HAEMATOLOGICAL VALUES, SEMEN CHARACTERISTICS AND GONADAL/EPIDIDYMAL SPERM RESERVES OF RABBIT BUCKS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

BY

IMABEN GRACE OPALUWA

DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION,
AHMADU BELLO UNIVERSITY ZARIA, NIGERIA

JULY, 2014

HAEMATOLOGICAL VALUES, SEMEN CHARACTERISTICS AND GONADAL/EPIDIDYMAL SPERM RESERVES OF RABBIT BUCKS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

BY

Imaben Grace OPALUWA, DVM (UNIMAID) 2009
(M.Sc/VET. MED./11938/2010-2011)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA,

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A DEGREE OF MASTERS OF SCIENCE IN THERIOGENOLOGY

DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION,
AHMADU BELLO UNIVERSITY ZARIA, NIGERIA

JULY, 2014

Declaration

I declare that the work in this thesis titled **"Haematological values, semen characteristics, gonadal/epididymal sperm reserves of rabbit bucks experimentally infected with *Trypanosoma brucei brucei*"** has been performed by me in the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria under the supervision of Dr. J.S. Rwuaan, Prof. P.I. Rekwot and Prof. Idris Lawal.

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

IMABEN GRACE OPALUWA		
NAME OF STUDENT	SIGNATURE	DATE

Certification

This thesis titled **"Haematological values, semen characteristics, gonadal/epididymal sperm reserves of rabbit bucks experimentally infected with *Trypanosoma brucei brucei*"** meets the regulations governing the award of masters of Science (M.Sc) of Ahmadu Bello University, Zaria and this is approved for the contribution of knowledge and development of sciences.

Dr. J.S. Rwuaan Chairman, Supervisory Committee	Signature	Date
Prof. P.I. Rekwot Member Supervisory Committee	Signature	Date
Prof. Idris Lawal Member Supervisory Committee	Signature	Date
Dr. A.I. Nwannenna Head, Theriogenology and Production Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.	Signature	Date
Prof.A.A.Joshua Dean School of Postgraduate Studies Ahmadu Bello University, Zaria.	Signature	Date

Dedication

This work is dedicated to God Almighty, the giver, author and finisher of my life, to whom all things are known, the discerning spirit which was my guide to the successful completion of this programme.

To my dear son, Daniel and to my lovely husband, Mr Kuzayed.

Acknowledgement

To God be all the glory, honour, praise, adoration and thanksgiving for sparing my life to have achieved all I did during the course of my studies and successful completion of this programme.

My sincere thanks to my supervisors, Dr. J.S. Rwuaan, Prof. P. I. Rekwot and Prof. Idris Lawal for their inspirational, challenging and encouraging advices, contributions, tireless assistance and painstakingly reading, correcting and proffering suggestions towards the compilation of this study.

Ultimate thanks to my parents Mr. and Mrs. G.I. Opaluwa, who cared, nurtured and trained me to this stage, for their love and magnanimity and above all, their advices which were words of wisdom.

Friendship they say is the wine of life. "A faithful friend is a strong defense, he that hath found such a one hath found a treasure" (Sirach 6:14). Mr. Kuzayed Duniya, you are a wonderful friend, loving husband and a Treasure.
To my lovely son, Daniel, you are a real friend, for your understanding, support and above all companionship during the course of this study; I pray earnestly that God blesses and keep you.

Worthy to note are the amiable and dedicated lecturers of the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria who worked tirelessly to see that knowledge is imparted.

My classmates and friends, Dr. Gloria Magaji, Dr. Julius Amabo, Dr. Ibrahim Shettima, Dr. Ruth Bassi, Dr. Bobwealth Omontese, Dr. Babashani, Dr. Bugau John and a host of others, you were all so wonderful.

Thanks also to all the staff of NAPRI especially of the Artificial Insemination Unit, Mal. Salihu Magaji, Mr. Philip Bassahwa, Mrs. Rahila Luka, Mr. Isaac Amutu, Mr. Jebo Jimen, Sarah Ibrahim and Mal. Isah Mohammed who assisted me in the laboratory work. Also Mr. Ayodele Kakason, Mr. and Mrs. Musa Tanko, God bless you. I am also very grateful to Miss.Yemisi Kate, Mal. Lawal of the Parasitology Department, A.B.U. Zaria.

Precious and lovely sisters, words cannot express my appreciation to you, Mrs. Ejura Jonah, Miss Lisa Opaluwa, Aunty Prophet (Melisa Opaluwa), Joyce Opaluwa over your patience, love and encouragement. God bless.

To all those who supported me in one way or the other, I am highly indebted to them. Thank you and God bless.

Abstract

This study was designed to determine the haematological values, semen characteristics, gonadal and epididymal sperm reserves of rabbit bucks experimentally infected with *T. brucei brucei.* Twenty healthy adult male rabbit bucks were randomly divided into two groups of ten each as control group A and infected group B. The rabbits were kept under intensive management and their pre-infection baseline data on haematological parameters and semen characteristics were taken twice weekly between Mondays and Wednesdays and weekly respectively, for two weeks prior to experimental infection. Stabilates were acquired from the Department of Parasitology and Entomology of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria who also sourced it from NITR, Kaduna State. Group B rabbits were infected with 1 ml of the parasites containing 1 X 10^6 trypanosomes, while group A bucks were left uninfected. Group B infected bucks were monitored for six weeks while the ten others served as control post infection. Blood was collected from each rabbit by venipuncture of the ear vein into heparinised capillary tubes thrice a week (Mondays, Wednesdays and Fridays) between the hours of 9.00 – 11.00 hrs from both groups and analyzed for packed cell volume, haemoglobin and plasma protein. The infected rabbit bucks had mean parasitaemia values of 1.99±0.25. There was progressive decrease in PCV giving total mean values of 40.07±0.49 % and 36.42±1.15 % for group A and B respectively, Haemoglobin concentration had mean values of 14.31±1.01 g/dl and 12.21±0.39 g/dl for group A and B, while plasma concentration had mean values for group A and B as 6.48±0.08 gms/100ml and 6.41±0.17 gms/100ml. Semen was collected once a week (Wednesdays) between the hours of 9.00 – 11.00 hrs from both groups. The semen samples were evaluated for colour, pH, motility, volume, morphology, concentration and spermatozoa live dead ratio. The control bucks had a semen volume total mean value of 0.52±0.05

mls, compared to the infected bucks with a total mean value of 0.43±0.08 mls. There was a consistent decrease in the semen motility from week six post infection giving mean values of 64.67±4.83 % for control group and 45.20±5.79 % for infected group. Spermatozoa concentration progressively decreased from five weeks post infection to ten weeks post infection. Control group had the highest mean spermatozoa concentration of 165.80±23.13 $X10^6$/ml at week five and lowest mean of 50.80±17.81 $X10^6$/ml at week one. Spermatozoa morphology had a mean value of 38.73±1.21 % for normal cells and 17.84±1.32 % for the infected group, detached heads had a mean value of 9.46±1.57 % and 3.85±0.40 % for control and infected groups respectively. Folded tails had mean values of 8.43±1.05 % for control group and 3.92±0.54 % for infected group. The mean values for bent tails for the control group was 11.35±0.90 % and 6.36±1.18 % for infected group. Percentage live sperm cell ratio control group had live mean values of 59.33±2.21 % compared to infected group that had live mean values of 48.11±5.86 %. At the end of the experiment, two bucks from each of the groups were sacrificed, their testes were harvested for the determination of gonadal and epididymal spermatozoa reserves. The mean gonadal spermatozoa reserves of group A and group B rabbit bucks were 247.6 ± 65.13 $X10^6$/g testis and 173.1 ± 26.88 $X10^6$/g testis, respectively. The mean epididymal spermatozoa reserves of group A and group B rabbit bucks were 96.50 ± 31.61 $X10^6$/g and 30.92 ± 12.94 $X10^6$/g, respectively. It is therefore concluded from this study that trypanosomosis has adverse effects on haematological values, semen characteristics and gonadal and epididymal sperm reserves of rabbit bucks.

This study has shown that *T. brucei brucei* is pathogenic to rabbit bucks causing serious reproductive disorders which may have a negative effect on rabbit production and the socio-economic wellbeing of the farmers and people in the tsetse endemic areas in the country where rabbit farming is important.

Table of Contents

Title

Title Page----------ii

Declaration----------iii

Certification----------iv

Dedication----------v

Acknowledgement----------vi

Abstract----------vii

Table of contents----------ix

List of figures ----------xiii

List of tables----------xiv

List of plates----------xv

List of Appendices----------xvi

Abbreviations----------xvii

CHAPTER ONE: INTRODUCTION

1.1 Background of the Study----------1

1.2 Statement of Research Problem----------4

1.3 Justification of Research Problem----------5

1.4 Aim----------6

1.5 Objectives----------6

1.5.1 Research Question----------7

CHAPTER TWO: LITERATURE REVIEW

2.1 Rabbit Production in Nigeria----------8

2.2 Breeds of Rabbit----------10

2.3 Problems of Rabbit Production in Nigeria----------11

2.3.1 Climate----------11

2.3.2 Diseases of Rabbits----------11

2.3.2.1 Non infectious conditions----------11

2.3.2.2 Infectious conditions- viral diseases----------12

2.3.3 Bacterial diseases----------12

2.3.4 Fungal diseases----------14

2.3.5 Parasitic diseases----------14

2.4 Reproduction in Rabbits----------20

2.4.1 Gonad development and puberty in rabbit bucks----------22

2.4.2 Spermatozoa production----------24

2.4.3 Methods of Semen collection----------35

2.4.3.1 The Artificial vagina----------36

2.4.3.2 Electroejaculator----------36

2.4.3.3 Rectal massage----------37

2.4.3.4 Abdominal massage/digital massage of the cloaca----------37

2.5 Semen Analysis----------38

2.6 Factors Affecting Semen Production----------42

2.6.1 Breed (Genetics)----------42

2.6.2 Nutrition----------42

2.6.3 Diseases (health)----------44

2.6.3.1 Effects of Trypanosomosis on Animal Reproduction----------46

2.6.3.2 Pathogenesis and clinical signs----------48

2.6.4 Season----------50

2.6.5 Management----------51

2.6.6 Collection frequency----------51

2.6.7 Age----------52

2.6.8 Temperature----------52

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Location----------54

3.2 Acquisition and Management of Experimental Animals----------54

3.3 Experimental Infection of the Animals----------55

3.4 Collection of Blood Samples----------55

3.4.1 Haematological analysis of the blood samples ----------56

3.4.2 Parasitological examination----------56

3.5 Semen Collection and Evaluation----------58

3.6 Gonadal Sperm Reserves----------60

3.7 Epididymal (extragonadal) sperm reserve----------61

3.8 Histopathological Examination----------62

3.9 Statistical Analysis----------62

CHAPTER FOUR: RESULTS

4.1 Parasitological Profile of The Infected Rabbits----------63

4.2 Clinical Signs----------66

4.3 Haematological Values----------70

4.4 Semen Characteristics----------79

4.5 Gonadal/Epididymal Weight (G), Length (Cm) and Spermatozoa Reserves—----------94

4.6 Histopathological Findings----------99

CHAPTER FIVE: DISCUSSION----------104

CHAPTER SIX: CONCLUSION AND RECOMMENDATION----------109

REFERENCES----------110

APPENDICES----------133

List of Figures

Figure 4.1: Mean (±SEM) Parasitological Profile of infected rabbit bucks post infection----------65

Figure 4.2: Mean (±SEM) Packed Cell Volume (%) of infected and non infected rabbit bucks pre and post infection----------72

Figure 4.3: Mean (±SEM) haemoglobin concentration of infected and non infected rabbit bucks pre and post infection----------75

Figure 4.4: Mean (±SEM) plasma protein concentration values of infected and non infected rabbit bucks pre and post infection----------78

Figure 4.5: Mean (±SEM) semen volume of infected and non infected rabbit bucks pre and post infection----------81

Figure 4.6: Mean (±SEM) spermatozoa motility (%) of infected and non infected rabbit bucks pre and post infection----------84

Figure 4.7: Mean (±SEM) spermatozoa concentration (X 10^6/ml) of infected and non infected rabbit bucks Pre and post infection----------87

Figure 4.8: Mean (±SEM) spermatozoa morphological defects of infected and non infected rabbit bucks pre and post infection----------90

Figure 4.9: Mean (±SEM) spermatozoa live ratio (%) of infected and non infected rabbit bucks pre and Post infection----------93

List of Tables

Table 4.1: Mean (±SEM) Parasitological profile (parasitaemia) of infected and non infected rabbit bucks pre and post infection----------64

Table 4.2: Mean (±SEM) Packed Cell Volume (%) of infected and non infected rabbit bucks pre and post infection----------71

Table 4.3: Mean (±SEM) Haemoglobin concentration (g/dl) of infected and non infected rabbit bucks pre and post infection----------74

Table 4.4: Mean (±SEM) Plasma Protein Concentration (gms/100ml) of infected and non infected rabbit bucks pre and post infection----------77

Table 4.5: Mean (±SEM) Semen Volume (mls) of infected and non infected rabbit bucks pre and post infection----------80

Table 4.6: Mean (±SEM) Spermatozoa motility (%) of infected and non infected rabbit bucks pre and post infection----------83

