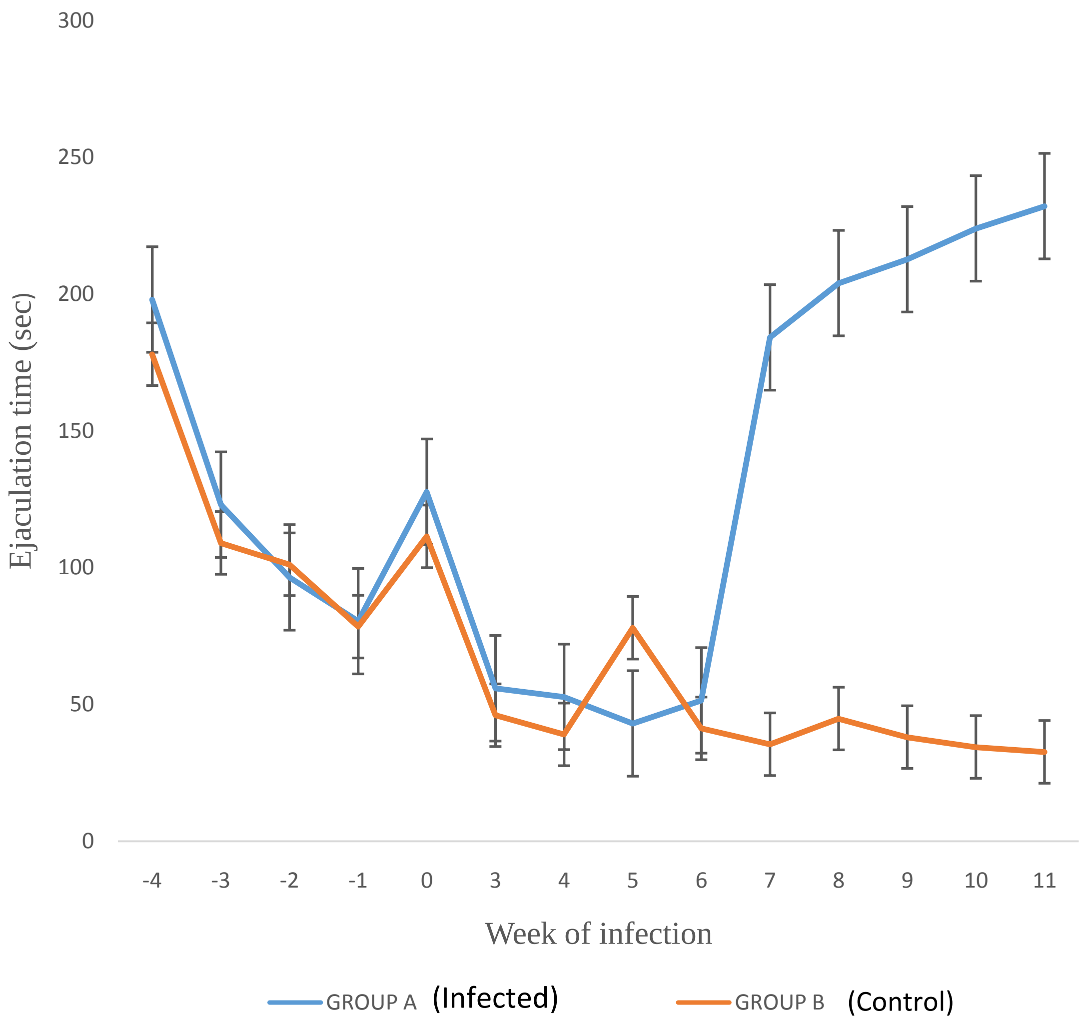


Fig. 4.5:Ejaculation time of Yankasa rams prior to and post experimental infection with *F*. *gigantica*.

4.3 Faecal Examination

*Fasciolagigantica*eggs were identified from faeces of experimental rams (Group A)at week 7 PI with a mean value (Mean ± SEM) of 2.4±0.3 eggs per gram (EPG) of faeces (Figure 4.4). The highest EPG recovered from the experimentally infected rams (Group A) was 12.9±0.9 eggs at week 9 PI(Figure 4.4). On the other hand, zero EPG was observed for rams in the control (group B).A continuous decline in EPG was observed from week 10 to end of the study (Week 12).

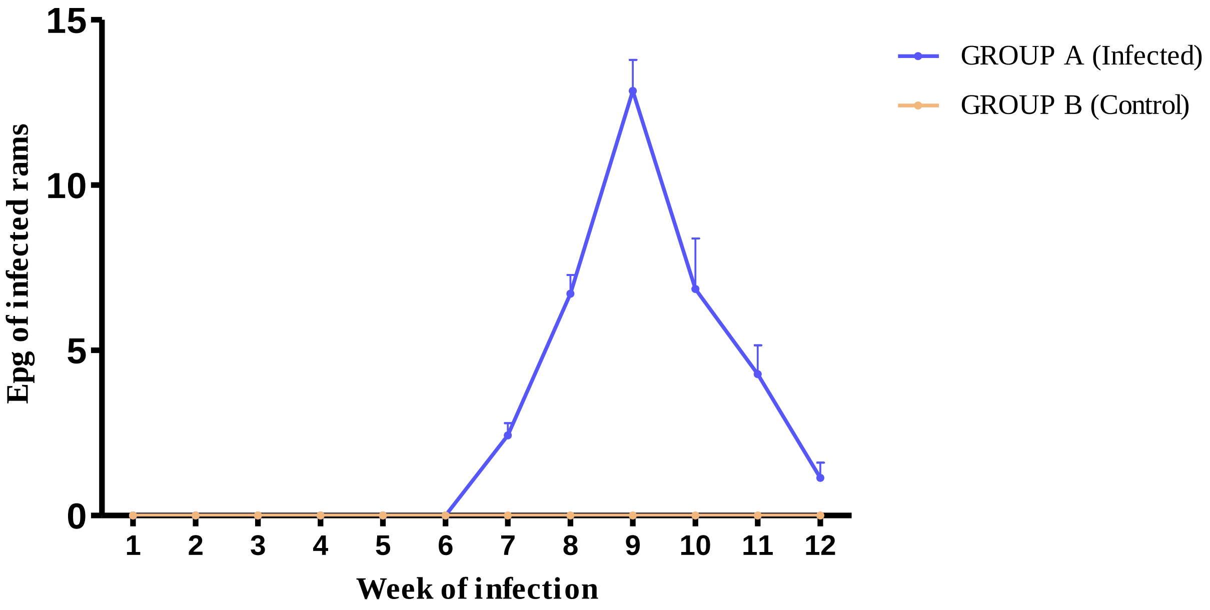


Figure 4.6:Eggspergram (EPG) of faeces of Yankasa rams following experimental infection with *F. gigantica*

4. 4 Haematological Parameters

The Packed Cell Volume (PCV) of rams in in group Aand B are presented in Table 4.1. In group A, there was a steady decrease in PCV from week 9 PI to the end of the study period (Table 4.2). There was significantly higher PCV values forrams in group B from week 3 PI compared to rams in group A (Table 4.2). The lowest PCV values (Mean± SEM) observed in this study was at week 12 (15.3±0.5 %) in group A (Table 4. 1).

Haemoglobin concentration differed significantly ($p<0.05$) between groups from week 5 PI and continued till end of the study (Table 4.2). Rams in group A showed a significant ($p<0.05$) decrease in haemoglobin concentrationof 11.4±0.6g/dL at week 3PI. Lowest haemoglobin concentration of 5.8±0.2 g/dLwas observed at week 12 PI. However, rams in group B showed marginal variations within the normal rangethroughout the study (Table 4. 2).

There were significant differences ($p<0.05$) in red blood cells (RBC)counts of rams in group A from week 3 PI (Table 4.2). RBC count steadily decreased from week 7 to the end of the study in group A reaching a low value of 3.1×10^{6} ml by week 12 PI. On the other hand, the RBC values remained fairly constant with marginal variation in group B throughout the study period (Table 4.2).

Similarly, there were significant differences ($p<0.05$) in white blood cells (WBC) of rams in group A from week 3 PI (Table 4.2) with WBC steadily higher in group A than B, although values were within the normal range (Table 4.2). The level of WBC fluctuated with no definite pattern within the study period. Differential leucocyte count revealed that neutrophils increased significantly in group a rams, with highest mean value of 28.1 % at week 8 PI (Table 4.3). Cumulative mean values for eosinophil were significantly higher in the infected (3.8 %) than in the control (2.1 %) group at the end of the study period (Table 4.3). No significant difference was observed in lymphocytes, monocytes and basophil percentages in both groups at the end of the st

Table 4.2: Mean (±SEM) of haematological parameter of Yankasa rams prior to and post experimental infection with *Fasciolagigantica*

parameters	Weeks														
	-1	-2	-3	-4	0-2	3	4	5	6	7	8	9	10	11	12
PCV(%)															
Group A	30.6±0.1	31.0±2.0	32.5±0.9	32.6±0.2	ND	33.6±1.8^{a}	33.0±1.5^{a}	29.1±1.2^{a}	27.9±0.8^{a}	25.3±0.8^{a}	22.1±0.6^{a}	19.1±0.3^{a}	17.9±0.3^{a}	16.8±0.3^{a}	15.3±0.5^{a}
B	31.6±1.3	30.0±1.4	34.2±1.0	30.9±0.1	ND	40.4±2.2^{b}	38.8±2.1^{b}	36.4±1.6^{b}	37.4±1.12^{b}	38.0±0.8^{b}	37.8±0.7^{b}	37.8±0.7^{b}	37.6±0.6^{b}	37.4±0.7^{b}	37.4±0.7^{b}
Hb conc (g/dl)															
Group A	10.3±0.0	10.3±0.0	10.8±0.3	10.8±0.1	ND	11.4±0.6^{a}	11.5±0.5	10.3±0.2^{a}	9.8 ± 0.2^{a}	8.9 ± 0.2^{a}	8.4 ± 0.1^{a}	7.8 ± 0.2^{a}	6.3 ± 0.2^{a}	5.6 ± 0.2^{a}	5.8 ± 0.2^{a}
B	11.2±0.0	11.3±0.0	12.7±0.0	10.3±0.3	ND	13.8±0.8^{b}	13.1±0.8	12.1±0.4^{b}	11.8±0.2^{b}	11.1±0.2^{b}	11.8±0.1^{b}	11.8±0.2^{b}	11.5±0.2^{b}	11.6±0.2^{b}	12.2±0.2^{b}
RBC ($x10^6$/ml)															
Group A	5.2 ± 0.1	5.3±0.1	5.22±0.1	5.4±0.2	ND	4.8 ± 0.4^{a}	6.5±0.9	5.3±0.2^{a}	5.1 ± 0.2^{a}	4.1±0.1^{a}	4.0 ± 0.1^{a}	3.5±0.1^{a}	3.2 ± 0.0^{a}	3.5 ± 0.3^{a}	3.1 ± 0.1^{a}
B	5.4±0.1	5.5 ± 0.1	5.5±0.2	5.5±0.2	ND	6.9±0.2^{b}	7.3±0.2	7.2±0.2^{b}	6.3 ± 0.3^{b}	6.1±0.3^{b}	5.7±0.1^{b}	5.6±0.0^{b}	5.5 ± 0.0^{b}	5.5±0.0^{b}	5.4 ± 0.0^{b}
WBC($x10^3$/ml)															
Group A	7.4±0.6	6.1±0.9	8.0±0.4	8.4±0.3	ND	6.8±0.3	7.8±0.1	7.8±0.1	5.5±0.7	6.6±0.3	6.7±0.2	6.5±0.0	6.4±0.0	6.4±0.1	6.7±0.3
B	6.5±0.5	5.7±0.3	6.8±0.8	7.1±0.3	ND	5.8±0.2	7.5±0.7	5.6±0.6	4.3±0.9	3.3±0.2	2.8±0.1	2.7±0.1	3.0±0.2	3.1±0.2	3.5±0.0

^{ab}Means in the same rows of each parameter with different superscript alphabets are statistically ($p<0.05$) different.