Table 4.7: Mean (±SEM) Spermatozoa Concentration (X 10^6/ml) of infected and non infected rabbit bucks pre and post infection----------86

Table 4.8: Mean (±SEM) % values of Spermatozoa Morphological Abnormalities of infected and non-infected Rabbit bucks pre and post infection----------89

Table 4.9: Mean (±SEM) Live Spermatozoa (%) of infected and non infected rabbit Bucks pre and post infection----------92

Table 4.10: Mean (±SEM) Testicular and epididymal weight of infected and non Infected Rabbit bucks post infection----------95

Table 4.11: Mean (±SEM) Testicular length of infected and non infected rabbit Bucks post infection----------96

Table 4.12: Mean (±SEM) Gonadal and extra gonadal spermatozoa reserve of infected and non infected rabbit bucks post infection----------98

List of Plates

Plate I: A. Photograph of the genitourinary tract of the control buck showing normal scrotal skin (A) and penile region (arrow).
B. Photograph of the genitourinary tract from the infected group. Note the scrotal dermatitis/orchitis (arrowed) and balanoposthitis (BP) at 13 days post infection-------67

Plate II: A. Photograph of the genitourinary tract of a buck with increased scrotal diameter on both sides (arrow), scrotal necrosis and excessive thickening of the scrotal corneum (SC) of the left side 29 days post infection.
B. Photograph of the genitourinary tract of a buck showing balanoposthitis (BP) 29 days post infection----------68

Plate III: Photograph of the testes and epididymis of a buck showing inflammation and haemorrhages (arrow) 29 days post infection----------69

Plate IV: Photomicrograph of the epididymal lumen of an infected buck. Note the infiltration of inflammatory cells around the lumen and the empty epididymal lumen (arrow). H & E stains X40----------100

Plate V: Photomicrograph of the epididymis of an infected buck. Note areas of necrosis (Arrow) H & E stain X 40 ----------101

Plate VI: A. Photomicrograph of normal testis from the control group. Note the intact seminiferous tubules with active spermatogenic activity
B. Testis from an infected buck showing necrotized seminiferous tubules (arrow) H and E stains X 40----------102

Plate VII: Photomicrograph of testis from an infected buck showing infiltration of inflammatory cells surrounding existing seminiferous tubules (Arrow) H & E stains X40----------103

List of Appendices

Appendix I: Photograph showing adapted Artificial vagina for rabbit bucks--------133

Appendix II: Blood picture showing *T. brucei* infection in rabbit bucks----------134

Abbreviations

AV	Artificial vagina
D	Days
E & N	Eosin and Nigrosin
G	Grammes
Hb	Haemoglobin
H & E	Haematoxylin and eosin
Kg	kilogram
M	meter
Min	minute5
Ml	milliliter
Mm	millimeter
°C	Degree Celsius
°E	Degree east
°N	Degree north
PCV	Packed cell volume
PATTEC	Pan African Tsetse and Trypanosomiasis Eradication Campaign
Pp	Plasma protein
PSS	Physiological saline solution
rpm	Rounds per mi
T	Trypanosoma
µ	Microns
µm	micrometre
SEM	Standard Error of the Mean
spp	Specie

SIT	Sterile Insect Technique
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The search for alternative sources of animal protein to meet up the population challenge is essential as the human population growth in developed countries is stabilizing while that of developing countries including Nigeria is on a rapid increase (Allen, 1993; Ahemen and Bitto, 2007). Economic indices indicate that as this population trend continues, more people are to be fed. Agricultural products need to be increased rather than through food importation into such countries (Allen, 1993).

Animal protein is very vital as it contains all the essential amino acids needed for the growth, development and maintenance of human life and a shortage of this protein is prevalent in most parts of Africa where it is estimated that on the average 10g of animal protein is consumed per day as compared to the recommended daily intake of 35g (FAO, 1986; Ugwu, 2007). Obioha (1992) and Egbunike (1997) observed that the level of consumption of meat and other sources of animal protein in Nigeria is estimated at about 6.0-8.4g per head per day which is about 20g less than the minimum requirement by the National Research Council of the United States of America and is also less than about 13.5g per day prescribed by the WHO.

To meet up with the fast growing population's demand for animal protein, viable options need to be explored and evaluated. This can be achieved by the use of livestock species that are yet to play a major role in animal production within these countries, increase in the output of animal products through the utilization of animals with short generation intervals such as the rabbits, poultry and pigs (Ozor and Maduekwe, 2001;

Adebayo and Sorungbe, 2002; Owen *et al.*, 2008). The camel which is usually used for a variety of purposes including draught and transportation in arid and desert regions of the world is now also an important source of animal production resource for meat, milk, hides and drought power (Tibary *et al.*, 2005). Poultry and pigs require food sources which are in direct competition with man while fortunately, rabbits can be produced from the enormous forages and feed material that freely abound in the tropics (Aduku and Olukosi, 1990; Biobaku, 1993).

Nigeria can boast of 13.9 million cattle, 34.5 million goats, 22.1 million sheep, 1.7 million rabbits and 0.5 million Guinea pigs (FDL, 1992; Adebowale, 2012) revealing that rabbits have a low population as compared with other livestock in the country. According to the Presidential Task Force on Alternative formulation of Livestock feeds (1992), rabbits are still the least populated livestock kept in the country.

Trypanosomosis is a group of protozoan infections of both man and animals caused by trypanosomes. The most occurring pathogenic species of trypanosomes consist of *Trypanosoma brucei, T. vivax, T. congolense, T. simiae and T. evansi* in animals (Sekoni, 1994; Pepin and Meda, 2001; Kamuanga, 2003; Bawa *et al.*, 2005; Courtin *et al., 2008*).

African animal trypanosomosis (AAT) also known as Nagana is a Zulu word meaning "to be depressed" (WHO, 2001; Courtin *et al., 2008*). It is a disease complex caused by tsetse-fly transmitted *Trypanosoma congolense, T. vivax, T. brucei* and *T. simiae* or mixed infections with one or more of these trypanosomes of the family Trypanosomatidae, genus *Trypanosoma* (Kamuanga, 2003; Courtin *et al., 2008*). Animal trypanosomosis in Africa poses one of the most serious veterinary problems in the world

causing a major constraint to the agricultural and socio-economic development of tropical Africa (Gasser, 1963; Ilemobade, 1981; Llewellyn *et al.,* 1987; Ikede, 1989). While most other animal diseases have been successfully controlled during this century, trypanosomosis continues to pose a major threat to animal production in sub-Saharan Africa (Pepin and Meda, 2001; WHO, 2001; Kamuanga, 2003; Bawa *et al.,* 2005), despite the age-long attempts to control it (Omotainse *et al.,* 2000).

Tsetse fly (Glossina), the vector of trypanosomosis occurs only in Africa and about forty countries in Africa are affected by the disease, where it has contributed to gross reduction in animal protein available to man (Sekoni, 1994). The risk of trypanosomosis in most of these areas prevents farmers from keeping cattle and small ruminants thereby accounting for Africa's low livestock productivity. Africa produces one-seventeenth of animal protein per one hectare, compared to the quantity Europe produces despite the abundance of livestock in Africa than Europe (ILRAD, 1990).

ILRAD (1990) reported that most of the agriculturally productive areas of Africa are infested by tsetse flies thereby making the negative impact of trypanosomosis on livestock enormous. Africa's cattle population and comparable numbers of small ruminants are at risk from the disease. Trypanosomosis results in emaciation with marked reduction in live weight changes, which is also associated with the production of poor quality ejaculates, reduced spermatozoa motility and spermatozoa concentration (Akpavie *et al.,* 1987), low foetal weights, premature births, neonatal losses and some other indices of poor reproductive performance.

Reports by Voh and Otchere (1989) have shown that ruminants in Nigeria kept under traditional management systems have lower productivity compared to those kept in

government ranches and other institutional farms. Their report therefore shows that the low level of animal protein production in Nigeria is not as a result of the numbers of animals, but their poor productivity due to management systems and disease factors.

1.2 Statement of Research Problem

Animals can host human pathogenic parasites, especially *Trypanosoma rhodesiense*. Domestic and wild animals are important reservoirs of the parasite. The disease in domestic animals, particularly cattle, is a major obstacle to the economic development of affected rural areas. Trypanosomosis remains a major public health problem to man and his livestock over much of tropical Africa. The disease is difficult to treat and is deadly, if untreated. It is difficult to estimate the overall burden of African trypanosomiasis because of under - diagnosis in the most heavily infected countries. However, it is believed to be in the vicinity of 100,000 new cases per year, with between one third and half of the cases remaining undetected and untreated (Pepin and Meda, 2001).

Chemotherapy is the most widely used means of controlling the disease. However, effectiveness of the drugs available is limited by a number of factors, which include increasing parasite resistance (Afewerk *et al.*, 2000), treatment failures, unacceptable toxicity (Akanji and Ngaha, 1989; Triolo, 1990; Onyekwelu, 1999), unavailability of drugs, logistics of administration, long period of treatment, and high cost.

Much of the studies on pathogenic animal trypanosomes have been conducted using either *Trypanosoma vivax* or *Trypanosoma brucei* and frequently in the rat, cattle, sheep and goat. Since the rabbit is a non-traditional meat source in many homes, its husbandry

is becoming increasingly important for various reasons including its low - venture starting capital and high multiplication rate. The present study was designed to determine the effects of trypanosome infection on haematological values, semen characteristics and gonadal and epididymal sperm reserves of rabbit bucks infected with *Trypanosoma brucei brucei*.

1.3 Justification of Research

Livestock production in Nigeria has not met the desired protein demand of the populace so far. Most people both in rural and urban areas seek alternative sources of meat such as wildlife, dog, donkey and camel meat (Ibe, 1999). There is need, therefore, to increase livestock production to meet the increasing demand for animal protein in Nigeria (Jibril *et al.*, 2010). Trypanosomosis is a limiting factor to livestock production in sub- Saharan Africa, despite all the attempts at controlling it (Kamuanga, 2003).

Since rabbit is now becoming a very important cheap source of meat coupled with its low venture starting capital and high multiplication rate, it is necessary to determine the haematological values, gonadal and epididymal sperm reserves and semen characteristics of rabbit bucks infected with *Trypanosoma brucei brucei.*

There is dearth of previous documentation on rabbit production in Nigeria therefore the need to identify the productivity potential of rabbit bucks infected with *Trypanosoma brucei brucei*.

This research becomes imperative because of the increasing rate at which animal pets that could act as reservoirs of zoonotic and non-zoonotic diseases are being imported and exported in and out of different countries.

1.4 Aim

The aim of this work was to determine the effects of *Trypanosoma brucei brucei* infection on haematological values, gonadal/epididymal sperm reserves and semen characteristics of rabbit bucks experimentally infected with *Trypanosoma brucei brucei*.

1.5 Objectives

The specific objectives were to:

- Determine the haematological values of rabbit bucks infected with *T. brucei brucei*.
- Determine the effects of *T. brucei brucei* infection on semen characteristics of rabbit bucks.
- Determine the gonadal and epididymal sperm reserves of rabbit bucks infected with *T. brucei brucei.*

1.6 Research Question

What is the effect of experimental *Trypanosoma brucei brucei* infection on the haematological values, semen characteristics, gonadal and extragonadal spermatozoa reserves of rabbit bucks?

CHAPTER TWO

LITERATURE REVIEW

2.1 Rabbit Production in Nigeria

Vietmeyer (1985) described rabbit (*Oryctolagus cuniculus*) as micro livestock species appearing to be one of the cheapest and fastest means of producing high quality animal protein and fast-growing livestock. Rabbits possess a number of features that might be of advantage in the small holder subsistence – type integrated farming in developing countries. Rabbit production is a veritable way of alleviating animal protein deficiency in Nigeria (Abdulmalik, 1994; Hassan and Owolabi, 1996; Ajala and Balogun, 2004).

Rabbit production in Nigeria could be described as rudimentary, developing or emergent as compared to countries such as France, Hungary, China and the United State, this is evident from the small rabbit keeping population in which gender bias and sociological status of rabbit farmers, weak inventory of rabbit keeping infrastructure, low consumption rate of rabbit meat, absence of an organized or thriving market for rabbit meat products and lack of governmental and institutional support limits the expansion of rabbit production (Onifade *et al.,* 1999).

Rabbit meat falls sixth after beef, fish, mutton, goat meat (chevon) and bush meat or game animals in the parametric assessment of meat animal production and consumption in Nigeria (Onifade *et al.,* 1999). Aduku and Olukosi (1990) and Onifade *et al.,* (1999) reported that rabbit production has a lot of benefits among which includes its high adaptability, easiness to handle and manage, high growth rate, high efficiency in converting forage to meat, short gestation period, very high prolificacy and fecundity levels, its returns for money, high quality meat products (it contains high protein level of

about 20.8%, low sodium, low fat and cholesterol levels which compares favorably with the local bush meat) its use for laboratory processes and as pets and its highly soil enriching manure, and its consumption is without cultural and religious biases (Biobaku and Oguntona, 1997; Omole *et al.,* 2005). The presence of caecal microbes enables the rabbit to digest large amounts of fibrous feed as most non ruminant species cannot (Taiwo *et al.,* 1999). High prices of beef, chevon, mutton, chicken and frozen fish is a good point for rabbit meat which is not only cheap to produce but saves cost of refrigeration as its meat is supplied in piece – meal suitable for a family's need or a small party (Ajala, 1990; Aduku and Olukosi, 1990).

Rabbit farming in Nigeria is faced with myriad of problems, which have resulted to a gross shortage of meat to meet up the population challenge in our country (Nworgu, 2007). The growth rate of the Nigerian agricultural sector is below the potentials of natural and human resources due to high cost of agricultural inputs, poor funding of agriculture, inadequate functional infrastructural facilities, inconsistencies of government agricultural policies, inadequate private sector participation, poor mechanized farming and little or no adoption of some simple agricultural technologies developed by scientists (Nworgu, 2007).

Ironkwe and Amaefule (2007) reported between 83% and 140% returns on capital investment on rabbit farming but despite these attractive characteristics, Nigerians still do not appreciate the rabbit meat which consequently resulting to its neglect.

2.2 Breeds of Rabbits

The United States Department for Agriculture (USDA) has classified rabbits according to size, weight and type of pelt and small rabbits weigh about 1.4 – 2.0 kg at maturity, medium breeds; 4.0 – 5.4 kg, and large breeds; 6.4 – 7.3kg (USDA, 1972). Based on this classification, there are two most popular breeds for meat production which includes the New Zealand White and the Californian. These breeds are most popular because they combine white fur and good growth characteristics. The New Zealand rabbits are slightly larger than the Californian, 4 – 5.9kg and 3.6 – 4.5kg, respectively. The New Zealand rabbit has a completely white, red or black body, whereas the Californian is white with colored nose, ears and feet. The two most popular rabbits for fur production are the Rex and the American Chinchilla. The Rex is slightly smaller than the American Chinchilla, 3.2kg and 4.5kg (USDA, 1972). At present there are many breeds of rabbit being used for both meat and skin production in developing countries. In Brazil there are the New Zealand White, Californian, Chinchilla, Palomino, Hollander, Rex, Dalmation, Flemish Giant, New Zealand Red, Barboleta, Champaigne, d'Argent. In Ecuador there are the New Zealand White, Blue Viennese, Silver German and Angora. In Malawi there are the New Zealand White, Californian, Angora, Rex. In Nepal there are the Californian Hybrids while in Ghana there are the Thuringa, Blue Viennese, Flemish Giant, Checkered Giant, Lop, Californian, Alaska, and the Yellow Silver (USDA, 1972). In Nigeria, the most common breeds include the New Zealand White, Californian, Angora, Rex amongst others (Aduku and Olukosi, 1990). All of these breeds of domestic rabbits are descendants of the European wild rabbit, Oryctolagus cuniculus (Aduku and Olukosi, 1990). Although many of these are breeding successfully in various countries, the most popular breeds are the New Zealand White and the Californians. These two

breeds are also the most popular in commercial rabbit industries in the developed countries. New Zealand Whites and Californians are amongst the best breeds available for meat production considering various production traits such as fertility, growth and feed conversion rates under commercial conditions, (Bombeke, *et al.*, 1975).

2.3 Problems of Rabbit Production in Nigeria

2.3.1 Climate

Heat is one of the most important climatic factors which may affect rabbit production in the tropics. The rabbit is very largely dependent on respiratory evaporation for the regulation of its body temperature and this confers only a limited power of adaptation to hot climates. Heat is also dissipated by radiation and convection, but these are somewhat restricted by the rabbit's furry covering. Johnson *et al.,* (1957) reported that short hair and larger ears helped the cooling process in New Zealand White rabbits.

2.3.2 Diseases of Rabbits

2.3.2.1 Non infectious conditions

The common non infectious conditions in rabbits include cannibalism, sore hocks, dental malocclusion, bloat and fur eating. However, cleanliness and good management, which not necessarily have to be sophisticated or involve the use of expensive drugs, can be extremely effective in the prevention of diseases (Stewart, 1974).

2.3.2.2 Infectious conditions - Viral diseases

Infectious myxomatosis is a fatal disease transmitted by mosquitoes, biting flies and by direct contact (Aduku and Olukosi, 1990). The virus of infectious myxomatosis of rabbits (Sanarelli) induces multiple lesions in the skin, lymph glands, tunica vaginalis, epididymis, testicle, spleen, and lungs. The virus causes growth and destruction of cells in the epidermis overlying the myxomatous masses leading to the formation of vesicles (Rivers, 1930). The disease is also characterized by conjunctivitis, listlessness, anorexia with high temperature. In severe outbreaks animals die within 48 hours after manifesting signs. The disease can be prevented by vaccination (Rivers, 1930).

Rabbit hemorrhagic disease (RHD), also known as rabbit calicivirus disease (RCD) or viral hemorrhagic disease (VHD) is a highly infectious and fatal disease affecting the wild and domestic rabbits. The infectious agent for the disease is rabbit hemorrhagic disease virus (RHDV), or rabbit calicivirus (RCV). The virus infects only rabbits (Mercks, 2006).

Other viral diseases include fibroma, herpes virus infection, viral enteric disease, oral papilloma, rabbit pox and papillomatosis both of which affect the skin and integuments (Aduku and Olukosi, 1990; Mercks, 2006).

2.3.3 Bacterial diseases

Pasteurellosis is a highly contagious disease caused by *Pasteurella multocida*. The disease manifests as inflammation of the mucus membranes of the air passages and lungs. Pasteurella specie can also cause abscesses which may be found on any part of the body (Mercks, 1998; Mcnitt *et al.,* 2000). Mastitis also known as “blue breast

disease" is caused by Streptococcus or Staphylococcus in lactating does. The mammary glands become hot, reddened and swollen and the doe may have fever as high as 40°C or more (Aduku and Olukosi, 1990). Enterotoxaemia is caused by gram positive bacteria, *Clostridium perfringes Type E, Clostridium spiriforme* and *Escherichia coli* which produce powerful enterotoxins. These bacteria normally reside in the gut of healthy rabbits in dormant states but are rapid multiplication and production of enterotoxins under ideal conditions causing enterotoxaemia. Rabbit's exhibit clinical signs of enterotoxaemia, acute diarrhea, lethargy, reduced feed intake, rough hair coat, soiling of the perineum, collapse and sudden death within 24 to 48 hours after the first symptoms are noticed (Cheeke, 1987; Mercks, 2006; Mcnitt *et al.,* 2000).

Colibacillosis is a common bacterial disease of young rabbits and other species. It is caused by the enteropathogenic strains of *Escherichia coli* in the small intestine and caecum. *E. coli* is usually present in the digestive tract of healthy rabbits and does not normally cause diarrhea. Enteropathogenic strains of *E.coli* could be transferred from the does to her kits through faecal contact. Proliferation of pathogenic strains of *E.coli* strains is triggered by factors such as concurrent diseases, stress and low fiber diets (Cheeke, 1987; Mercks, 2006; Mcnitt *et al.,* 2000).

Rhinitis (Snuffles or nasal catarrh) is an acute, subacute or chronic inflammation of the mucous membrane of the air passages and lungs induced primarily by Pasteurella but Pseudomonas, *Bordetella bronchiseptica*, staphylococcus and streptococcus have also been isolated. Initial sign is a thin serous exudates from the nose and eyes that later becomes purulent (Cheeke, 1987; Mercks, 2006; Mcnitt *et al.,* 2000).

Otitis media or interna ("wry neck" or head tilt) results from *Pasteurella multocida* or *Encephalitozoon cuniculi* infection. There is an accumulation of pus or fluid in the

middle or inner ear causing the rabbit to twist its head (Cheeke, 1987; Mercks, 2006; Mcnitt *et al.,* 2000).

Listeriosis is a sporadic septicaemic disease characterized by sudden death or abortions is most common in does in advanced pregnancy caused by *Listeria monocytogenes* spreads through the blood to the liver, spleen and gravid uterus (Cheeke, 1987; Mercks, 2006; Mcnitt *et al.,* 2000).

Other bacterial diseases are pneumonia, conjunctivitis (weepy eye), spirochetosis (vent disease), salmonellosis, staphylococcosis, orchitis, septicaemia and enteritis.

2.3.4 Fungal diseases

Two main groups of fungi, Trichophyton and Microsporum, are found on rabbits and produce disease of the skin and fur under certain conditions. Ringworm is caused by *Trichophyton mentagrophytes* and is characterized by lesions which may occur in any area of the rabbit's skin with loss or thinning of fur. The affected area may be inflamed or capped with white bran-like flaky material (Mercks, 1998).

2.3.5 Parasitic diseases

Coccidiosis is a major disease problem in rabbit production. This disease is caused by protozoan parasite *Eimeria stadia, E. magna, E. irresiduna, E. media,* or *E. perforans.* Affected rabbits exhibit diarrhea, anorexia, rough hair coat and unthriftiness (Aduku and Olukosi, 1990). Mange is also a major cause of poor production in the rabbit industry. The disorder may be caused by *Sarcoptes scabiei* or *Notoedres cati*. Pruritis and alopecia are major clinical signs (Cheeke, 1987; Mercks, 2006).

Encephalitozoonosis caused by Encephalitozoon cuniculi is a protozoan parasite and is the cause of a mild but long standing disease in rabbits (Cheeke, 1987; Mercks, 2006). Only one roundworm presents a problem in domestic rabbits. Several more have been reported in wild rabbits but these rarely occur in domestic rabbits. The pinworm, *Passalurus ambiguous*, is a very common parasite of domestic rabbits, but it does not affect other animals or people (Cheeke, 1987; Mercks, 2006). Tapeworms also occur in rabbits as adults in the intestine and as larval forms in the liver and abdominal cavity. The rabbit tapeworm *Citotaenia variablis* is uncommon in domestic rabbits. When many tapeworms are present, diarrhea and emaciation might occur while rabbits harboring a few tapeworms show no signs of the disease (Cheeke, 1987; Mercks, 2006). Rabbits are intermediate hosts to some intestinal tapeworms such as *Taenia pisiformis* and *Multiceps seralis*. Also pinworm and ear mite infections are common to the rabbit (Rai, 1988). Babesiosis in rabbits is transmitted by ticks such as the *Amblyomma specie* and *Dermacentor specie* in wild rabbits. Babesia species are an erythrocytic protozoan parasite of wild lagomorphs worldwide. Babesia species pathogenic to rabbits are *Babesia bovis* (Yabsley *et al.,* 2006); lagomorphs often serve as reservoirs host for the parasite, which normally infects cattle and humans (Holman *et al.*, 2005).

Trypanosomes are extracellular haemoprotozoan that survive in the blood stream of the host by complex evasion mechanism, including antigenic variation of the variant surface glycoprotein (VSG) and immunosuppression (Taylor, 1998). The resultant effects are inability of the host to clear the trypanosomes from its body, even after administration of trypanocides (Osmar *et al.,* 1992) making the host more susceptible to secondary infections (Nantulya *et al*., 1982).

Trypanosoma brucei brucei resides in the subgenus *Trypanozoon* and is an extremely polymorphic trypanosome occurring as short, stumpy organisms without flagella, long slender organisms with distinct flagella, and intermediate forms that are usually short flagellated (Barrett *et al.,* 2003).

T. congolense resides in the subgenus Nannomonas, a group of small trypanosomes with medium–sized marginal kinetoplasts, no free flagella and poorly developed undulating membranes (WHO, 2001). In Africa, *T. congolense* is considered to be the single most important cause of African animal trypanosomosis.

T. vivax is a member of the subgenus Duttonella, a group of trypanosomes with large terminal kinetoplasts, distinct free flagella, and inconspicuous undulating membranes. It is a large (18–26μm long) monomorphic organism that is very active in wet-mount blood smears (WHO, 2001).

The subgenus Trypanozoon which is the brucei group comprises five members: *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. evansi* and *T. equiperdum*. The three subspecies of *T. brucei* are normally transmitted by tsetse flies (in contrast to *T.* evansi (surra) and *T.* equiperdum (dourine) transmitted through copulation) and are exactly similar in morphology, but only *T. brucei gambiense* and *T. brucei rhodesiense* are the cause of human sleeping sickness, the former mainly in West and Central Africa and the latter in eastern and southern Africa. *T. brucei brucei* is not infective to humans (Coetzer *et al.,* 1994; Hide and Mottram 1996; Geerts and Holmes 1997; OIE, 1997).

T. brucei is polymorphic, with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane which are the long slender forms (23–30 μm in length), short stumpy forms (17–22 μm in length) and the intermediate forms varying in length between the two previously mentioned types.

During the course of the infection, there is a change in the trypanosome population from the long thin forms, through the intermediate, to the short stumpy, and this altered appearance is accompanied by a change in the type of respiration, as the trypanosome prepares for its period within the tsetse fly. The short stumpy forms are adapted to living and developing in the tsetse, while long thin forms are the true mature blood forms which die in the gut of the insect. (Similar metabolic changes also occur in other trypanosome species, but there are no such obvious morphological changes associated with them as in *T. brucei*) (Hide and Mottram 1996; Geerts and Holmes 1997; OIE, 1997).

Insects are usually involved in the natural transmission of the African pathogenic trypanosomes; the life cycle has two phases, one in the insect vector and one in the mammalian host. Transmission by insects may be cyclical by tsetse flies, Glossina species, or mechanical by other biting flies (but apart from transmitting trypanosomes cyclically, tsetse flies can also act as mechanical vectors) (Hide and Mottram 1996; Geerts and Holmes 1997; OIE, 1997).