Table 4.3: Cumulative (Mean±SEM) of differential leucocyte counts and total protein levels of Yankasa rams following experimental infection with *F. gigantica*.

Parameter	Group A	Group B
Neutrophils (%)	28.1± 1.8	25.7 ± 1.1
Lymphocytes (%)	68.1 ± 1.8	71.1 ± 1.8
Monocytes (%)	2.5 ± 0.3	2.5 ± 0.5
Eosinophils (%)	3.8 ± 0.6^{a}	2.1 ± 0.3^{b}
Basophils (%)	1.9 ± 0.3	1.3 ± 0.2
Total protein (g/dl)	5.49 ± 0.3^{a}	6.56 ± 0.4^{b}

^{a,b}Means values in the same rows of each parameter with different superscript are statistically ($p<0.05$) different

4.5 Semen Characteristics

Thesemen characteristics of both groups A and B prior to experimental infection are presented in Table 4.4.There was no significant difference between the semen characteristics of both groups prior to infection and up to week 3 PI (Table 4.4).

Significant decrease in semen volume was observed in group A from week 3 PI with significant ($p>0.05$) difference 0.4±0.1 ml at week 4 PI, and continued to decline steadily to 0.01 ± 0.01 ml by week 12 (Table 4.4). However, semen volume in group B (control) increased gradually from 1.1±0.3 ml at week 3 PI to 1.6±0.1 ml by week 11 PI.

There was no significant difference in sperm motility of both groups prior to infection (Table 4.4). Rams in group A showed significant ($p>0.05$)consistent decrease in spermmotility from week 4 PI with the highest and lowest sperm motility of 51% and 0.7% at week 3 and 10 PI, respectively. Unexpectedly, there was marked increase in sperm motility (10.0±3.9 %) at week 11 PI (Table 4.4). Rams in group B showed consistent increase in sperm motility from week 4 PI with highest and lowest semen motility of 94% and 64% at week 12 and 3 PI respectively (Table 4. 4).

There was progressive increase in sperm concentration in both groups prior to experimental infection (Table 4. 4).However, sperm concentration progressively decreased from week 5 PI(208.0±7.2 $\times 10^6$/ml) significantly ($p>0.05$)till the end of the study, with lowest sperm concentration of 120.0$\times 10^6$/ml at week 12 PI. On the other hand, sperm concentration decreased minimally 436.0±9.3 $\times 10^6$/ml at week 10 PI but was within the normal range throughout the study period for rams in group B.

The mean percentage of live spermatozoa during the pre-infection phase was 54.5±0.5 % and 54.5±0.3 %for group A and B, respectively (Table 4.4). A progressive decrease in percent live spermatozoa of rams in group A was observed from the pre-infection values of 61.4±0.3 % to 9.00 ±0.3 % by week 6 PI, with significant ($p>0.05$) difference (36.0±12.0 %) at week 3 PI compared (66.0±14.0 %) to group B. Thereafter, it fluctuated minimally to reach a value of 3.0±0.2 % at end of the study (week 12). On the other hand, progressive increase in percent live spermatozoa was observed for ram ingroup B throughout the study (Table 4.4).

Average morphological defect in semen of rams pre-infection was 10.3±3.5 % and 11.1±3.4 % in group A and B, respectively, with no significant differences observed (Table 4.5). Sperm defects in group A increased significantly by week 3 PI to a value of 12.3±1.4 (Table 4.5). There was no consistent pattern for the morphological defects observed from week 3 to 12 PI, although, values were higher than pre-infection values. Similarly, no specific pattern in sperm morphological defects were observed for rams in group B, although, fluctuations observed were within the normal range established for the species (Table 4.5).

Table 4.4: Means (±SEM) Semen characteristics of Yankasa rams prior to and post experimental infection with *Fasciola gigantica*

								Weeks							
Parameters	-4	-3	-2	-1	0 -2	3	4	5	6	7	8	9	10	11	12
Semen pH															
Group A	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	ND	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.3±0.0	7.3±0.0
B	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	ND	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0
Semen colour															
Group A	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	ND	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
B	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	ND	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Semen volume															
Group A	0.3±0.1	0.6±0.2	0.4 ± 0.1	0.5± 0.2	ND	0.5 ± 0.2	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.2 ± 0.0^{a}	0.5 ± 0.1^{a}	0.05 ± 0.0^{a}	0.06 ±0.01^{a}	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}
B	0.3±0.1	0.5±0.2	0.6 ± 0.2	0.5 ± 0.1	ND	1.1 ± 0.3	1.6 ± 0.3^{b}	1.6 ± 0.4^{b}	1.0 ± 0.2^{b}	1.4 ± 0.1^{b}	1.6 ± 0.1^{b}	1.6 ± 0.1^{b}	1.6 ± 0.1^{b}	1.6 ± 0.1^{b}	1.5 ± 0.12^{b}
Sperm motility															
Group A	22.0 ± 7.3	57.0 ± 13.9	31.0 ± 11.9	78.0 ± 9.6	ND	51.4 ±10.4	35.7 ± 5.6^{a}	37.1 ± 9.4^{a}	37.9 ± 2.9^{a}	13.6 ± 3.7^{a}	7.1 ± 4.2^{a}	3.6 ± 2.4^{a}	0.7 ± 0.7^{a}	10.0 ± 3.9^{a}	6.4 ± 3.2^{a}
B	29.0 ± 19.1	46.0 ± 19.2	49.0± 13.7	81.8 ± 7.4	ND	64.0 ± 7.5	74.0 ± 5.1^{b}	81.0 ± 3.7^{b}	83.0 ± 3.7^{b}	91.0 ± 1.9^{b}	86.0 ± 2.9^{b}	93.0 ± 1.2^{b}	93.0 ± 1.2^{b}	94.0 ± 1.0^{b}	94.0 ± 1.0^{b}
Sperm Concentration															
Group A	263.6 ± 36.6	334.2 ± 25.4	224.6 ± 44.8	387.0 ± 12.5	ND	218.7 ± 12.4^{a}	222.1 ± 11.7^{a}	208.0 ± 7.2^{a}	207.4 ± 5.3^{a}	191.0 ± 2.3^{a}	179.0 ± 3.5^{a}	157.1 ± 4.2^{a}	143.6 ± 6.4^{a}	130.0±7.6^{a}	120.0 ±7.6^{a}
B	139.6 ± 40.4	300.8±47.1	276.0 ± 64.9	350.6±27.9	ND	402.0±8.6^{b}	497.0±8.6^{b}	456.8±11.6^{b}	446.0±6.1^{b}	444.0±13.4^{b}	444.0±10.8^{b}	440.0±13.0^{b}	436.0 ± 9.3^{b}	432.0±9.7^{b}	432.0±9.7^{b}
Sperm viability															
Group A	49 8±0.7	52.8±0.3	51.1±0.5	61.4±0.3	ND	36.0±12.0^{a}	31.0 ± 6.0^{a}	14.0 ± 4.0^{a}	9.0 ± 3.0^{a}	11.0 ± 3.0^{a}	11.0 ± 3.0^{a}	6.0 ± 3.0^{a}	4.0 ± 2.0^{a}	6.0 ± 2.0^{a}	3.0 ± 2.0^{a}
B	50.0±0.4	50.0±0.2	58.0±0.4	60.0±0.3	ND	66.0 ±14.0^{b}	86.0± 2.0^{b}	88.0 ± 4.0^{b}	90.0 ± 3.0^{b}	90.0 ± 3.0^{b}	86.0 ± 4.0^{b}	94.0 ± 2.0^{b}	96.0 ± 2.0^{b}	90.0 ± 3.0^{b}	94.0 ± 2.0^{b}

^{a,b}Means in the same rows of each parameter with different superscript alphabets are statistically ($p < 0.05$) different
ND = Not determined.

Table 4.5a: Semen morphological abnormalities of Yankasa rams prior to and post experimental infection with *Fasciolagigantica*.

Parameters	Weeks													
	-4	-3	-2	-1	0-2	3	4	5	6	7	8	9	10	11	12
DMP %															
Group A	0.1±0.1	0.9±0.6	0.6±0.3	0.8±0.4	ND	3.1±0.7^{a}	1.1±0.3	0.7 ±0.3	1.3 ±0.4	0.7± 0.2	2.9±0.5^{a}	3.5±0.7^{a}	3.7±0.3^{a}	2.7±0.5^{a}	0.7±0.2
B	0.6±0.4	1.0±0.5	1.4±0.5	1.4±0.2	ND	1.0±0.5^{b}	0.6±0.4	0.6 ±0.4	1.3± 0.1	0.6 ±0.2	0.6±0.2^{b}	1.0±0.5^{b}	0.6±0.4^{b}	0.5±0.2^{b}	0.4±0.2
CT %															
Group A	3.1±0.9	1.3±0.7	1.4±0.8	0.8±0.4	ND	3.1±0.3^{a}	3.3±0.7	1.6±0.5	1.9±0.5	3.4±0.5^{a}	3.9±0.6^{a}	3.1±0.3^{a}	1.6 ± 0.4	3.2±0.6^{a}	1.6±0.5
B	3.0±1.4	0.8±0.4	1.6±1.1	1.6±0.5	ND	1.1±0.4^{b}	3.2±0.3	1.2 ±0.5	0.8 ±0.3	1.6±0.2^{b}	1.6±0.2^{b}	1.1±0.3^{b}	1.2 ± 0.5	1.7±0.2^{b}	0.7±0.2
BT %															
Group A	2.4±0.5	2.1±0.9	3.4±0.9	1.8±0.5	ND	4.1 ± 0.6	7.1±0.4^{a}	4.4±0.7^{a}	2.4±0.4	5.1±0.6^{a}	6.7±0.7^{a}	3.1 ± 0.6	4.7±0.7^{a}	6.7±0.6^{a}	7.7±0.7^{a}
B	1.0±0.3	3.4±0.9	2.0±1.3	1.6±1.0	ND	1.4 ± 0.5	3.4±1.1^{b}	2.0±0.4^{b}	1.0±0.6	1.2±0.2^{b}	1.3±0.2^{b}	1.4 ± 0.5	1.0±0.4^{b}	1.2±0.2^{b}	0.6±0.4^{b}

^{ab}Means in the same rows of each parameter with different superscript alphabets are statistically ($p<0.05$) different.