Trypanosomes can infect all domesticated animals; clinical cases have been described in cattle, water buffalo, sheep, goats, camels, horses, donkeys, alpacas, llamas, pigs, dogs, cats and other species. In parts of Africa, cattle are the main species affected, due to the feeding preferences of tsetse flies; in effect, they can shield other domesticated animals such as goats and pigs from the effects of trypanosomiasis (WHO, 2001). More than 30 species in the wild or zoos, including ruminants such as white-tailed deer, duikers, antelope and African buffalo, as well as wild Equidae, lions, leopards, warthogs, capybaras, elephants, nonhuman primates and various rodents are also known to be susceptible to infection. *T. vivax* DNA has been found by Polymerase Chain Reaction in crocodiles and monitor lizards (*Varanus ornatus*) in Africa, but whether this organism

can become established in reptiles or it is merely inoculated transiently by insects' remains to be determined (WHO, 2001). Experimental infections can be established in laboratory animals including mice, rats, guinea pigs and rabbits. The host preferences of each trypanosome species may differ, but *Trypanosoma congolense*, *T. vivax* and *T. brucei brucei* have a wide host range among domesticated animals. *T. godfreyi* and *T. suis* occur in pigs. *T. simiae* appears to be most important in pigs, but it has also been reported by Polymerase Chain Reaction in camels, horses and cattle (WHO, 2001).

Horses, dogs, cats, camels and pigs are very susceptible to *T. brucei brucei* infection. Infection of cattle, sheep, goats and sometimes pigs results in mild or chronic infection (WHO, 2001).

African animal trypanosomiasis is a parasitic disease that causes serious economic losses in livestock from anemia, loss of condition and emaciation. Many untreated cases are fatal. In male camels, trypanosomosis has been associated with severe testicular degeneration following bouts of fever and development of immune complexes that compromise sertoli cell function (Omer *et al*., 1998; Al-Qarawi *et al.*, 2004). The parasite has also been associated with impairment of pituitary function that may contribute to testicular degeneration and poor **semen quality**. Effects of trypanosomosis on testicular function disappear within 75-90 days after recovery of the male (Al-Qarawi *et al*., 2004; Kaufmann, 2005). However, infertility may persist beyond this period or become permanent in severe cases. African animal trypanosomiasis is found mainly in those regions of Africa where its biological vector, the tsetse fly, exists. Protecting animals from trypanosomosis is difficult in endemic areas, as bites from tsetse flies and a variety of other insects must be prevented. A tsetse fly eradication program being conducted in Africa may help control this disease, as well as other forms of

trypanosomosis that affect humans (Omotainse *et al.,* 2000; Pepin and Meda, 2001; WHO, 2001; Kamuanga, 2003; Bawa *et al.,* 2005).

Haematological, pathological and serum biochemical disorders are characteristics of trypanosomosis in domestic animals and man, the severity of which is often determined by the strain of the infecting trypanosomes and host (Anosa 1988a; b). Anaemia and leucopenia which are the consistent haematological features in trypanosomosis are normocytic normochromic in nature in *T. congolense* infected cattle (Sadique *et al*., 2001; Biryomumaisho and Katunguka, 2007) with the leucopenia characterized by neutropaenia, eosinopaenia and lymphopaenia in cats experimentally infected with *T. brucei*, as well as in cattle infected with *T. congolense* (Nfon, 2000; Biryomumaisho and Katunguka, 2007). The haematological disorders could be responsible for the immunosuppression which renders the animals more susceptible to secondary infection (Nantulya, 1982). Biochemical changes reported in trypanosomosis include hypoglycaemia (Anosa, 1988b), increased plasma bilirubin in *T. brucei* infected dogs (Omotainse *et al.,* 1994), and rabbits and in *T. congolense* infected dogs (Gow, *et al.*, 2007). An increase in serum urea in rats experimentally infected with *T. brucei* has also been reported (Egbe-Nwiyi *et al.,* 2005). Impairment of reproduction by these organisms is achieved by their ability to cause pathological changes in the endocrine glands and gonads (Ikede, 1979; Sekoni *et al*., 1990; Sekoni, 1992; Sekoni, 1994). Other reproductive pathologies reported in association with trypanosomosis in both man and animals include irregular oestrus, sterility, intrauterine infections and abortions in the female (Reincke *et al.*, 1998), and also testicular degeneration, aspermatogenesis and various sperm abnormalities in the male (Akpavie *et al*., 1986; Sekoni *et al*., 1990; Sekoni, 1993; Omer *et al*., 1998; Al-Qarawi *et al*., 2004). It has also been observed that

following chemotherapy of infected animals, regeneration of seminiferous epithelium occurred at a rate which depended on the severity of initial lesions (Ikede and Akpavie, 1982; Sekoni, 1990; Al-Qarawi *et al*., 2004; Kaufmann, 2005). Recent studies show that they cause a wide range of reproductive disorders in animals, including degeneration of the hypothalamus, pituitary glands and gonads with consequent disruptions in the secretions and plasma concentrations of the hormones, necessary for normal reproductive processes in both sexes (Sekoni *et al.,* 1990; Sekoni, 1994). The incubation period for *T. congolense* varies from 4 to 24 days; for *T. vivax* from 4 to 40 days; and for *T. brucei brucei*, from 5 to 10 days.

Most trypanosomes must develop for one to a few weeks in tsetse flies (*Glossina* spp.), which act as biological vectors. When an infected tsetse fly bites an animal, the parasites are transmitted in the saliva. Trypanosomes can also be spread by fomites and mechanical vectors including surgical instruments, needles, syringes and various biting flies including horse flies (Tabanidae). *T. vivax* in South America does not require tsetse flies to develop; it is transmitted mechanically with the most important vectors thought to be Tabanidae (Hide and Mottram 1996; OIE, 1997).

2.4 Reproduction in Rabbits

In the male, the oval-shaped testes within the scrotum remain in communication with the abdominal cavity, where they were at birth. The testicles descend at about two months. (Hoffman and Smith 2005; Kemppainen, 2007; Smith and Boyer, 2008).

In the rabbit doe, ovulation does not occur spontaneously, but has to be induced through a neurohormonal reflex, which is initiated during mating and occurs 10 – 16 hours after mating (Hafez, 1993; Jones *et al.,* 1976). There have been some common incidences of failure to ovulate which appears to vary with the season of the year (Adams, 1972). When using artificial insemination, in the absence of a male, ovulation has to be induced by artificial methods which include treatment with luteinizing hormone, human chorionic gonadotrophin injection (HCG), copper salts or other hormonal means (Adams, 1972). The ovulation inducing method most frequently used is an intramuscular injection of Gonadotropin releasing hormone or its synthetic analogues (Foote *et al.,* 1963; Michelmann and Paufler, 1973; Theau-Clément *et al.,* 1990; Battaglini, 1982), since they are molecules with a small molecular weight (gonadoreline is a decapeptide and buserelin a nonapeptide), they can also be absorbed subcutaneously or across mucous layers (Camier *et al.,* 1989; Donnez *et al.,* 1989). This has been shown to induce ovulation in rabbit does with results similar to those obtained by natural mating. Alternatively, Luteinizing Hormone or human chorionic gonadotropin can be used to induce ovulation in rabbit does (Foote *et al.,* 1963), however, the repeated injection of these hormones can result in failure of the does to ovulate due to antibody formation (Adams, 1972). Gonadotropin releasing hormone can be repeatedly injected without antibody formation (Adams, 1981). Vasectomized males have been used to induce ovulation in does prepared for artificial insemination (Khalifa, 1994; Khalifa *et al.,* 2000), but the method is time-consuming and some females refuse to mate, but ovulate when given Luteinizing Hormone or Gonadotropin releasing hormone.

Giojalas *et al.,* (2004), comparing human and rabbit spermatozoa, suggested that the timing and duration of the capacitation is programmed according to the egg availability

in the oviduct: long in periodic ovulators and short in induced ovulators, such as rabbits. Indeed, Brackett *et al.*, (1982) reported that *invitro* capacitation of raw rabbit semen is long and difficult whereas Percoll ®-selected spermatozoa (without granules) show a faster rate of capacitation. This circumstance maximizes the possibilities that an ovulated egg would meet spermatozoa in the best functional state (capacitated).

2.4.1 Gonad development and puberty in rabbit bucks:

In mammals, gonad differentiation is initiated early during fetal life. Genital crests arising from the adjacent mesonephros are colonized by germ cells (Daniel *et al.*, 2013). Somatic cells and germ cells then differentiate in a coordinated manner: somatic cells differentiate into cells expressing steroids or cells acting as nursing or supporting cells for germ cells. At the same time, germ cells differentiate to acquire the ability to enter meiosis (Daniel *et al.*, 2013).

Rabbit gestation is 30 days, though short but still enough to enable regular and systematic sampling throughout fetal life. Meiosis occurs at birth in females and not during fetal life, as in the case in numerous other mammalian species (Daniel *et al.*, 2013). Moreover, there is a 15 days delay between the appearance of genital crests and the onset of meiosis around births in the female, while this interval is only 3 days long in the mouse. The gonads begin to differentiate on the 16th day after fertilization (Daniel *et al.*, 2013). After birth the testes develop less quickly than the rest of the body. From the age of five weeks they begin to grow very rapidly. Accessory glands undergo a similar development, but at a more even rate and are less precocious (Daniel *et al.*, 2013). Spermatogenesis begins between days 40 and 50. The testicular tubes become

active at about 84 days. The first spermatozoa are present in the ejaculate at about 110 days (Hoffman and Smith, 2005; Kemppainen, 2007; Smith and Boyer, 2008).

Sexual maturity, defined as the moment when daily spermatozoa production ceases to increase, is reached at 32 weeks by New Zealand White rabbits in temperate climates (Lombardi and Fernandez, 2003; Rodel and Bora, 2004). However, a young buck in these same conditions can be used for reproduction from the age of 20 weeks. Indeed the first manifestations of sexual behavior appear at days 60 to 70 when the rabbit makes its first attempts at riding. Coitus may occur for the first time at about 100 days, but the viability of the spermatozoa are very weak or nil in the first ejaculates so first mating should be timed for age 135 to 140 days (Kemppainen, 2007; Smith and Boyer, 2008).

The onset of puberty varies from breed to breed, but environmental conditions in the rabbitry also play an essential role, particularly feeding, which is even more important than climate (Lombardi and Fernandez 2003; Rodel and Bora 2004).

Does generally reach puberty when they have grown to 70 to 75 percent of their mature weight. However, it is usually preferable to wait until they reach 80 percent of their mature weight before breeding them. These relative weights should not be considered absolute thresholds for all rabbits, but rather limits applicable to the population as a whole. Sexual behaviour (acceptance of mating) appears long before the ability to ovulate and bear a litter.

2.4.2 Spermatozoa production

Reproductive inefficiency is the most limiting constraint to efficient rabbit production in the tropics (Gbadamosi and Egbunike, 1999).

The efficiency of sperm production, libido and quality of spermatozoa tend to remain uniform throughout the reproductive life of an animal but may be significantly altered by age, nutrition, environment, health status, drugs, and chemicals (Togun and Egbunike, 2006). Among these factors nutrition is the most prominent management factor affecting sexual maturity, and improved feeding can optimize spermatozoa cell production and the survival of an animal (Salisbury *et al.,* 1978; Rekwot *et al.*, 1987, 1988).

The unavailability of grains and high cost of imported feed ingredients have made the price of commercial animal feed go up. These problems remain the most important constraints to the expansion of commercial livestock production in Nigeria.

Undernutrition poses adverse effect on the reproductive capacity of males, therefore, successful reproduction requires complete provisions of macro- and micronutrients, including zinc (Hidiroglou and Knipfel, 1984), vitamin A (retinol) (Chung, 2009), vitamin B12 (Abdu, 2008), vitamin B9 (Abdu, 2008), vitamin E (Abdu, 2008), vitamin D (Chinoy and Ahmedabad, 1998), folate (Ebisch, *et al.,* 2007), selenium (Hawkes and Turek, 2001), nickel (Yokoi *et al.,* 2003), manganese (Lee, 2006), chromium (Tuormaa, 2000), copper (Tuormaa, 2000), fatty acid (Aksoy, 2006), protein (Zambrano, 2005), arginine (Wu *et al.,* 2009), and carnitine (Ng *et al.,* 2004).

Nutrition is a major factor in the effectiveness of reproductive function. It may affect the efficiency of related hormone production and the growth of reproductive organs

(Almeida, 2007). In addition, delayed appearance of motile sperm in the ejaculate, retarded testicular growth, decreased size of Leydig cells and low differentiation of the seminiferous tubules and of the interstitial tissue is observed in the male gonads of underfed maturing bull-calves (Almeida, 2007). Restricted diets significantly decrease the number of cells of the spermatogenic series at all stages and the number of sertoli cells. Thus, it is apparent that the cellularity of the tubular epithelium is significantly lower in low nutrition animals compared to those of high nutrition (Almeida, 2007).

Vertebrate male reproductive endocrine systems commonly comprise hypothalamus, anterior pituitary gland and testes, which form the hypothalamic-pituitary-gonadal (HPG) system. The male reproductive tract mainly consists of testes, epididymis and ductus deferens (Emanuele and Emanuele, 1998; Foley, 2001). The major function of the male reproductive tract is to produce spermatozoa and hormones. The anterior pituitary gland produces two hormones: follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Emanuele and Emanuele, 1998).

The hypothalamic–pituitary–gonadal axis (also HPG axis) refers to the effects of the hypothalamus, pituitary gland, and gonads as if these individual endocrine glands were a single entity as a whole. These glands often behave in cooperation (Emanuele and Emanuele, 1998).

The hypothalamic–pituitary–gonadal axis is a critical part in the development and regulation of a number of the body's systems, such as the reproductive and immune systems. Fluctuations in the hormones cause changes in the hormones produced by each gland and have various widespread and local effects on the body (Emanuele and Emanuele, 1998).

This axis controls development, reproduction, and aging in animals. The hypothalamus produces gonadotropin-releasing hormone (GnRH). The anterior portion of the pituitary gland produces luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and the gonads produce estrogen and testosterone (Emanuele and Emanuele, 1998).

In oviparous organisms (e.g. fish, reptiles, amphibians, birds), the HPG axis is commonly referred to as the hypothalamus-pituitary-gonadal-liver axis (HPGL-axis) in females. Many egg-yolk and chorionic proteins are synthesized heterologously in the liver, which are necessary for oocyte growth and development. Examples of such necessary liver proteins are vitellogenin and choriogenin (Emanuele and Emanuele, 1998).

The hypothalamus is located in the brain and secretes GnRH (Millar *et al.*, 2004). GnRH travels down the anterior portion of the pituitary through the hypophyseal portal system and binds to receptors on the secretory cells of the adenohypophysis (Charlton, 2008). In response to GnRH stimulation these cells produce Luteinizing Hormone and Follicle Stimulating Hormone, which travel into the blood stream (Vadalladath and Atwood, 2005).

These two hormones play an important role in communicating with the gonads. In females Luteinizing Hormone and Follicle Stimulating Hormone, act primarily to activate the ovaries to produce estrogen and inhibin and to regulate the menstrual cycle and ovarian cycle. Estrogen forms a negative feedback loop by inhibiting the production of GnRH in the hypothalamus. Inhibin acts to inhibit activin, which is a peripherally produced hormone that positively stimulates GnRH-producing cells (Meethal *et al.*, 2009). Follistatin, which is also produced in all body tissue, inhibits activin and gives the rest of the body more control over the axis. In males luteinizing hormone stimulates

the interstitial cells located in the testes to produce testosterone, and Follicle Stimulating Hormone plays a role in spermatogenesis. Only small amounts of estrogen are secreted in males. Recent research has shown that a neurosteroid axis exists, which helps the cortex to regulate the hypothalamus's production of GnRH (Meethal *et al.,* 2009).

One of the most important functions of the HPG axis is to regulate reproduction by controlling the uterine and ovarian cycles (Katja *et al.,* 2007). In females, the positive feedback loop between estrogen and luteinizing hormone help to prepare the follicle in the ovary and the uterus for ovulation and implantation. When the egg is released, the ovary begins to produce progesterone to inhibit the hypothalamus and the anterior pituitary thus stopping the estrogen- luteinizing hormone positive feedback loop. If conception occurs, the fetus will take over the secretion of progesterone; therefore the mother cannot ovulate again. If conception does not occur, decreasing excretion of progesterone will allow the hypothalamus to restart secretion of GnRH (Katja *et al.,* 2007). These hormone levels also control the uterine (menstrual) cycle causing the proliferation phase in preparation for ovulation, the secretory phase after ovulation, and menstruation when conception does not occur. The activation of the HPG axis in both males and females during puberty also causes individuals to acquire secondary sex characteristics (Katja *et al.,* 2007).

In males, the production of Gonadotropin releasing hormone, luteinizing hormone and follicle stimulating hormone are similar, but the effects of these hormones are different (Veldhuis *et al.,* 2009).

Follicle stimulating hormone stimulates sustentacular cells to release androgen-binding protein, which promotes testosterone binding. Luteinizing hormone binds to the interstitial cells, causing them to secrete testosterone. Testosterone

is required for normal spermatogenesis and inhibits the hypothalamus. Inhibin is produced by the spermatogenic cells, which, also through inactivating activin, inhibits he hypothalamus After puberty these hormones levels remain relatively constant (Veldhuis *et al.*, 2009).

The activation and deactivation of the HPG axis also helps to regulate life cycles (Katja *et al.*, 2007). At birth FSH and LH levels are elevated, and females also have a lifetime supply of primary oocytes. These levels decrease and remain low through childhood. During puberty the HPG axis is activated by the secretions of estrogen from the ovaries or testosterone from the testes. This activation of estrogen and testosterone causes physiological and psychological changes. Once activated, the HPG axis continues to function in men for the rest of their life but becomes deregulated in women, leading to menopause. This deregulation is caused mainly by the lack of oocytes that normally produce estrogen to create the positive feedback loop. Over several years, the activity of the HPG axis decreases and women are no longer fertile (Downs and Wise, 2009).

Although males remain fertile until death, the activity of the HPG axis decreases. As males age, the testes begin to produce less testosterone, leading to a condition known as post-pubertal hypogonadism (Veldhuis *et al.*, 2009). Post-pubertal hypogonadism results in progressive muscle mass decrease, increase in visceral fat mass, loss of libido, impotence, decreased attention, increased risk of fractures, and abnormal sperm production.

Semen is a mixture of spermatozoa suspended in the seminal plasma. It is a representative of the combined secretions from different locations by the epididymis

and various glands, such as the seminal vesicles, bulbourethral gland and prostate, which, at the time of ejaculation, are combined (Boiti *et al.*, 2005).

The relative contributions of these different glands vary between species (Morrel, 2011). The accessory sex gland secretions act as a vehicle for the sperm cells, as nutrients and as an aid to neutralizing acidity in the female genital passages (Biljani, 2012; Edmondson *et al.*, 2012). Spermatogenesis is a precisely organized process by which normal diploid cells turn into sperm which occurs within the seminiferous tubules of the testis (Nuti, 2012). Three distinct phases can be divided in the process of mammalian spermatogenesis which are: proliferation of diploid spermatogonia, meiosis of spermatocytes into haploid spermatids, and differentiation of haploid spermatids during spermiogenesis (Chung, 2009). Round spermatids are differentiated into elongated spermatozoa with midpiece and tail at the final phase of spermiogenesis.

As a result of extensive chromatin remodeling and compaction during spermiogenesis, spermatozoa with highly condensed nucleus are formed (Chung, 2009). Spermatozoa are released into seminiferous tubules, spermatogonia and immature spermatocytes gather at the basal compartment of the seminiferous epithelium. The seminiferous tubule is the main compartment of the testes and also the site of sperm cell formation and maturation (Chung, 2009; Jones, 1999).

After completing mitosis and meiosis, haploid spermatids enter spermiogenesis, a process of physical and physiological maturation. During spermiogenesis, spermatids acquire tail and progressive motility with a developed mid- piece which connects the head compartment with the tail. This physical maturation process mostly happens in the seminiferous tubule. Sperm maturation and storage occurs in the epididymis (Jones, 1999). Epididymis also forms the blood epididymal barrier which allows the epididymis to regulate and modify the luminal fluid contents. Different epididymal fluid contents in

caput epididymis, corpus epididymis and cauda epididymis change the surface of the sperm plasma membrane into a fully matured condition and provide spermatozoa with motility and capacity for fertilization as the immotile immature spermatozoa transits through the epididymis. Therefore, it is shown that the luminal microenvironment of the epididymis is vital for successful sperm maturation (Foley, 2001). Finally, after a series of morphological changes, mature spermatozoa are stored in the epididymis in a quiescent state. Seminal plasma is also very important in sperm nutrition and function, including activation, protection against bacteria or immune cells and preparation of spermatozoa for fertilization (Wong, 2001). Therefore, the wellness of the seminiferous tubule and the epididymis is particularly important in assisting spermatids to undergo maturation successfully. Sertoli cells, namely nurse cell lines with the inner walls of the seminiferous tubules and participate partly in the process of differentiation by providing structural and nutritional support for developing germ cells. The materials secreted by Sertoli cells provide transportation for non-motile spermatozoa from testes into the efferent duct system. There are different spermatogenic cell types in the seminiferous tubules as the timing of spermatogenesis is under rigid control, this phenomenon is known as the seminiferous epithelial cycle (Emanuele and Emanuele, 1998; Foley, 2001). Besides Sertoli cells, the cell type that is interspersed within the seminiferous tubule, which are the interstitial cells, are known as Leydig cells. Leydig cells which produce the male sex hormone, testosterone are the major sites of steroidogenesis in the testes. They also play a crucial role in sperm maturation (Foley, 2001). During spermiogenesis, the potential for forward motility starts as the spermatozoa pass through the epididymis, while the capacity for spermatozoa to move forward is acquired in different regions of that organ. At the beginning of epididymal maturation, the spermatozoa in epididymides caput are immotile. As the spermatozoa travels from the

caput of the caudal epididymides the membrane of the spermatozoon tail obtains new properties, the undulating movements of spermatozoa alter gradually (Foley, 2001). Besides factors from the caudal epididymal plasma, the induction of forward motility requires also an increased cyclic adenosine monophosphate level and calcium ion. During epididymal transit, the intrasperm level of cyclic adenosine monophosphate and the adenosine triphosphatase activity increases which is essential for the induction of sperm motility. A specific glycoprotein which involves the induction of forward movement binds to the spermatozoa and it is also present in the testicular, epididymal and seminal fluids. Even though the presence of calcium ions is not always necessary depending on species, calcium ion is crucial for the initiation of sperm motility. It is reported that sperm motility is proportional to the free calcium level found in the sperm of the caudal epididymis (Kann and Serres, 1980). Trace minerals and vitamins also influence sperm motility.

After ejaculation into the female genital tract, spermatozoa need to get activated before they obtain the ability to fertilize an oocyte. This activation process is known as capacitation. The ultimate control of capacitation lies within the sperm plasma membrane. As the outer surface of the plasma membrane, the glycocalyx undergoes several biochemical changes; a depression of a set of preprogrammed cellular events takes place and hyperactivates the spermatozoa. These modifications include alterations in protein and lipid composition. The microenvironment of the oviduct where bicarbonate levels are much higher than in the epididymis permits capacitation.

In order to accomplish fertilization accurately and efficiently, it is crucial for sperm to be progressively motile and membranes to be intact. Fertilization will be impaired if either of the two conditions are not fulfilled. Peroxidative damage to the sperm plasma causes membranes to lose their fluidity and integrity, which consequently impairs the

competence of spermatozoa to involve in membrane fusion events, like the acrosome reaction linked with fertilization. As a result of exposure to high levels of oxidative stress, fertility of spermatozoa is significantly affected, such as by low sperm motility (Kothari, 2010).

Sperm quality and quantity is also closely associated with semen quality. The various contents in semen play a significant role in sperm survival and semen quality is a parameter of male fertility (Mason *et al.,* 1982; Yue, 2010). Semen is formed during the process of ejaculation, when sperms pass through the ejaculatory ducts and combine with fluids secreted by seminal vesicles, prostate and bulbourethral glands (accessory glands).

The estimated total duration of spermatogenesis in the rabbit depends on the point chosen as the onset of spermatogenesis. If spermatogenesis is considered to begin with the first of the series of spermatogonial divisions leading to the production of primary spermatocytes then about four cycles of the seminiferous epithelium or $4 \times 10.9 = 43.6$ days are required. However, if one assumes that spermatogenesis starts with the formation of spermatogonial stem cells and that the lifespan of these stem cells is one cycle of the seminiferous epithelium then spermatogenesis extends over about 4.75 cycles, or 51.8 days (Swierstra and Foote, 1965). Since the duration of spermatogenesis is about 7-8 weeks, a minimum period of 10 weeks is recommended to determine the effect of certain exogenous factors (Boiti *et al.*, 2005).

Rabbit semen consists of two main parts which are the gelatinous and the fluidy parts. Following ejaculation, the gelatinous part fills the lumen of the vagina as a copulation plug (bouchon vaginal). This plug prevents the semen from running out of the vagina after ejaculation (Engle, 1926).

Ejaculated rabbit semen is a heterogeneous mixture consisting of seminal plasma in which the mature spermatozoa are suspended along with numerous membrane-surrounded granules (Eghianruwa and Anika, 2011). These vesicular formations, which have a predominantly round shape and a diameter ranging from 0.5 to 4 μm, are produced and secreted by the anterior part of the prostate (pro-prostate) as shown by their presence in vasectomised rabbit bucks (Cardinali *et al.,* 2007). Similar multi-lamellar granules have also been isolated from human semen and from the semen of other animal species as prostasomes and prostasomelike vesicles, respectively (Arienti *et al.,* 1998; Kravets *et al.*, 2000; Ghaoui *et al,* 2004; Siciliano *et al.*, 2008). The physiological roles of these prostatic secretory granules (PSGs) have been thoroughly debated and several hypotheses have been put forward (Kravets *et al.,* 2000). In rabbits, prostatic secretory granules are able to inhibit spermatozoa capacitation, delaying the acrosome reaction, and they promote spermatozoa motility during the first few hours after mating (Cardinali *et al.,* 2007; Castellini *et al.,* 2008).