Table 4.5b: Semen morphological abnormalities of Yankasa rams prior to and post experimental infection with *Fasciolagigantica*.

parameters	weeks														
AC %															
Group A	0.9±0.3	3.3±0.9	1.7±0.5	1.8±0.6	ND	8.9±1.0^{a}	19.4±1.1^{a}	13.6±1.1^{a}	4.3±1.8^{a}	18.1±0.6^{a}	25.1±2.3^{a}	18.9±1.0^{a}	15.6±1.1^{a}	25.1±2.3^{a}	23.5±1.0^{a}
B	0.3±0.3	3.4±1.0	2.2±1.1	2.0±0.5	ND	1.4±1.4^{b}	0.6±1.5^{b}	2.1±0.7^{b}	1.0±0.3^{b}	8.2±0.7^{b}	1.2±0.7^{b}	2.4±1.4^{b}	3.0±0.7^{b}	5.2±0.7^{b}	1.0±0.3^{b}
FT %															
Group A	2.0±0.8	1.4±0.7	0.7±0.4	1.2±0.5	ND	5.0±0.4^{a}	4.0 ± 0.4	1.8 ± 0.4	4.0±0.8^{a}	3.4±0.7^{a}	6.4±0.7^{a}	5.0±0.4^{a}	1.6± 0.4	6.5±0.8^{a}	1.7± 0.4
B	4.2±1.2	1.0±0.3	1.3±0.4	2.8±0.6	ND	1.4±0.4^{b}	1.2 ± 0.5	1.3 ± 0.9	0.8±0.4^{b}	3.2±0.3^{b}	2.3±0.4^{b}	1.4±0.4^{b}	1.2± 0.9	1.2±0.4^{b}	0.6 ±0.4
DH%															
Group A	2.6±1.8	2.0±0.6	2.4±0.9	1.0±0.5	ND	5.0 ±0.7^{a}	3.8± 0.5	5.3 ±0.7^{a}	10.1±0.5^{a}	2.5± 0.4	5.6 ±0.8^{a}	5.8 ±0.7^{a}	5.2 ±0.7^{a}	5.3 ±0.8^{a}	5.4±0.7^{a}
B	3.8±0.1	1.8±0.6	1.6±0.1	0.6±0.2	ND	2.0±0.5^{b}	1.2± 0.3	1.9±0.4^{b}	2.3±1.4^{b}	1.7 ± 0.2	1.6±0.2^{b}	2.0±0.5^{b}	1.8±0.4^{b}	1.6±0.2^{b}	1.0±0.4^{b}
Mean ABS															
Group A	11.1±4.4	11.0±4.4	10.5±3.7	7.4±2.9	ND	29.2±3.7^{a}	35.2±2.5^{a}	27.4±3.7^{a}	24.1±2.2^{a}	36.9±1.6^{a}	50.9±2.3^{a}	39.1±1.0^{a}	32.4±1.1^{a}	49.9±2.3^{a}	40.4±1.0^{a}
B	12.9±3.7	10.5±3.7	10.2±3.8	10.0±2.1	ND	9.3±3.7^{b}	10.2±4.1^{b}	10.0±0.7^{b}	7.5±1.3^{b}	8.9.±0.7^{b}	8.8±0.7^{b}	9.6±1.4^{b}	8.0±0.7^{b}	8.8±0.7^{b}	7.8±0.6^{b}

^{ab}Means in the same rows of each parameter with different superscript alphabets are statistically ($p<0.05$) different

4.6 Testosterone Concentration

Testosterone average mean values at different period of the week in both groups prior to infection was 3.05±0.6 ng/ml and 3.07±0.9 ng/ml for group A and group B rams respectively, with no significant difference observed (Appendix V). There were significant ($p<0.05$) decreased in testosterone levels observed in group Arams at different period 2.3 ng/ml at week 3 PI.There were, steady decreased in (Mean±SEM) 0.9±0.4 ng/ml testosterone levels were observed at week 5 PI. Thereafter, progressive decreased were observed 1.3 ng/ml and 1.6 ng/ml at week 7 and 9 PIrespectively. While in group B rams the testosterone levels progressively increased 3.4 ng/ml and 7.5 ng/ml at week 7 and 9 PI respectively (Figure 4. 5).

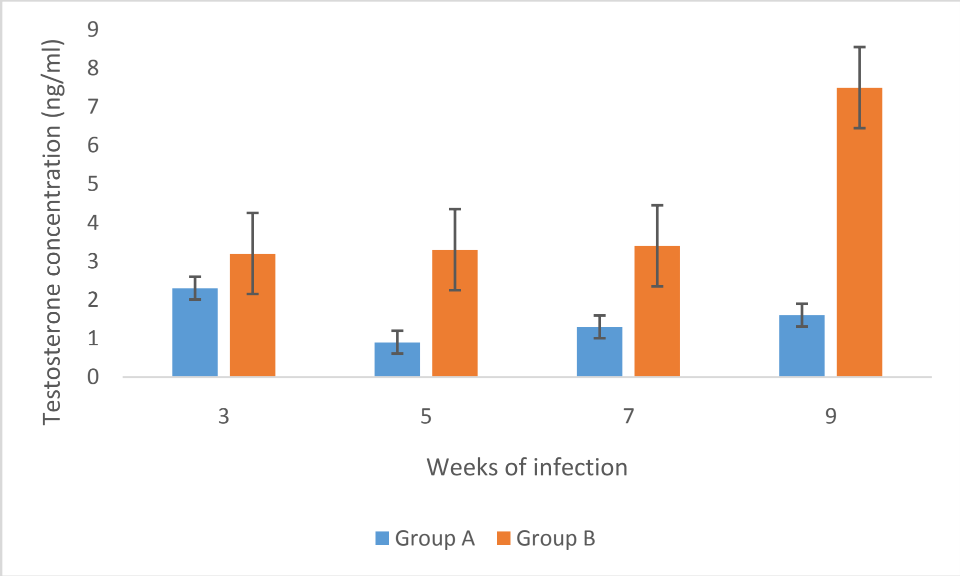


Figure 4.7:Testosterone concentration (ng/ml) of Yankasa rams post experimental infection with *Fasciolagigantica* at week 3, 5, 7 and 9.

4.7 Gonadal and Epididymal Dimensions and Sperm Reserves of Yankasa rams

The length and weight of the testicles and epididymides are presented in Table 4.6. The mean testicular weight of group A rams (88.1±54.3 g)was significantly ($p<0.05$) lower than that of group B rams (210.0±0.0 g). Similarly, testicular length of group A rams(6.8±1.8 cm)was significantly ($p<0.05$)shorter than that of Group B rams (12.6±0.0 cm).

The gonadal and epididymal sperm reserves of group A and B rams are presented in Table 4.7.The Meanvaluesof gonadal sperm reserves of rams in group A($136.0\pm70.0\times10^{6}$/gm) wassignificantly ($p<0.05$) lowerthan that of rams in group B ($377\pm0.0\times10^{6}$ /gm).

Table 4.6: (Mean ±SEM) of gonadal and epididymal dimensions of uninfected and *F. gigantica* infected Yankasa rams.

Variables	Group A	Group B
Paired testicular weight (g)	88.1 ± 54.3^{a}	210.0 ± 0.0^{b}
Right Testis weight (g)	53.4 ± 33.4^{a}	110.0 ± 0.0^{b}
Left Testis weight (g)	34.7 ± 20.9^{a}	100.0 ± 0.0^{b}
Paired epididymis weight(g)	12.8 ± 5.9^{a}	43.1 ± 0.0^{b}
Right epididymis weight (g)	7.1 ± 3.1^{a}	22.5 ± 0.0^{b}
i. Right Caput weight (g)	2.2 ± 0.6^{a}	5.3 ± 0.0^{b}
ii. Right Corpus weight (g)	0.5 ± 0.2^{a}	1.6 ± 0.0^{b}
iii. Right Caudal weight (g)	4.1 ± 2.2^{a}	15.3 ± 0.0^{b}
Left epididymis weight (g)	6.4 ± 3.1^{a}	20.1 ± 0.0^{b}
i. Left Caput weight (g)	1.7 ± 0.8^{a}	5.2 ± 0.0^{b}
ii. Left Corpus weight (g)	0.5 ± 0.2^{a}	1.4 ± 0.0^{b}
iii. Left Caudal weight (g)	3.8 ± 1.9^{a}	14.2 ± 0.0^{b}
Paired testicular length (cm)	6.8 ± 1.8^{a}	12.6 ± 0.0^{b}
Paired epididymis length (cm)	13.5 ± 3.7^{a}	24.7 ± 0.9^{b}
Paired caput length (cm)	3.0 ± 1.0^{a}	7.6 ± 0.7^{b}
Paired corpus length (cm)	6.5 ± 1.7^{a}	11.5 ± 1.3^{b}
Paired caudal length (cm)	4.0 ± 1.0	3.9 ± 0.5

^{a,b}Means in the same rows for each parameter in both groups with different superscript are statistically ($p<0.05$) different.

Table 4.7: (Mean ±SEM) Gonadal epididymal sperm reserves of uninfected and *F. gigantica* infected Yankasa rams.

Variables	Group A	Group B
gonadal sperm reserves ($x10^6$/gm)	136.0 ± 70.0^a	377.0 ± 0.0^b
epididymal sperm reserves ($x10^6$/gm)	55.4 ± 43.5^a	284.7 ± 0.0^b
i caput	53.5 ± 53.5^a	310.0 ± 0.0^b
ii corpus	16.0 ± 8.0^a	140.0 ± 0.0^b
iii cauda	95.0 ± 69.0^a	404.0 ± 0.0^b

^{a,b}Means in the same rows for each parameter with different superscript between group A and B are statistically ($p<0.05$) different.