Both spermatozoa and prostatic secretory granules are characterized by a unique lipid and fatty acid composition (Castellini *et al*., 2006; Mourvaki 2010). The lipid bilayer of the rabbit spermatozoa membrane is mainly composed of phospholipids (69% of the total amount of lipids) (Castellini *et al.,* 2006), with phosphatidylcholine being the major one, followed by phosphatidyl-ethanolamine and lysophosphatidylcholine. Sterols are the second major component of rabbit spermatozoa and are considered to be important modulators of membrane fluidity and the acrosome reaction (Go and Wolf 1983). Prostatic secretory granules have a dissimilar lipid composition compared with the spermatozoa plasma membrane (Castellini *et al.,* 2006). In particular, they contain twice as many sterols as phospholipids and have a considerable amount of sphingomyelin.

The unusual abundance of these two lipids is thought to be responsible for the ordered structure of the prostatic secretory granules membrane and also for the role of prostatic secretory granules in reversing or blocking spermatozoa capacitation (Cardinali *et al.*, 2007, Castellini *et al.*, 2008; Castellini *et al.*, 2006). Sterols and phospholipids are essential constituents of mammalian semen and have an important role in the fertilizing capacity of sperm cells. Various proportions of this unusual sterol have also been reported in human semen (Lalumiere *et al.,* 1976) and in the semen of other mammalian species such as the rhesus monkey (Lin *et al.*, 1993), hamster (Bleau and Vandemheuvel, 1974) and boar (Nikolopoulou *et al.,* 1986). Monkey spermatozoa is also richer in desmosterol than in cholesterol (Lin *et al.,* 1993), while the opposite has been observed for human (Sion *et al.,* 2001) and boar spermatozoa (Nikolopoulou *et al.,* 1986). It is speculated that these differences depend on the levels of Δ-24 reductase and or on the presence or absence of an inhibitor of this enzyme among the various species (Lin *et al.,* 1993).

As found for monkey spermatozoa (Connor *et al.,* 1998), desmosterol–cholesterol ratio has been found to be higher in the tail than in the head of rabbit spermatozoa. This asymmetric desmosterol–cholesterol composition between the spermatozoa head and tail has been suggested to be responsible for the different functions of these two spermatozoa subcellular parts. In particular, desmosterol has an additional double bond with respect to cholesterol and adequately modulates the membrane fluidity, which is necessary for spermatozoa motility (Connor *et al.,* 1998). Unlike the semen from other mammals, rabbit semen contains discrete amounts of sterol esters and the presence of desmosterol esters (representing about 44% of total sterol esters). Sterol esterification occurs during spermatozoa transit through the epididymis in the ram (Quinn and White, 1967), and this could also be true for the rabbit. In hamster and monkey spermatozoa,

desmosterol is also present chiefly in the free form, and only a minor proportion is conjugated with a thiol group, exhibiting several structural (spermatozoa membrane stabilizer) and functional (modulator of capacitation, acrosin inhibitor) roles (Bleau and Vandemheuvel, 1974, Connor *et al.,* 1998). The presence of desmosterol and or cholesteryl sulphate in rabbit semen remains to be verified. Desmosterol has not been found in significant quantities elsewhere in the body except in the brain (Edmond *et al.,* 1990) and testis, and it has been proposed that it is a biochemical marker of puberty in the monkey (Connor *et al.,* 1997). Although there is no direct evidence, the presence of this sterol in the PSGs of rabbit semen, which are specifically produced by the prostate, is a unique biological finding and may indicate an active desmosterol synthesis in this accessory sex gland.

The shape of rabbit spermatozoa is similar to that of other mammals. The dimensions of the ovoid head are about 7 × 4 × 0.5 cm. The length of the tail is 45 cm. The acrosomal cap is situated on the top of the head for three-quarters of its extent and presents an increase along its edge. The longitudinal section of the rabbit spermatozoa head shows a very compact nuclear chromatin surrounded by the acrosomal complex (Boiti *et al.,* 2005).

2.4.3 Methods of Semen Collection

There are three most common and recent methods of semen collection which are; the artificial vagina, electro – ejaculator and the rectal massage. Abdominal massage, penile and cloacal massage could also be used (Morrel, 2011). The use of any particular method depends on the type and condition of specie. The ideal method of semen

collection will be the one which is safe for the sire and the collector (Morrel, 2011), and where the semen obtained is a real representative of a normal ejaculate, free of contamination and should eventually be protected from thermal shock.

2.4.3.1 The Artificial vagina

This is a quick method of semen collection in animals. The artificial vagina consists of the rubber casing, a rubber liner, two rubber bands, rubber funnel, graduated test tube, protective jacket and a two way valve. When the artificial vagina is coupled, the space between the inner rubber liner and rubber casing is filled with glycerol, after which the coupled artificial vagina is inserted into hot water at a temperature of 40 - 55°C; the warm glycerol simulates the vagina of the doe. A latex rubber collection cone is placed in the artificial vagina and a graduated collection tube is placed on the end of the cone. Lubrication with non perfumed petroleum jelly in the end where intromission of the penis occurs is essential (Dorji, 2012). The rabbit doe is restrained firmly in place allowing the male to exhibit his courting behavior. The buck is allowed few false mounts, after which the person with the artificial vagina collects the ejaculate by directing the penis into the artificial vagina. The test tube containing the ejaculate is then carefully handled against foreign bodies, contamination and direct sunlight and cold temperatures (Nuti, 2012).

2.4.3.2 Electroejaculator

This is used by inserting a probe or electrode into the male's rectum stimulating the ampulla and sacral nerves plexus of the reproductive tract (Carter *et al.*, 1990; Dorji,

2012). The electro ejaculator could either be operated only on battery or battery-cum-electric transistorized circuits. This method is used where the artificial vagina is not practical in semen collection from males for artificial insemination purposes, situations like when the male is extremely slow in serving the artificial vagina or physically incapable of mounting. The experience with the use of electro ejaculator is usually aversive.

2.4.3.3 Rectal massage

This is achieved by massaging the vesicular glands and ampullae of the ductus deferens through the rectal wall (Barth and Oko, 1989; Morell, 2011). Semen is then collected by gentle massage resulting into stimulation and protrusion of the glans penis from the prepuce (Sekoni, 1990). This method has a rapid semen collection characteristic when animals cannot mount, but contamination with urine may occur (Morell, 2011).

2.4.3.4 Abdominal massage/digital massage of the cloaca

Massaging the back of a bird and stroking gently towards the tail with the thumb and forefinger, stimulates erection of the copulatory organ. Gentle pressure is exerted on the ejaculatory ducts and semen is collected into a calibrated tube. This method is most preferred in poultry (Burrows and Quinn, 1937). The tail feather is held to express the vent of the bird. The vent (cloaca) of the male bird (cock or tom) is cleaned with warm sterile normal saline and gently massaged.

2.5 Semen Analysis

Ejaculated semen sample is subjected to routine semen evaluations which include semen volume, pH, colour, spermatozoa concentration, spermatozoa motility, live dead ratio and spermatozoa morphology (Zemjanis, 1970).

The volume of the 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 influences the volume obtained, its gross appearance and spermatozoa concentration. The approximate average ejaculate volumes are about 0.3 to 0.6 ml in the rabbit bucks though ejaculate volume is liable to vary.

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). The colour is coded 1, 2 and 3 in relation to creamy, milky and watery colour.

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 (x 40). 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 (Al-Ghalban *et al.,* 2004). 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).

Progressive motility of individual sperm is determined by diluting a drop of semen with 4 drops of normal saline on a clean pre-warmed 37 ^{0}C glass slide and covered with a clean cover slip and examined at X 40 magnification (Al-Ghalban *et al.,* 2004). The percentage of motile sperm is broadly categorized as very good, good, fair and poor corresponding to about 80-100 %, 60-80 %, 40-60 % and 20-40 % motile cells respectively. Generally, for semen to be considered satisfactory it should have at least 60 % moderately active spermatozoa (Mickelsen *et al*., 1991; Brito *et al*., 2002).

Concentration of spermatozoa is determined by the use of the improved Neubaeur haemocytometer which has 25 large squares or 400 small squares. Semen is pipette to the 0.5 mark using the blood cell pipette and this is made up to 1.0 mark with normal saline. The normal saline serves both as diluents for the semen and fixes the spermatozoa present. The pipette is introduced into a pipette shaker and allowed to mix. About 2 to 3 drops of fluid is discarded from the pipette before being introduced into the counting chamber of the haemocytometer. Sperm cells are counted diagonally from the left top to the right bottom in five large squares or a total of 80 small squares according to the method of Coffin, (1953), Rekwot *et al*., (1994, 1997). Spermatozoa output is determined by multiplying semen volume and spermatozoa concentration. Other methods commonly used are spectrophometer and computer assisted analysis.

The live/dead ratio was done by mixing one drop of semen with one drop of Eosin-nigrosin on a warm clean slide. A thin smear was then made, air dried and observed under the microscope. The ratio of the *in vitro* dead sperm cells was observed and it is

based upon the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm cells repel the stain (Johnson, 1994; Esteso *et al.*, 2006; Koonjaenak, 2006).

Two individuals were involved in the counting using the tally system, clicking on type of cell seen totaling 100 cells after which the amount clicked is recorded.

The sperm morphology was assessed using Eosin-nigrosin stained smear of semen samples on a clean warm glass slide. The smear was air dried and observed under light microscope. The defective sperm cells can either be primary, secondary and tertiary or head, mid-piece and tail abnormalities. The spermatozoa abnormalities were determined as described by (Koonjaenak, 2006).

Species differences exist with respect to the fine points of sperm morphology but all sperm cells have the same basic structure (Koonjaenak, 2006). The percentage of abnormal spermatozoa and their types were recorded after observing 600 cells. Abnormalities are conveniently divided into head, mid piece and tail problems. Abnormalities 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 defect is 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, 1989). Secondary defects include tailless heads protoplasmic droplets on the mid piece and bent or broken tails or heads (Amann, 1998).

Tertiary abnormalities develop *in vitro* 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 products 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).

2.6 Factors affecting Semen Production

Seminal characteristics are affected by many factors (breed, feeding, health status, rearing condition, season and collection frequency, etc.) causing a wide variety in semen traits (Alvarino, 2000; Boiti *et al*., 2005).

2.6.1 Breed (Genetics)

This was observed in animals exposed to various strict protocols of rearing (Theau-Clement *et al.,* 2003; Moce *et al.,* 2005). Viudes *et al.,* (2004), and Brun *et al.,* (2002) observed differences in semen characteristics for males from different genetic lines and from crossbred and purebred males.

2.6.2 Nutrition

Nutrition is a very important factor. It can act not only as a paramount ultimate factor, but as a proximate factor as well in many cases. Furthermore, if food availability falls below a threshold that may vary according to species and populations, an animal will not breed. Moreover, changes in food availability can influence seasonal breeding patterns. This effect has been extensively studied in sheep, and marked differences in sensitivity to food availability have been described. Generally speaking, the effect of food availability on seasonal breeding is much stronger in breeds originated from Mediterranean or tropical climates than in breeds originated from temperate latitudes (Martin and Walkden-Brown, 1995, Boukhliq *et al.*, 1996, Bielli *et al.*, 1999, Zarazaga *et al.*, 2005). Conversely, food availability or even some increase in nutrient availability can be the proximate factor triggering breeding activity.

Luzi *et al.* (1996) showed that a restricted dietary protocol reduces libido and some seminal traits. However, the most important factor is not the amount of feed given but its chemical characteristics. An inverse relationship exists between growth rate and age at puberty (Waddad and Gaili, 1985).

Testicular spermatozoa /spermatid reserves and epididymal sperm reserves in bulls on the high plane of nutrition were significantly higher than those for the bulls on the low and medium planes of nutrition (Rekwot *et al.,* 1994).

There is also an influence of different levels of energy and protein on age at puberty, libido and quality of semen as low protein feeding has been shown to delay puberty (Rekwot *et al.,* 1987), poor testicular development and smaller ejaculates compared to those having adequate protein (Rekwot *et al.,* 1988). Older bulls are also susceptible to low dietary' protein showing a decreased testicular size and reduced fertility (Rekwot *et al.,* 1988). Energy levels of the diet fed to breeding bulls have marked effect on fertility. A high level of energy (TDN 115% or 130% of normal diet) tends to reduce age at the onset of semen production (Flipse and Almaquist, 1961) and increases total spermatozoa output per ejaculate. However, high energy diet in adults tends to increase fat deposition and reduce sexual drive. Protein deficient bulls produced significantly smaller volume of semen and less number of spermatozoa per ejaculate. Libido is also adversely affected by protein deficiency and bulls failing to mount due to weakness. Adequate ration for breeding bulls can be made up of any of the standard roughage or roughage combination provided the feeds are of good quality. It is routine management practice in most breeding farms to give a portion of the ration in the form of grain mixture to the breeding bulls.

Feeds high in calorific value through its specific dynamic action may contribute to the thermoregularity mechanism of the animals during acute winter months resulting in enhanced number of spermatozoa and improved progressive motility (Salisbury *et al.*, 1978).

According to Bhosrekar and Rajdan (1973) bulls fed on 100% dried crude protein level had the least abnormalities and dead spermatozoa. Different dried crude protein level (80%, 100% and 120%) with constant energy level did not cause any, significant difference in the quality of semen.

However, different periods of feeding in different seasons have been shown to significantly affect the semen quality and composition (Bhosrekar and Rajdan, 1973). Diet with varying, energy levels (80, 100 and 120%) did not influence in morphological and biochemical qualities of semen (Bhosrekar and Rajdan, 1973) whereas, 120% energy level caused significant increase in primary spermatozoa abnormalities (Bhosrekar and Rajdan, 1973).

Specific recommendations on feeding rations for rabbit bucks are not available, and only some specific requirements have been established (de Blas and Wiseman, 1998).

2.6.3 Diseases (health)

It is widely known that inflammation of the male reproductive apparatus (O'Bryan *et al.*, 2000) worsens various testicle functions and seminal characteristics by affecting biosynthesis of pro- inflammatory eicosanoids (prostaglandins and leukotriens) and cytokines (Knapp, 1990).

Pasteurellosis, a bacterial infection caused by Pasteurella multocida is common in domestic rabbits. It is highly contagious and is transmitted by direct contact, although

transmission by coughing or sneezing may also occur. Signs of pasteurellosis include rhinitis (stuffy, runny nose), pneumonia, abscesses, reproductive tract infections and inflammation of the reproductive tract which is usually seen in adult rabbits (Mercks 2011). Does are more often infected than bucks, if both horns of the uterus are affected, often the doe becomes sterile; if only one horn is involved, a normal litter may develop in the other. The only sign of an infection in the uterus may be a thick, yellowish gray vaginal discharge. Bucks may discharge pus from the urethra or have an enlarged testicle. Long term infection of the prostrate and seminal vesicles is likely. Infection can be passed during breeding and so infected animals should not be bred (Mercks 2011).

Ketosis (pregnancy toxaemia) is a rare disorder that may result in death of does 1 to 2 days before giving birth. The disease is more common in first-litter does (Mercks 2011).

Treponematosis (vent diseases, rabbit syphilis) is a venereal disease of rabbits caused by Treponema bacteria. It occurs in both sexes and is transmitted through sexual intercourse and from the doe to her offspring. It is closely related to the organism causing human syphilis, the bacteria is not transmissible to other domestic animals or humans. It is characterized by smalle blisters orslow healing sores which become covered with heavy scabs. These sores are generally confined to the genital region but the lips and eyelids may be involved. Infected rabbits should not be mated (Mercks 2011).

Listeriosis is a bacterial infection of the blood characterized by sudden death or abortions, seen occasionally and is most common in does near the end of pregnancy. The bacteria causing the disease spread by way of the blood to the liver, spleen and uterus. It can infect many animals including humans (Mercks 2011).

Trypanosomosis: Trypanosomes are classified in the phylum Sarcomastigophora, the order Kinetoplastida and the family Trypanosomatidae. The trypanosome consists of a single cell varying in size from 8 to over 50 μm. There are distinct differences in

appearance, shape and size between the various species of trypanosomes, allowing specific identification (Uilenberg, 1998). The salivaria group of trypanosomes may or may not have a free flagellum, the kinetoplast is terminal or sub-terminal, and the posterior end of the body is usually blunt. They develop as trypomastigotes within the mammalian host and are usually pathogenic.

There are distinct differences in appearance, shape and size between the various species of trypanosomes, allowing specific identification. Trypanosomes are not rigid and continuously change their shape slightly (Coetzer *et al.*, 1994; Hide and Mottram 1996; Geerts and Holmes 1997; OIE, 1997).

2.6.3.1 Effect of Trypanosomosis on Animal Reproduction

Trypanosomosis causes severe degenerative changes in the spermatogenic epithelium and the interstitial sells of Leydig which may disrupt steroidogenesis and may explain reduced libido in infected cattle (Sekoni *et al.,* 1988). Increased scrotal diameters, scrotal inflammations, scab formation with alopecia as well as peri orchitis, epididymitis, severe testicular degenerations, abnormal spermatogenesis in bucks (Sekoni *et al.,* 1991; Sekoni., 1992; Omer *et al.*, 1998; Al-Qarawi *et al.*, 2004) and preputial haemorrhages have been reported in rabbits infected with *T. brucei* (Ikede and Akpavie, 1982). Some of the effects of trypanosomosis on the male gonads are through the indirect effects of pyrexia, accompanying waves of increasing parasitaemia during infection and increased scrotal temperature (Mutayoba *et al.*, 1995b). Testosterone synthesis and secretion may be altered due to circulatory disturbances in the scrotum and testis as a result of elevated body temperature and the inevitable reduction in the

temperature gradient between the testes and the body (Jeffcoate and Holmes, 1997). Morphometric analysis on the testes of any species or breed is necessary in assessing and estimating quantitative changes in testicular components and spermatogenic function arising from factors such as age, season, temperature and diseases (Egbunike *et al*., 1976).

The effects of Trypanosomosis on female reproduction of the various breeds of cattle (Ogwu *et al*., 1986; Ogwu and Njoku, 1987), sheep (Bawa *et al*., 1990), and goats (Mutayoba *et al*., 1988) have been reported. It has been reported that extravascular trypanosomosis (*T. brucei* infection) has effects on female reproduction such as causing abortions in pregnant ewes and cattle and also crossing the placenta to cause the death of the neonate (Ogwu and Nuru, 1981). Much of these abortions has not been clearly ascertained, but may have to do with transplacental transmission (Ogwu and Nuru, 1981), foetal pathology (Ogwu and Nuru, 1981) and placental damage. Ogwu and Nuru (1981) reported anoestrus of up to six months in zebu heifers, chronically infected with *T. vivax*. Mutayoba *et al., (*1988) described an increase in the numbers of small antral follicles and occasional cystic follicles in infected goats with *T. congolense* consistent with history of anoestrus. It has also been reported that reproductive problems such as abortions during various stages of pregnancy, birth of weaklings, premature births, births of anaemic offspring, low birth weights and neonatal calf mortalities have been observed with *T. vivax* and *T. congolense* infected cattle (Ogwu *et al.,* 1986; Ogwu and Njoku, 1987). Ogwu *et al.*, (1984) reported ovarian function arrested with no functional structure in the ovaries of *T. vivax* infected zebu heifers and they also observed atrophic ovaries with multiple follicular cystic degeneration and total absence of corpora lutea in zebu heifers. Mutayoba *et al., (*1989) observed a persistent increase in circulating prostaglandin metabolite levels in *T. congolense* infected goats, associated with reduced

progesterone levels, short cycles and eventual anoestrus and suggesting prostaglandins as the cause of cyclic disturbances.

2.6.3.2 Pathogenesis and Clinical Signs

Initial replication of trypanosomes happens at the site of inoculation in the skin; this causes a swelling and a sore (chancre). Trypanosomes then spread to the lymph nodes and blood and continue to replicate and are disseminated throughout the body by the circulating blood leading to parasitaemia (Coetzer *et al.,* 1994; Hide and Mottram 1996; Geerts and Holmes 1997; OIE, 1997). The level of parasitaemia reflects the virulence of the organism (Losos, 1986). *T. congolense* localizes in small blood vessels and capillaries. *T. brucei brucei* and *T. vivax* localize also in tissue. Antibodies developed to the glycoprotein coat of the trypanosome lyses the trypanosome and result in the development of immune complexes. Antibody, however, does not clear the infection, for the trypanosome has genes that can code for many different surface-coat glycoproteins and change its surface glycoprotein to evade the antibody. Thus, there is a persistent infection that results in a continuing cycle of trypanosome replication, antibody production, immune complex development, and changing surface-coat glycoproteins (Blood and Radostits, 1989).

The clinical signs and severity of the disease caused by these organisms vary according to the trypanosome species, the virulence of the particular isolate and the species of host infected. Acute disease is characterized by anaemia which is a cardinal clinical sign observed in African Animal Trypanosomosis, within a week of infection with the haematic trypanosomes (*T. congolense* and *T. vivax*) there is usually a pronounced

decrease in packed cell volume (PCV), haemoglobin and red blood cells, and within 2 – 3 months the PCVs may drop to below 30 percent of their preinfection values (Sekoni *et al*., 1990b).

Other clinical signs are an intermittent fever, lymphadenopathy and weight loss which can be so severe. Animals lose condition and become progressively emaciated. Milk yield may be decreased in dairy animals. Neurological signs, dependent edema, cardiac lesions, diarrhea, keratitis, lacrimation, appetite loss and other clinical signs have also been reported. Effects on reproduction include abortions, premature births and perinatal losses, as well as testicular damage in males. Deaths are common among chronically infected animals, and animals that recover clinically may relapse when stressed. Sudden deaths have been reported in small ruminants infected with *T. vivax*. Trypanosomes can cause immunosuppression, and concurrent infections may complicate this disease (Coetzer *et al.*, 1994; Hide and Mottram 1996; Geerts and Holmes 1997; OIE, 1997). Animals that survive are often infertile and of low productivity. In some instances, infected animals show no overt signs of disease but can succumb if stressed by work, poor nutrition or concurrent disease (Taylor and Authie, 2004), pregnancy, milking or adverse environmental conditions (Luckins, 1988). However, the clinical signs are so varied and the ecological conditions under which trypanosomiasis occur so diverse that, in terms of identifying animals with active infections, clinical diagnosis is too imprecise a procedure to use as a basis for the control of trypanosomiasis, and other means of diagnosis must be employed.

An acute hemorrhagic syndrome has been reported among cattle infected with *T. vivax* in Africa. Affected animals have enlarged lymph nodes and signs of severe anemia, and they develop widespread visceral and mucosal hemorrhages, particularly in the gastrointestinal tract. In one outbreak, the main hemorrhagic sign was bleeding from the

ears (Coetzer *et al.*, 1994; Hide and Mottram 1996; Geerts and Holmes 1997; OIE, 1997).

Few clinical cases have been described in dogs and cats (Coetzer *et al.*, 1994; Hide and Mottram 1996; Geerts and Holmes 1997; OIE. 1997).

2.6.4 Season

It is well known that the expression of a given trait (phenotype) in an animal depends on the combined influence of both, genetic factors (genotype) and environmental factors. The main environmental factors influencing animal reproduction are temperature, humidity, amount and distribution of rainfall, solar radiation and photoperiod, nutrition, productive system management, social interactions among individuals within the same population, predator-prey interactions, parasite- and pathogen- host interactions (Sadleir, 1968; Giwercman and Giwercman, 2011, Taberlet *et al.*, 2011, Burns *et al.*, 2010).

Most wild mammals, especially those of greater size and longevity, are to some extent, seasonal breeders (Goldman *et al.*, 2004). Such animals limit their mating activity and offspring births to well defined seasons of the year. However, some domestic species such as cattle, pigs and rabbits exhibit no seasonal breeding if they are raised in environments with mild climatic changes throughout the year.

Light length affects the hypothalamus-pituitary axis and consequently hormonal release and spermatozoa production. A daily constant 16 : 8 D light program increases spermatozoa production (qualitative and quantitative aspects) compared with a shorter light length (8L:16D, Theau-Clément *et al.*, 1994).

Semen quality is subjected to seasonal variation and the fall of semen quality during winter could be attributable to the feed ration (Valge, 1970, Sekoni *et al*., 1988).

2.6.5 Management

Animals live in environments that are both complex and continually changing, so they have to respond to short- and long-term variations in a wide range of factors, such as photoperiod, nutrition and sociosexual signals. Before they were domesticated, animals developed reproductive strategies that coped with these changes and often took advantage of them (Martin *et al.,* 2004). The physiological processes that implement these strategies have been modified to some extent during several millennia of controlled breeding, but most persist. Thus, many genotypes still exhibit profound responses to external inputs, such as the induction of ovulation by sociosexual signals and the doubling of litter size by a change in nutrition (Martin *et al.,* 2004). The complexity in these responses is now becoming clearer. Similarly, the impact of nutrition has been extended beyond the control of puberty and the production of gametes to include phenomena such as 'fetal programming', with its potentially profound effects on the life-long performance of the animals (Martin *et al.,* 2004).

2.6.6 Collection frequency

This has an important effect on semen characteristics: two ejaculates collected once a week (with an interval of at least 15 min) allows for the best semen production both in terms of quality and quantity (Bencheikh, 1995; Moce *et al.,* 2000). Conversely, too long collection frequency (every 14 d) exerts a depressive effect on spermatozoa output

probably for the scarce sexual stimulus followed by androgen reduction (Castellini *et al.*, 2006).

Collection frequency affects not only spermatozoa production but also the concentration of seminal granules: daily collection, in comparison to a collection every week, reduced the spermatozoa and granule concentration even if granules showed a more stable and higher production (Castellini *et al.*, 2006).

2.6.7 Age

Sexual maturity occurs approximately at 5 months (depending on the strain) and semen quality generally decreases in older rabbit bucks. Recently, some authors showed that the spermatozoa chromatin structure of the semen of rabbits between 5 and 28 months of age significantly changed. Changes in chromatin structure suggested a relatively high stability of spermatozoa chromatin in the rabbit. The lowest percentage of spermatozoa with damaged chromatin (1.7-2.4%) was observed between 6 and 16 months of age. Decreased spermatozoa chromatin stability was found in ejaculates taken from male rabbits less than 5 months and more than 20 months of age (Gogol *et al.*, 2002).