4.8 Gross Pathology and Histopathology.

Gross pathology of group A rams revealed pale and emaciated carcasses with enlarged liver marked by rounded/blunt edges. Hepatic petechial haemorrhages were observed in addition to adhesion of liver with the diaphragm. Also, distended gall bladder was observed in the carcasses of group A rams during post-mortem examination. Other pathologies observed during post-mortem examination of rams in group A were the presence of haemorrhagic fluid measuring up to 3 litres in the abdominal cavity, pericapsular haemorrhage in the testicles, presence of adult *F. gigantica*flukes and juvenile larvae in the bile duct and hepatic parenchyma respectively (plate III and IV). Post-mortem examination of rams in group B revealed no pathology in all the organs (plate III and IV).

Histological examination of the liver of ramsin group A revealed loss of hepatic architecture and obliteration of sinusoidal spaces (plate V). There was diffuse haemorrhage in the liver, degenerated hepatocytes and hepatic cords, with extensive proliferation of fibrotic tissues. Furthermore, the testes revealed degeneration of spermatogonial cell and seminiferous tubules devoid of spermatids(plate VII). There was significant degeneration of Leydig cells and interstitial cells, with widened intertubular spaces and collapse of some seminiferous tubules (plate VII). No significant histopathological finding was observed in the kidneys and spleens of rams in group A. No histological pathology was observed in the kidney, spleen, testes and liver of rams in Group B.

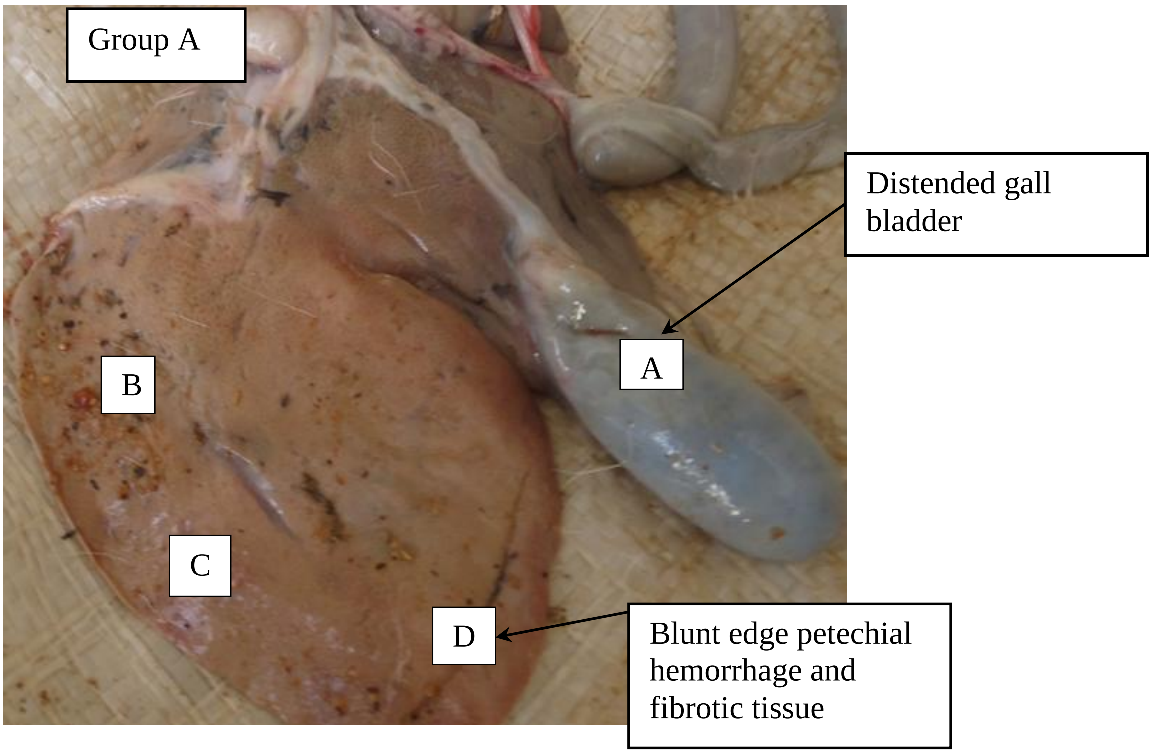
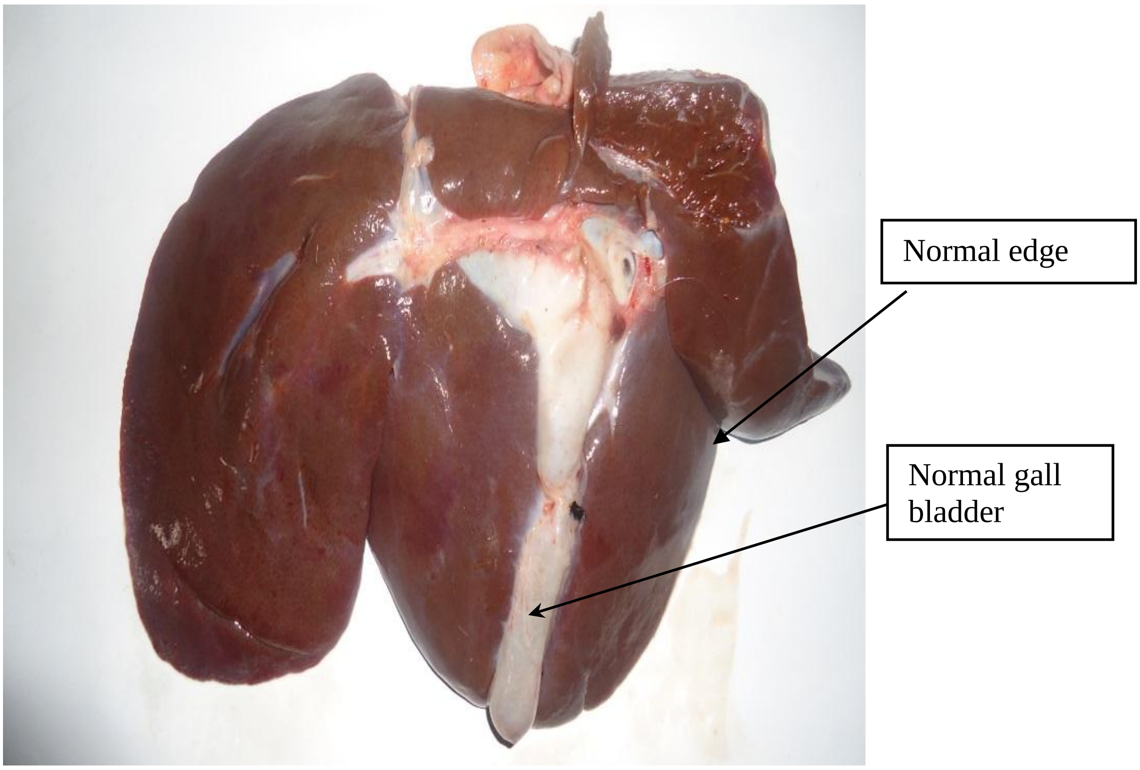


Plate I. The liver of a ram 12 weeks post *F. gigantica* infection, showing hepatomegaly with,(A) distended gall bladder, (B)petechial haemorrhage, (C) fibrosis, and (D) blunt shape.



PlateII.Liver from uninfected ram,12 weeks post experiment showingnormal liver and gall bladder.

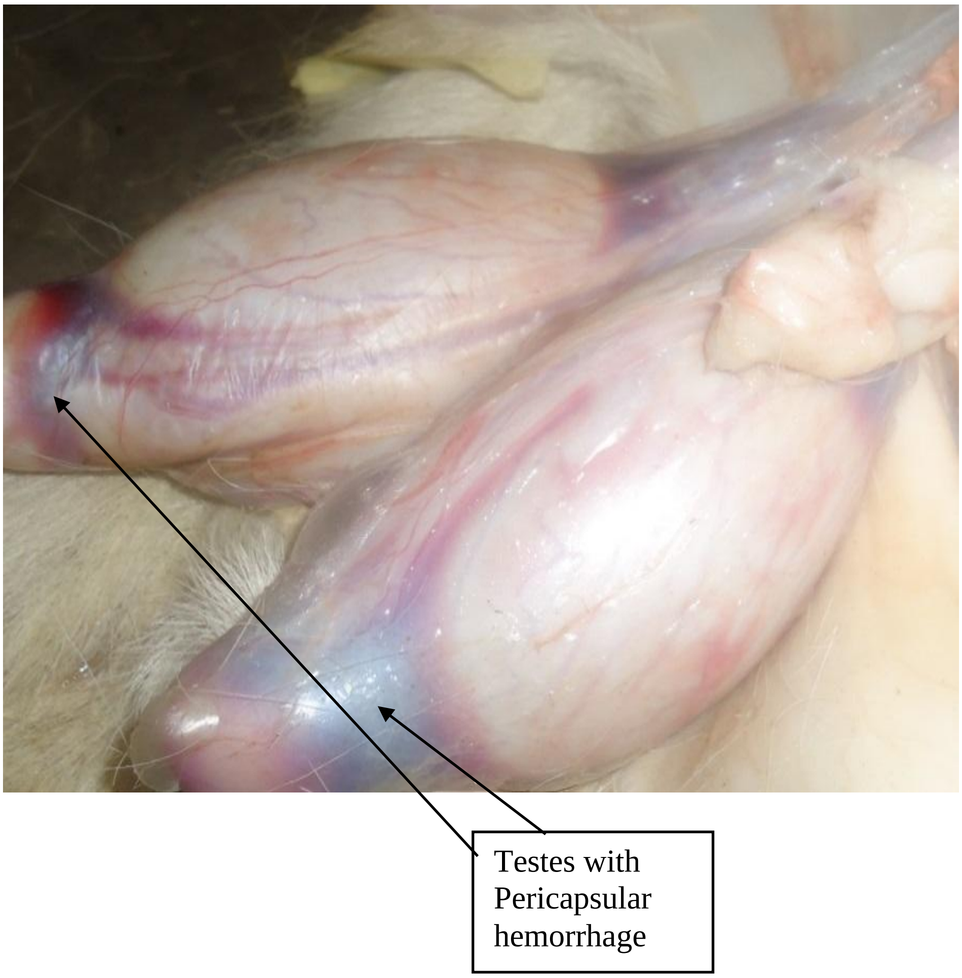


Plate III. Testes of a ram, 12 weeks post *F. gigantica* infection showing pericapsular haemorrhage.

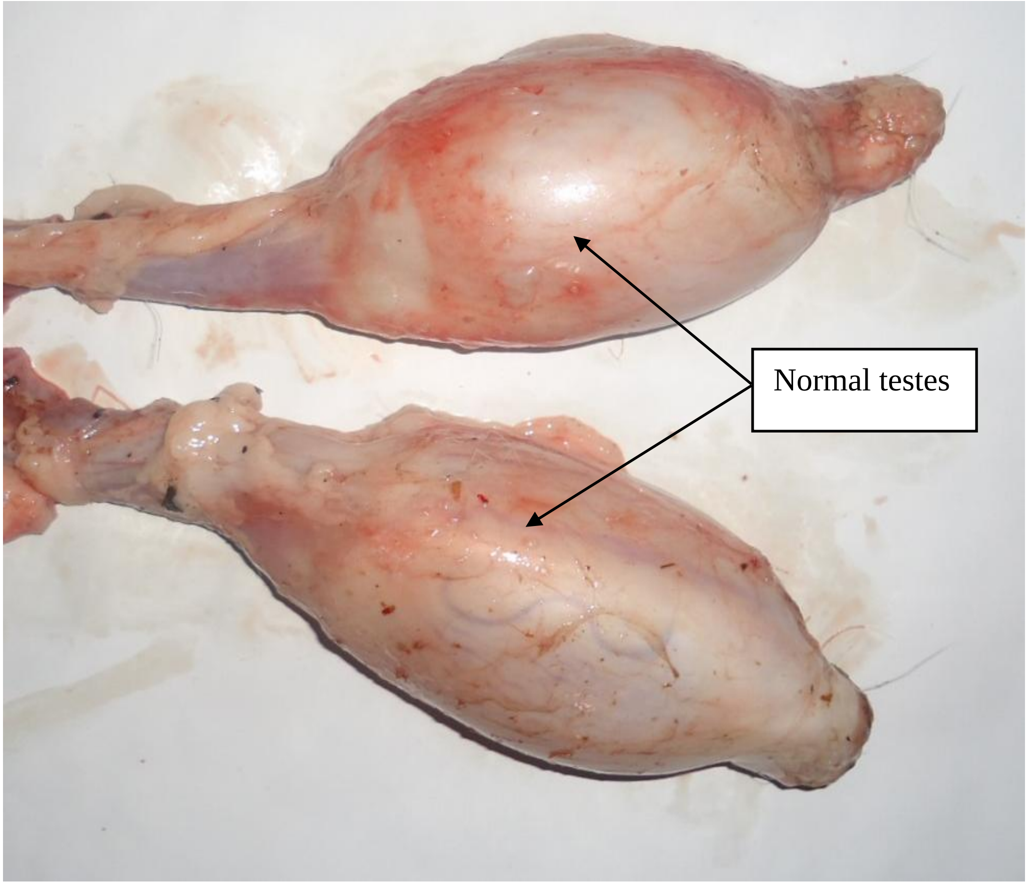


Plate IV. Testesfrom an uninfected ram showing no pathology.

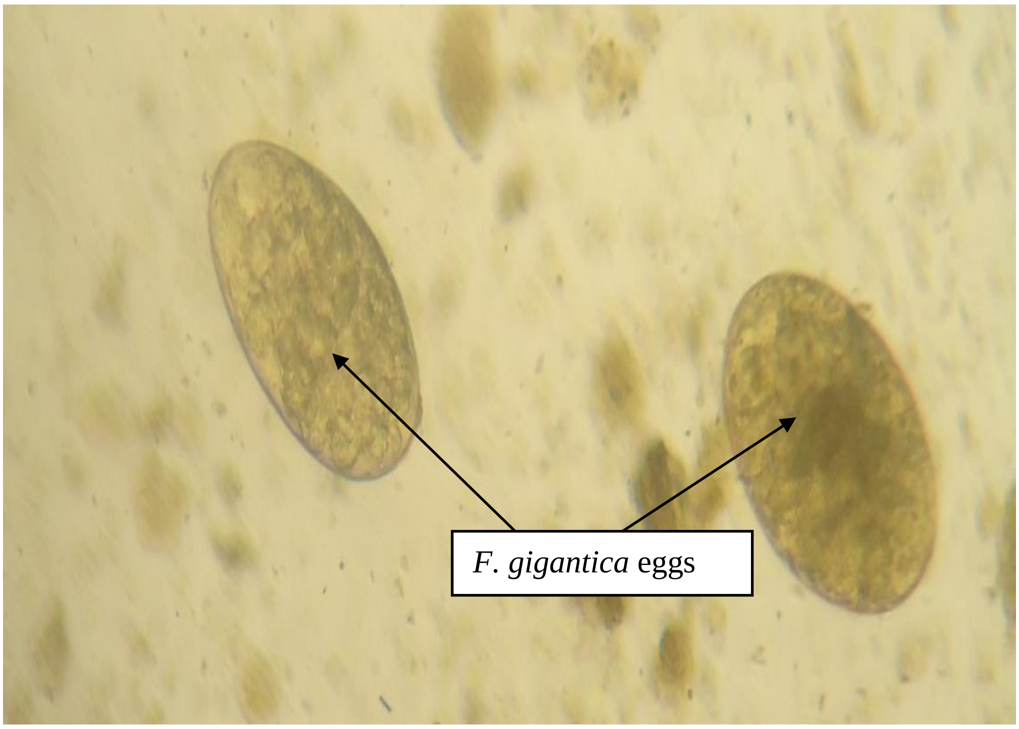


Plate V. *Fasciola* eggs from faecesof infected ram 8 weeks post *F. gigantica* infection under microscope at x40 magnification

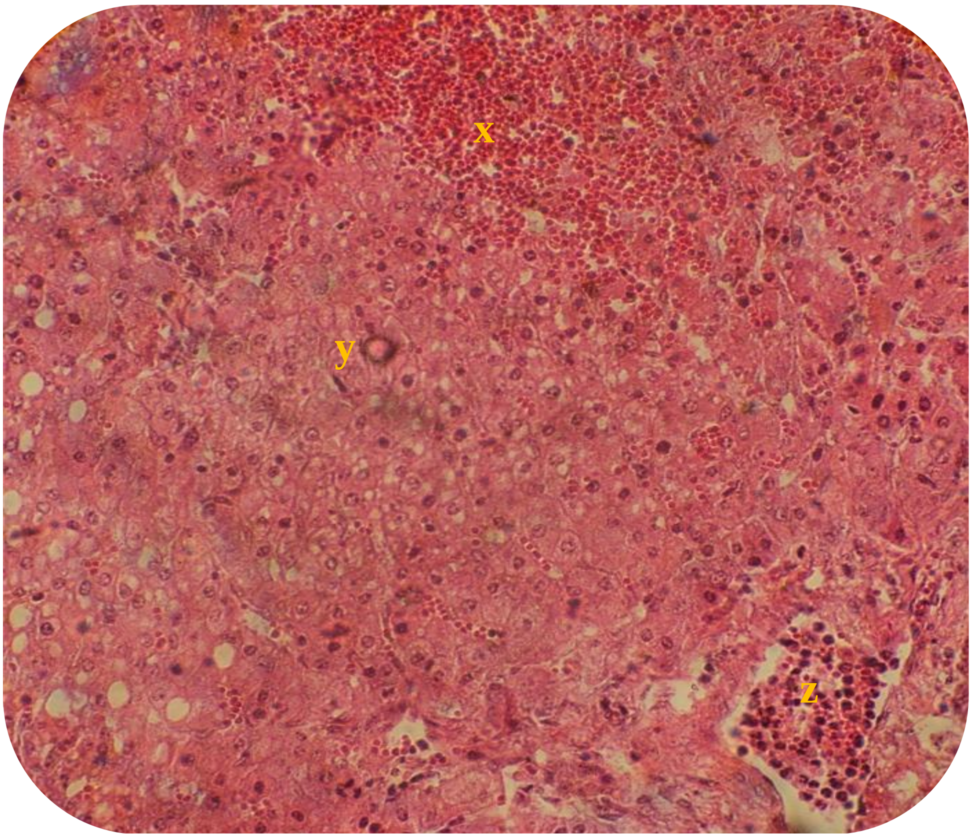


Plate VI. Photomicrograph section of a liver 12 weeks post experimental infection showing(x) haemorrhage (y) degeneration of hepatocytesand (z)congested hepatic cords. (H and Ex400).

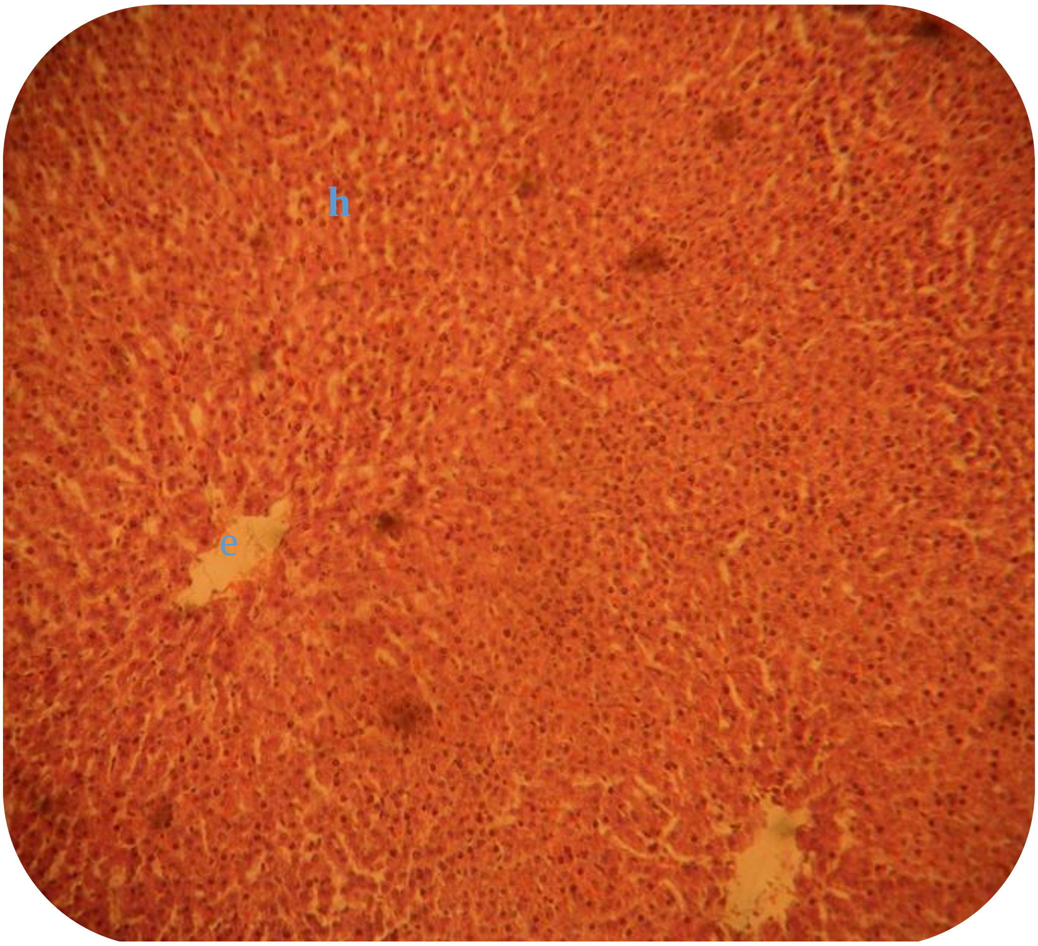


Plate VII. Photomicrograph section of a liver of uninfected ram,12 weeks post experiment showing (h) normal hepatocytesand (e)empty central vein. (H and Ex400).

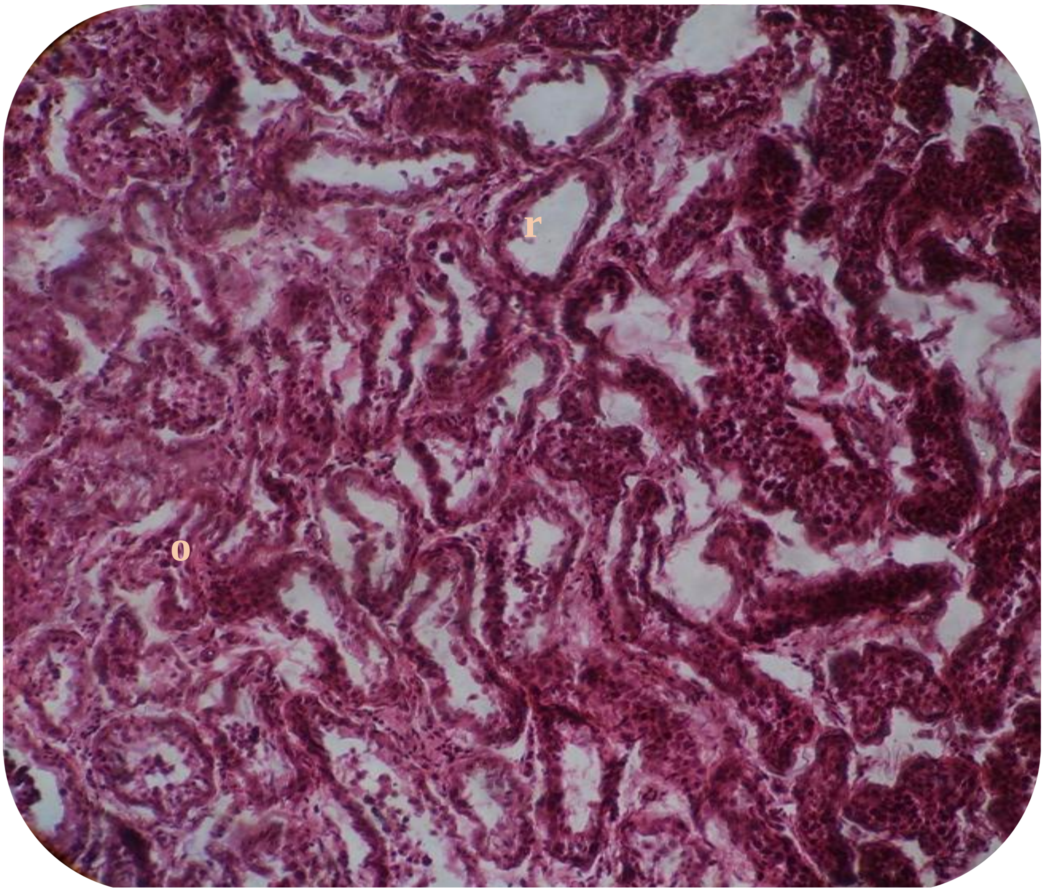


Plate VIII. Photomicrograph of a section of the testis of a ram, 12 weeks post infection with *F. gigantica* showing (o) collapsed of some seminiferous tubules and (r) absence of spermatids. (H and Ex400).

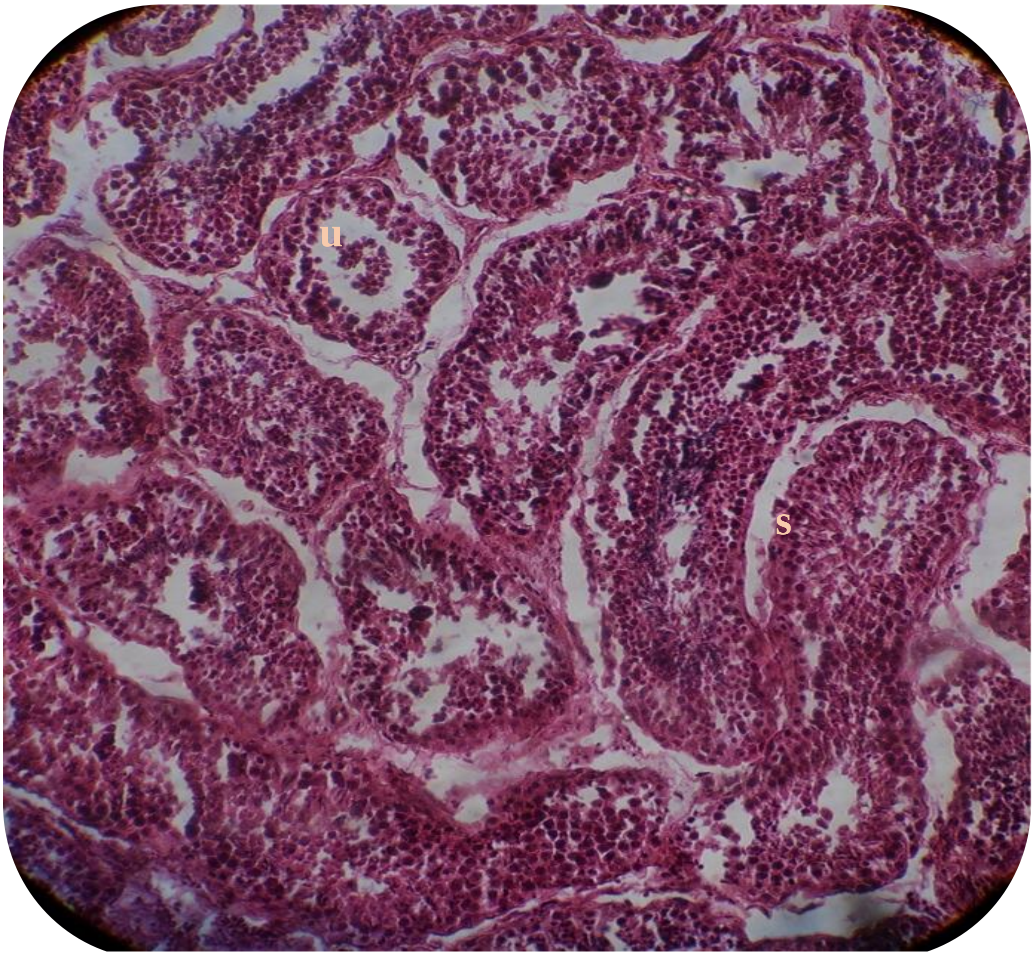


Plate IX. Photomicrograph of a section of the testis of a ram uninfected showing (u) spermatids in some seminiferous tubules and (s) different stages of spermatogenesis. (H and Ex400).

CHAPTER FIVE

DISCUSSION

All infected rams developed a patent infection and started showing clinical signs as early as 3 weeks after infection even though no eggs were found in the faeces. The clinical signs included pyrexia, anemia, ascites, emaciation, anorexia, bilateral muco purulent nasal and ocular discharge were also reported by Ahmed *et al*. (2007).

Fasciola eggs were recovered in the faeces between 7-8 weeks PI contrary to the earlier report by Wiedosari and Copeman, (1990) they are found in feces at 9-10 week PI. This can be attributed to higher dose of metacercariae used in this study and immune status of the animals.

Pyrexia is common in *Fasciola*infection in rams (Ahmed *et al*., 2007), and the elevated body temperature observed in infected rams may affect semen quality. Elevated temperature is known to impair hormonal function in the testes thus, adversely effecting spermatogenesis (Barth *et al*., 2008). Both the interstitial cells of Leydig and the germinal epithelium are Optimally active at a temperature 3-5 ^{0}C below the body temperature (Oyeyemi *et al*., 2009).The pyrexia observed from the 6 week of infection in this study may have been responsible for the diminished semen quality observed during the cause of this research.Marai *et al*. (2007) reported that persistent increase in body temperature may result to heat stress that can affect the process of spermatogenesismay render bucks or rams infertile. Hoogenboezem and Swanepoel, (2000) reported thattesticular degeneration might be due to nutritional deficiencies and management-related factors.Degenerative changes in the seminiferous tubules, testicular degeneration, low semen output, low sperm concentration, high percentage of dead spermatozoa, and sperm abnormalities have also been reported to be caused by heat stress (Kumi-Diaka *et al*., 1981; Sekoni *et al*., 1991; Mamabolo, 1999; Hoogenboezem and Swanepoel, 2000).

Anaemia and eosinophilia as observed in this study are features of *F. gigantica* infection in ruminants and has been reported by other authors (Dargie, 1987; Ahmed *et al*., 2007). The anaemia has been attributed to the direct blood feeding activity of the flukes and trauma caused on the liver parenchyma, distention of the bile ducts and enlargement of the liver was due to the migrations of immature flukes within the liver (Dargie, 1987; Ahmed *et al*., 2007, Yüksek *et al*., 2013). The eosinophilia may be associated with the host response to the tissue migratory phase of the parasite, and increases rapidly persisting until after the flukes enter the bile ducts (Yüksek*et al*., 2013). Fasciolosis induced liver damage, is associated with reduced liver synthesis capacities (Yüksek*et al*., 2013). Therefore the hypoproteinemia observed in the present study may be related to this effect since albumin is only synthesised in the liver (Wada *et al*., 2009). Hypoalbuminemia has been reported in liver flukeinfections in all host species (Behm and Sangaster, 1999; Dargie, 1987; Yüksek*et al*., 2013). Albumin is also the major plasma protein, and its decrease may lead to pot belly and a decrease in the total protein values as seen in this study.

However, this may be obvious since hyperglobulinaemia, as a result of host immune response to infection also occurs in fasciolosis (Dargie, 1987; Chauvin *et al*., 1995; Raodostits *et al*., 1995; Kaneko *et al*., 1997; Behm and Sangaster, 1999; Raaddsma *et al*., 2007). Consequently, because of hypoalbuminemia on one hand and hyperglobulinaemia on the other hand, hyper or hypoproteinemia may result (Dargie, 1987; Yüksek*et al*., 2013) as well as a normal proteinemia (Raodostits *et al*., 1995), may occur dependingupon the severity and length of infection (Radostits *et al*., 1995). The variationsof proteinemia in the present study were in agreement with these previousstudies and did not differ significantly between the infected and control rams.

Decreased body weight in infected rams in this study could be attributed to loss of appetite observed in the rams as has been reported by other workers (Dargie, 1987; Ahmed *et al*., 2007). Ferre *et al.* (1994) demonstrated that the decrease in food intake accompanying fasciolosis is related to the liver injury and compromised synthesis capacities of the liver, and subsequent decrease in serum glucose concentrations. A number of authors have reported an energy deficiency, ketosis and depression in hepatic glycogenic pathways and gluconeogenesis in fasciolosis (Ferre *et al*., 1995; Phiri *et al*., 2005). They postulated that migrating flukes cause severe liver pathology and death of hepatocytes which may potentially reduce glycogen reserves and consequent reduction in available liver glycogen that leads to an increased mobilization of free fatty acids and a secondary increased serum concentration of ketone bodies (Raadsma *et al*., 2007).

A negative energy balance has been found to affect reproduction. Kaneko *et al*. (1997) suggested that chronic hepatitis and liver trauma from parasitic infection could damage the Growth hormone (GH) and GH receptor interactions, resulting in increased circulatory concentrations of GH and increased concentrations of Immunoglobulin GF-1 (IGF) IGF-1. Ferre*et al*. (1994),explained in their studies that metabolic clearance rate of cyclical hormones might be impaired in cirrhosis. Leptin is synthesised and secreted by adipose tissue and is thought to play a key role in the hypothalamus-pituitary-gonadal–axis. If adipose tissue deposition is delayed due to parasitic infections leading to decreased body condition, this can cause delay in attainment of puberty due to depressed serum leptin concentration (Ferre*et al*., 1994).

Effects of *Fasciolagigantica* infection in male reproduction has not been extensively studied but the effects of increased ejaculation time, testicular degeneration and poor semen characteristics observed in this study may be likened to some effects of trypanosomiasis on reproduction in bulls. The role of trypanosomiasis in alteration of reproductive function in sheep and goats has been adequately reported and reviewed (Anosa and Isoun, 1983; Ogwu, 1986; Sekoni, 1990 and Sekoni *et al*., 1994). These include highly elevated ejaculation time and poor semen characteristics due to severe degeneration of the testes and epididymides as revealed by the histopathology in this study.

The semen volume of the infected rams was adversely affected by fasciolosis, with drastic drop in the ejaculate volume toward end of the study.The reason for this progressive drop in ejaculate volume is probably due to anorexia and pyrexia associated with fasciolosis, itmay also be attributed to the deleterious effect of fasciolosis on the reproductive organs, particularly the accessory sex glands responsible for the bulk of the semen. This agrees with the findings of Sekoni *et al*. (2003) and Ahmed *et al*. (2007). The semen volume of the control group continued to increase probably due to improved nutrition and this agreed with the findings of Rekwot *et al*.(1987a).

The sperm motility was adversely affected by fasciolosis and this agrees with findings of Ahamed *et al*. (2007). This can be attributed to the fact that *F. gigantica* was able to totally disrupt energy supply required for sperm motility due to the destruction caused on the liver, this may affect accessory sex glands secretion that is rich in carbohydrate or by their toxic by-product. This agrees with findings of others (Sekoni *et al*., 1991; Ahmed *et al*., 2007). The sperm motility in group B rams continued to increase due to improved nutrition and this agreed with the findings of Rekwot *et al*.(1987a).

Semen concentration was adversely affected by fasciolosis in group A rams. The progressive decrease in sperm concentration in the infected rams can be attributed to faulty spermatogenesis due to persistent pyrexia, testicular degeneration and anorexia observed in this study. This agrees with the findings of same workers (Sekoni *et al*., 2003; Ahmed *et al*., 2007 and Kumar *et al*., 2008).

The sperm morphological defects found in this study were those of the head, body and tail. The head defects involvedacrosome abnormalities and detached head which were noticed inGroup A rams.There wasa progressive increase in sperm morphological defects throughout the investigation period in group A rams, this agrees with the findings of same workers (Sekoni *et al*., 2003; Ahmed *et al*., 2007). It may be possible fasciolosis disrupted the epididymal function of sperm maturation which involves the movement of proximal droplets to the distal position in the initial segment of the epididymis (Sekoni *et al*., 1991; Ahmed *et al*., 2007).

The incidence of sperm middle-piece detached and tail defects following *F. gigantica* infection are possibly similar to those already discussed. The reason for the high incidence of sperm tail defects following the infection could be due to elevated pyrexia and functional damage done to the testes as revealed in histology of this study. This agreed with findings of Roberts, (1971) and Sekoni *et al*. (1991).Equally, Sekoni *et al*.

(1991)reported high incidence of mid piece abnormalities in bovine semen following experimental and natural cases of bovine ephemeral fever.The sperm morphological abnormalities in control group remained within acceptable range throughout the study.

The sperm viability was affected by fasciolosis in group A rams. This can be attributed to decreased appetite. Also interference with post-absorptive metabolism of protein, carbohydrates and minerals had significant effect on semen production as reported by Kumar*et al*. (2008). Structural abnormalities occur in the development of spermatozoa as a result of faulty spermatogenesis which compromise the physiology of the spermatozoa (Salisbury *et al*., 1978). But extreme in temperature, heat stress and malnutrition have been reported to affect the testicular function (Rathore, 1970). Ibrahim*et al*. (1998) reported that anorexia in bucks result in lower ejaculate volume, sperm motility and increase in the percentage of abnormal sperm and dead ratio.

The low testosterone concentration found in this study may have been due to liver destruction during fluke migration in the liver. The liver is the central metabolic organ and as has been postulated by other workers(Fleming and Fetterer, 1986;López-Díaz *et al.* 1998), trauma from migrating flukes can lead to an alteration in the metabolic clearance rate of estrogen or testosterone. The high concentrations of these hormones suppress the release of GnRH as a result of a negative feed-back mechanism,and even when only small liver areas are damaged this may result in significant disturbances or changes in components of blood. Hepatic pathology due to fasciolosis affect mitochondrial bioenergetics metabolism of carbohydrate, protein, lipid and steroid metabolisms as well as bile flow and its composition. Degeneration of hepatocytes was revealed in histological findings of this study.

The low levels of plasma testosterone found in infected rams could also be attributed to the degenerative changes of the interstitial cells and Leydig cells within the testes which caused reduction of testosterone levels. Low levels of plasma testosterone have also been reported in bucks and bulls infected with trypanosomes (Masake, 1980;Sekoni, 1991). The testicular histological changes were attributed to pituitary lesion that interfered with endocrine function along pituitary- gonadal axis. Pituitary gland were not studied in this investigation. The testosterone concentration with respect to time is showing the pulsatile and episodic profiles and this agreed with the finding of Rekwot *et al*. (1997) the difference in testosterone concentration in both groups signified the sexual and behavioral state of the rams.

There was decrease in testicular and epididymal weights and length of Group A rams.This can be attributed to the minimal function of the organs which resulted to disuse atrophy. Kaneko (1984) reported decreased in testicular weight of deer mice (*Peromyscusmaniculata*) experimentally infected with *T. brucei* with marked testicular degeneration and collapse seminiferous tubules, this agrees with the finding of this study as revealed in the histopathology. The weight of the testes and epididymis obtained in control group B of this study are higher compared to the weights reported for other breeds in Nigeria within similar age range in West African dwarf rams, (Ahemen and Bitto, 2007). Kwari, (2001) reported higher testicular and epididymal weight and length in Balami rams. Iheukwumere *et al*. (2008) reported lower testicular weight in some Yankasa rams which differ from the findings of this study for group B rams. This can be attributed to individual genotype and size of the Yankasa rams. The

Balami rams had been reported as one of the largest in size of all indigenous breeds of sheep in Nigeria (Iheukwumere *et al*., 2008).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The result of this study have revealed that fasciolosis due to *F. gigantica* in Yankasa rams is a threat to the reproductive indices of the rams. The need for animal protein is increasing in Nigeria due to our fast growing population,unfortunatelyfasciolosismayrender infected rams infertile hence they cannot reproduce and might be one of the challenges in small ruminants industries. The major challenge is on the socioeconomic aspect of the farmers, infected rams progressively lost weight and become physically weak and anaemic.This has considerable impact on national economy as infected rams can die and the meat productshas poor carcass quality as revealed during post-mortem.

6.2 Recommendations

Semen evaluation should be done from time to time in breeding flocks in fluke-endemic areas. This is to enable identification and exclusion of infertile rams from the breeding flock. This will decongest the flock and allow fertile ram: ewe ratio, that can provide successful breeding and more returns after birth with profits for the farmer.

Federal government should give adequate attention to the control of fasciolosis through awareness campaign and incorporating the preventive measures into regular livestock immunization schedules in Nigeria. Livestock owners and all stakeholders should be educated about the importance of prevention of fasciolosis in male animals in particular by routine deworming prior to breeding periods. There is a need to further study the infertility acquired due to fasciolosis whether either reversible or not in Yankasa rams.

There is a need to employ the use of modern diagnostic aids like ultrasound to ascertain the level of damage caused on the liver with respect to the testicular function.

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APPENDIX I

MEAN (SEM±) CHANGE IN RECTAL TEMPERATURE, BODY WEIGHT AND SCROTAL CIRCUMFERENCE OF YANKASA RAMS PRIOR TO EXPERIMENTAL INFECTION WITH *F. gigantica*.

Parameters	Group	Week-1	Week-2	Week-3	Week-4
Temperature (^{0}C)	A	38.8±0.2	38.0± 0.3	39.1± 0.2	38.8 ± 0.2
	B	38.8 ± 0.1	38.4± 0.2	39.2± 0.1	38.8 ± 0.1
Body weight (kg)	A	16.0 ± 1.0	16.4 ± 0.9	15.6± 0.8	18.0 ± 1.3
	B	17.6± 0.9	17.6 ± 0.9	18.0 ± 1.1	20.4± 0.7
Scrotal circumference (cm)	A	19.4± 1.1	18.8 ± 1.3	19.4 ± 1.3	21.0 ± 1.0
	B	21.0±1.6	20.8± 1.3	22.6 ± 1.8	23.2 ± 1.6

APPENDIX II

RECTAL TEMPERATURE OF YANKASA RAMS PRIOR TO AND POST EXPERIMENTAL INFECTION WITH *Fasciolagigantica*

weeks	Group A	Group B
-1	38.8±0.2	38.8 ± 0.1
-2	38.0± 0.3	38.4± 0.2
-3	39.1± 0.2	39.2± 0.1
-4	38.8 ± 0.2	38.8 ± 0.1
0	38.1± 0.2	38.2± 0.2
1	38.2 ± 0.2^{a}	38.8 ± 0.1^{b}
2	38.6 ± 0.2	38.1 ± 0.1
3	38.6 ±0.1^{a}	38.2 ±0.1^{b}
4	39.5 ±0.1^{a}	38.5 ±0.1^{b}
5	39.5 ± 0.1^{a}	38.8±0.1^{b}
6	39.6 ± 0.1 ^{a}	38.5 ±0.1^{b}
7	39.6 ± 0.2^{a}	38.5 ± 0.0^{b}
8	38.9 ± 0.1^{a}	38.5 ± 0.1^{b}
9	40.1 ± 0.1^{a}	38.7 ±0.1^{b}
10	39.7 ± 0.1^{a}	38.4 ± 0.1^{b}
11	39.0 ± 0.1^{a}	38.6 ± 0.1^{b}

^{a,b}Mean in the rows with different superscript alphabets between group A and B are statistically ($p<0.05$) different.

APPENDIX III

LIVE WEIGHT OF YANKASA RAMS PRIOR TO AND POST EXPERIMENTAL INFECTION WITH *Fasciolagigantica*.

Weeks	Group A	Group B
-1	16.0 ± 1.0	17.6± 0.9
-2	16.4 ± 0.9	17.6 ± 0.9
-3	15.6± 0.8	18.0 ± 1.1
-4	18.0 ± 1.3	20.4± 0.7
0	22.3 ± 0.7	21.8 ± 1.9
1	22.3 ± 0.7	21.8 ± 1.9
2	21.0 ± 0.6	22.8± 1.5
3	20.0± 0.5^{a}	23.0 ± 1.3^{b}
4	20.7± 0.7^{a}	24.0± 1.5^{b}
5	20.6 ± 1.0^{a}	22.8 ± 1.5^{b}
6	19.4 ± 1.0^{a}	23.0 ± 1.1^{b}
7	20.6 ± 1.1^{a}	25.0± 1.0^{b}
8	19.4 ± 1.1^{a}	25.0± 0.9^{b}
9	19.0± 1.1^{a}	25.2 ± 1.1^{b}
10	18.3 ± 1.0^{a}	25.2 ± 1.1^{b}
11	17.7 ± 0.9^{a}	26.0 ± 0.7^{b}

^{a,b}Mean in the rows with different superscript alphabets between group A and B are statistically ($p<0.05$) different

APPENDIX IV

MEAN (SEM±) HAEMATOLOGICAL PARAMETERS OF YANKASA RAMS PRIOR TO EXPERIMENTAL INFECTION WITH *F.gigantica*.

Haematological parameter	Group	Week -1	Week -2	Week -3	Week -4
PCV (%)	A(n=7)	32.6±0.2	32.5±0.9	31.0±2.0	30.6±0.1
	B(n=5)	30.9±0.1	34.2±1.0	30.0±1.4	31.6±1.3
Haemoglobin Conc	A(n=7)	10.8± 0.1	10.8±0.3	10.3±0.0	10.3±0.0
	B(n=5)	10.3± 0.3	12.7±0.0	11.3±0.0	11.2±0.0
RBC	A(n=7)	5.4± 0.2	5.22±0.1	5.3± 0.1	5.2 ± 0.1
	B(n=5)	5.5± 0.2	5.5± 0.2	5.5 ± 0.1	5.4± 0.1
WBC $x10^6$/ml	A(n=7)	8.4±0.3	8.0±0.4	6.1±09	7.4±0.6
	B(n=5)	7.1±0.3	6.8±0.8	5.7±0.3	6.5±0.5
Neutrophils %	A(n=7)	40.8±2.1	36.8±1.9	40.0±2.4	29.6±1.4
	B(n=5)	40.5±2.5	31.0±1.0	34.5±2.5	37.0±2.0
Lymphocytes %	A(n=7)	57.8±2.3	64.4±2.0	69.4±2.9	58.6±2.7
	B(n=5)	61.4±3.1	68.8±1.2	67.0±0.8	62.4±1.1
Monocytes %	A(n=7)	0.6±0.6	0.0±0.0	1.5±1.5	2.0±0.0
	B(n=5)	0.0±0.0	1.0±0.0	2.0±0.0	0.0±0.0
Eosinophils %	A(n=7)	1.0±0.0	0.0±0.0	0.0±0.0	2.0±0.0
	B(n=5)	2.0±0.0	1.0±0.0	0.0±0.0	0.0±0.0
Total protein (g/dl)	A(n=7)	6.0±0.3	5.9±0.4	6.8±0.2	6.8±0.3
	B(n=5)	6.2±0.1	6.4±0.1	6.4±0.4	7.0±0.7

Key:- PCV- Packed Cell Volume, RBC- Red Blood Cell, WBC- White Blood Cell

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APPENDIX V

MEAN (±SEM) TESTOSTERONE LEVEL OF YANKASA RAMS PRIOR TO EXPERIMENTAL INFECTION WITH *Fasciolagigantica*

Groups	weeks	8:00 am	8:30 am	9:00 am	Mean
A (n=7)	-4	4.2±0.3	3.7±0.7	1.0±0.7	3.0±0.6
B (n=5)		3.4±0.7	4.5±1.6	1.3±0.3	3.1±0.8
A (n=7)	-3	3.1±0.8	3.0±0.2	2.8±0.4	3.0±0.5
B (n=5)		3.3±0.6	3.7±1.5	1.8±0.1	2.9±0.7
A (n=7)	-2	3.8±0.8	3.4±0.1	2.1±0.4	3.1±0.4
B (n=5)		3.6±1.4	3.8±1.1	2.7±1.6	3.4±1.4
A (n=7)	-1	3.6±0.5	3.1±0.3	2.6±0.1	3.1±0.3
B (n=5)		3.1±1.0	3.2±1.2	2.5±0.3	2.9±0.8

[a,b]Mean in the same rows with different superscript alphabets between group A and B are statistically ($p<0.05$) different.

APPENDIX VI

MEAN (±SEM) EJACULATION TIME OF YANKASA RAMS PRIOR TO AND POST EXPERIMENTAL INFECTION WITH *Fasciolagigantica*

Weeks	Group A(n=7)	Group B(n=5)
-1	198.0 ± 29.3	178.0 ± 12.4
-2	123.0 ± 11.1	109.0 ± 5.6
-3	96.4 ± 24.6	101.2 ± 6.4
-4	80.4± 10.0	78.4± 3.4
0-2	NB	NB
3	127.7 ± 36.0	111.4 ± 39.8
4	55.9 ± 8.7	46.0± 15.1
5	52.7±9.2	39.0±13.1
6	43.0 ± 7.8^{a}	78.0 ± 14.9^{b}
7	51.4±7.2	41.2±1.2
8	184.1 ± 23.5^{a}	35.4 ± 3.2^{b}
9	204.0 ± 4.2^{a}	44.8 ± 2.8^{b}
10	212.7 ± 3.8^{a}	38.0 ± 3.5^{b}
11	224.0 ± 2.7^{a}	34.4 ± 3.7^{b}
12	232.1 ± 3.6^{a}	32.6 ± 2.7^{b}

^{ab}Mean in the column with different superscript alphabets between group A and B are statistically ($p<0.05$) different.

APPENDIX VII
MEAN (SEM±) SEMEN CHARACTERISTICS OF YANKASA RAMS PRIOR TO EXPERIMENTAL INFECTION WITH *F. gigantica*.

Parameters	Group	Week -1	Week -2	Week -3	Week -4
Semen volume (mls)	A	0.3 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.5± 0.2
	B	0.3 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.1
Semen motility (%)	A	22.0 ± 7.3	57.0 ± 13.9	31.0 ± 11.9	78.0 ± 9.6
	B	29.0 ± 19.1	46.0 ± 19.2	49.0± 13.7	81.8 ± 7.4
Semen concentration ($x10^6$/ml)	A	263.6 ± 36.6	334.2 ± 25.4	224.6 ± 44.8	387.0 ± 12.5
	B	139.6 ± 40.4	300.8 ± 47.1	276.0 ± 64.9	350.6 ± 27.9
Semen PH	A	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0
	B	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0
Semen colour	A	1.2 ± 0.2	1.0±0.0	1.0±0.0	1.0±0.0
	B	1.4± 0.2	1.0±0.0	1.0±0.0	1.0±0.0
Sperm morphological abnormalities (%)					
MPD	A	0.1±0.1	0.9 ±0.6	0.6 ±0.3	0.8 ±0.4
	B	0.6±0.4	1.0 ±0.5	1.4±0.5	1.4 ±0.2
CT	A	3.1±0.9	1.3 ±0.7	1.4 ±0.8	0.8±0.4
	B	3.0±1.4	0.8 ±0.4	1.6 ±1.1	1.6 ±0.5
BT	A	2.4±0.5	2.1 ±0.9	3.4 ±0.9	1.8 ±0.5
	B	1.0±0.3	4.3±0.9	4.0±1.3	1.6±1.0
AC	A	0.9±0.3	3.3±0.9	1.7±0.5	1.8±0.6
	B	0.3±0.3	3.4±1.0	2.2±1.1	2.0±0.5
FT	A	2.0±0.8	1.4±0.7	0.7±0.4	1.2±0.5
	B	4.2±1.2	1.0±0.3	1.3±0.4	2.8±0.6
DH	A	2.6±1.8	2.0±0.6	2.4±0.9	1.0±0.5
	B	3.8±0.1	1.8±0.6	1.8±0.1	0.6±0.2
Total sperm	A	11.1±4.4	12.8±4.4	10.2 ±3.0	7.4±2.1
abnormalities (%)	B	11.9±3.7	12.5±3.7	10.0±3.4	10.0±2.9

Key: - MPD- mid peace detached, CT- coiled tail, BT- bent tail, AC- abnormal acrosome, FT- flat tail, DH- detached head

APPENDIX VIII

METACERCARIAE UNDER x40 MAGNIFICATION