2.6.8 Temperature:

Ambient temperature can also be a proximate factor, since warmer temperatures could trigger reproductive activity. Conversely, too high temperatures could limit reproductive activity (Haim *et al.*, 2005). Under humid tropical climatic conditions, high temperature and relative humidity are major environmental factors that result in heat stress which influences the productivity and physiological development of animals.

In such an environment, rabbits are susceptible to heat stress (McNitt *et al*., 2000). In Nigeria, rabbit rearing is mainly based on the use of local population which needs a better knowledge of its biological capabilities as well as adaptability to the rearing and environmental conditions. Most of the stocks of rabbits used in developing countries including Nigeria are heterogeneous resulting from years of planned and unplanned crosses among different exotic and or indigenous breeds of rabbits introduced to most developing nations (Somade and Adesina, 1990; Odubote *et al*., 1995). In this regard, there is need to identify the productivity potential of these stocks of rabbit. Reproductive performance of rabbit does, growth rate as well as mortality are the factors that define the productivity potential of rabbit (Rebollar *et al*., 2009). The feeling of warmth under hot ambient temperature increases with high relative humidity, although the temperature is interrelated with other climatic factors. Such relationship (between ambient temperature and relative humidity) as was developed by Marai *et al*. (2001) for rabbits as small animals to propose a measurement of the level of severity of heat stress by combining temperature and relative humidity in an index called the temperature-humidity index (THI), to account for the thermal comfort or distress (Finzi *et al.*, 1994).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

The study was carried out at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria which is located in the Northern Guinea Savannah zone of Nigeria on latitudes 11°3N and 12°N and longitudes 7°42E, and 8°E at an elevation of 650 m above sea level. It has an average annual maximum and minimum temperature of 31.0 ±3.2^{o}C and 18.0 ±3.7^{o}C, respectively. Zaria has an average annual rainfall of 1100 mm usually lasting from May to October with a mean relative humidity of 72 %. The dry season lasts from November to April with mean daily temperatures ranging from 15 – 36 °C and mean relative humidity of between 20 – 37 %.

3.2 Acquisition and Management of Experimental Animals

Twenty (20) domesticated adult rabbit bucks weighing between 1.2 and 2.8 kg were used for this study. The rabbit bucks were acquired from a rabbitry within Zaria metropolis. The animals were examined, screened, dewormed with Levamisole Hcl and treated for other diseases such as *Eimeria staedia* with Embazine® forte before the commencement of the experiment.

They were kept in fly proof cages and given access to fresh grass, growers mash and water was provided *ad libitum*. They were allowed to acclimatize for 14 days within which period, the bucks were trained for semen collection and baseline data was

established. Two ejaculates each were obtained from the rabbit bucks within the 14 days of acclimatization.

3.3 Experimental Infection of the Animals

The rabbits were divided into two groups of A and B of ten per group. Group A served as the control and Group B were infected. The two groups A and B were housed and fed separately. Stabilates of *T. brucei brucei* were acquired from the Department of Parasitology and Entomology of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, who also sourced it from NITR, Kaduna state. Before infecting the rabbits, the trypanosomes were maintained by serial syringe passages in white rats, and periodically checked for the viability of the parasite. Blood was obtained from the passaged rats by tail bleeding into normal saline and the parasitaemia adjusted to 1×10^6 trypanosomes per milliliter (ml) by the method of (Herbert and Lumsden, 1976). Each rabbit in Group B was inoculated intraperitoneally with 1ml of saline diluted blood containing 1×10^6 trypanosomes *T. brucei brucei*, while Group A rabbits served as uninfected control. Parasitaemia was monitored throughout the course of the experiment using Haematocrit Centrifugation Technique and Thin blood smear. At the end of the experiment, the remaining rabbits from the infected group were treated with Diminazene aceturate (0.1ml/kgb.w).

3.4 Collection of Blood Samples

Blood was collected from all the rabbits by venipuncture of the ear vein. The site for the venipuncture was aseptically prepared and swabbed with methylated spirit and blood

was collected into heparinised capillary tubes, about 30 % of each capillary tube was left unfilled and the open end of each of the tubes was carefully sealed with flame. The tubes were then loaded into a microhaematocrit centrifuge (Hawksley, England) and centrifuged at 15,000 rpm for 3 minutes.

3.4.1 Haematological analysis of the blood samples

The packed cell volume (PCV) was estimated using the microhaematocrit method and haemoglobin concentration was analyzed by the cyanmethaamogblobin method as described by Benjamin (1978), Kelly, (1984) and Baker and Silverton, (1985) once a week initially pre-infection, thrice a week post-infection, thereafter for nine weeks during the course of the experiment.

The blood was collected via venipuncture of the ear vein and capillary tubes were filled directly. The total plasma protein was determined using the hand refractometer as described by Benjamin (1985). The total plasma protein was taken once a week initially pre-infection, thrice a week post-infection, thereafter for nine weeks during the course of the experiment.

3.4.2 Parasitological examination

Direct methods: Blood sample was obtained from the infected rabbit bucks and a wet blood film (fresh blood preparation) was examined between a coverslip and a slide under the microscope, using x40 objective lens. Trypanosomes were detected and estimated using a rapid approximation method (Herbert and Lumsden, 1976) by matching the microscopic appearance of a wet film of infected blood with a precalculated count.

Parasitaemia was determined thrice weekly initially for 2 weeks post infection and thereafter weekly for five weeks.

Thin blood smears. A drop from the blood was put on a clean glass slide and these were made as in the case of blood smears. The slides were fixed in methanol for 3-5 minutes and stained with Giemsa stains for 25-30 minutes then were read using an oil immersion objective at x100 for identification of trypanosomes (OIE, 1997).

Concentration methods: Buffy coat examination (also called the Woo method, OIE, 1997). The microhaematocrit heparinised capillary tubes opened at both ends. Blood was taken up at one end by capillary attraction, until about three-quarters of the length of the tube is filled. The other end of the tube was then sealed over a burner (taking care not to char the blood) or by the use of plasticine. The tubes were then placed in the grooves of the rotor plate, with the sealed end outwards (to prevent the blood from being thrown out during centrifugation); the cover was closed and screwed down, and the timing set and spun at high speed (15,000 rpm) for three to five minutes. After centrifugation, the tubes were removed, care being taken that it remains known to which animal each of the tubes corresponds (OIE, 1997).

The tubes were then examined for the presence of trypanosomes by placing them on a microscope slide in a slot form. Immersion oil was placed on the capillary tube, over the region of the tubes where the parasites, if present, are concentrated and will be visible (buffy coat, and buffy coat/plasma junction) (OIE, 1997).

3.5 Semen Collection and Evaluation

Bucks were on a once a week semen collection regimen for two weeks pre-infection, thereafter twice weekly (Mondays and Wednesdays) post infection during the course of the experiment using a specially designed artificial vagina for rabbits by IMV Technologies 2911 model. The rabbit artificial vagina consists of the rubber casing, a rubber liner, two rubber bands, rubber funnel, graduated test tube, protective jacket and a two way valve. When the artificial vagina is coupled, the space between the inner rubber liner and rubber casing is filled with glycerol, after which the coupled artificial vagina is inserted into hot water at a temperature of 40 - 55°C; the warm glycerol simulates the vagina of the doe. A latex rubber collection cone is placed in the artificial vagina and a graduated collection tube is placed on the end of the cone. Lubrication with non perfumed petroleum jelly in the end where intromission of the penis occurs is essential (Dorji, 2012). The rabbit doe is restrained firmly in place allowing for the male exhibit his courting behavior. The buck is allowed few false mounts, after which the person with the artificial vagina collects the ejaculate by directing the penis into the artificial vagina. The test tube containing the ejaculate is then carefully handled against foreign bodies, contamination and direct sunlight and cold temperatures (Nuti, 2012). The ejaculated sample was subjected to routine semen evaluations such as semen volume, pH, colour, spermatozoa concentrations, motility, live/dead ratio and morphology (Zemjanis, 1970).

The volume of the ejaculate is easily measured with a calibrated tube which may be incorporated into the collection receptacle (Mickelsen *et al*., 1991).

The opacity and colour of the samples are usually recorded. The colour was coded 1, 2 and 3 in relation to creamy, milky and watery colour (Brito *et al*., 2002).

Sperm motility was assessed subjectively for meaningful results (Brito *et al*., 2002). Progressive motility of individual sperm is determined by diluting a drop of semen with 4 drops of normal saline on a clean pre-warmed 37 ^{0}C glass slide and covered with a clean cover slip and examined at X 40 magnification (Al-Ghalban *et al.*, 2004). Gross motility was assessed based on the swirling activity observed in a drop of semen on a pre warmed clean glass slide and viewed at a low power magnification (x 40) (Al-Ghalban *et al.*, 2004).

Concentration of spermatozoa was determined by the use of the improved Neubaeur haemocytometer which had 25 large squares or 400 small squares. Semen was pipette to the 0.5 mark using the blood cell pipette and this was made up to 1.0 mark with normal saline. The normal saline serves both as diluents for the semen and fixes the spermatozoa present. The pipette was introduced into a pipette shaker and allowed to mix. About 2 to 3 drops of fluid was discarded from the pipette before being introduced into the counting chamber of the haemocytometer allowing for even concentration of the sperm cells. Other methods commonly used are spectrophometer and computer assisted analysis. Sperm cells were counted diagonally from the left top to the right bottom in five large squares or a total of 80 small squares according to the method of Coffin, (1953), Rekwot *et al*., (1994, 1997). Spermatozoa output is determined by multiplying semen volume and spermatozoa concentration.

The live/dead ratio was done by mixing one drop of semen with one drop of Eosin-nigrosin on a warm clean slide. A thin smear was then made, air dried and observed

under the microscope. The ratio of the *in vitro* dead sperm cells was observed and it is based upon the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm cells repel the stain (Johnson, 1994; Esteso *et al.,* 2006; Koonjaenak, 2006).

Two individuals were involved in the counting allowing for accuracy, using the tally system, clicking on type of cell seen totaling 100 cells after which the amount clicked is recorded.

The sperm morphology was assessed using Eosin-nigrosin stained smear of semen samples on a clean warm glass slide. The smear was air dried and observed under light microscope. The defective sperm cells can either be primary, secondary and tertiary or head, mid-piece and tail abnormalities. The spermatozoa abnormalities were determined as described by (Koonjaenak, 2006).

3.6 Gonadal Sperm Reserves

Gonadal and epididymal spermatozoa reserves were determined 7 days post parasitaemia and 21 days post parasitaemia, two weeks apart as described by Alabi, 2005 and Ogunlade *et al.*, 2006, two bucks each from each of the treatment groups were sacrificed, the length, weight and volume of each testis was determined using a measuring tape and the digital weight balance (essae®) respectively and recorded after which the *tunica albuginea* was removed from each testis. The epididymis was separated from each testis and divided into caput, corpus and cauda which were measured and weighed using a measuring tape and the digital weight balance (essae®). Thereafter, testicular and epididymal spermatozoa numbers were determined by homogenization (Igboeli and Rahka, 1971; Egbunike *et al.,* 1976). Each fraction was homogenized in 25

ml of physiological saline solution using a mortar and pestle. Antibiotics, (Streptomycin sulphate 1 mg/ml and Sodium penicillin G 100 iu/ml) were added to the saline. The homogenate volume was measured after rinsing the mortar with 10 ml of physiological saline solution and adding the effluent. 2.5 ml of the homogenate was transferred into a conical flask and further diluted with 40 ml of saline. The diluted testicular homogenate sample was stored overnight at 5^{o}C and filtered through gauze, and the filtrate volume measured. Spermatozoa/spermatids concentrations were determined using a haemocytometer according to the method of Kwari and Waziri (2001). The determination of spermatozoa and spermatid reserves was done according to the standard method of Zemjanis (1970) and Rekwot *et al.* (1994).

3.7 Epididymal (Extragonadal) Sperm Reserves

The epididymis was carefully separated from the testis with a scalpel and the lengths and weights of the caput, corpus and cauda portions were determined using a measuring tape and the digital weight balance (essae®) respectively and recorded. The caput, corpus and cauda epididymides were separated using a sharp scissors, minced separately in 20 ml of saline and stored overnight at 5^{o}C then filtered through gauze and the volume of the filtrate was measured. 1ml of epididymal filtrate was diluted with 2ml of saline and the concentration of spermatozoa was determined with a haemocytometer under a light microscope as described by Kwari and Waziri, (2001).

3.8 Histopathological Examination

A total of eight rabbits comprising two rabbits from each of the groups were sacrificed 7 days post parasitaemia and 21 days post parasitaemia, two weeks apart. The sacrificed rabbits were autopsied immediately and samples from various body tissues (liver, kidney, testis, epididymis) were immersed in 10 % formalin dehydrated in graded concentration of absolute alcohol and xylene and embedded in paraffin. Thin sections (5 µ) mounted on a clean glass slide were stained routinely for histopathological examination by light microscopy using haematoxylin and eosin (H & E) standard protocols.

3.9 Statistical Analysis

Data generated on haematological parameters and semen characteristics (colour, volume, pH and spermatozoa concentration were expressed as mean ± standard error of the mean (SEM). Data on motility and percent live/dead of the spermatozoa were expressed as percentages and mean ± SEM. In all cases, unpaired t-test was used to test for differences between groups using Graph pad prism version 5.0. Values of P□ 0.05 were considered statistically significant.

CHAPER FOUR

RESULTS

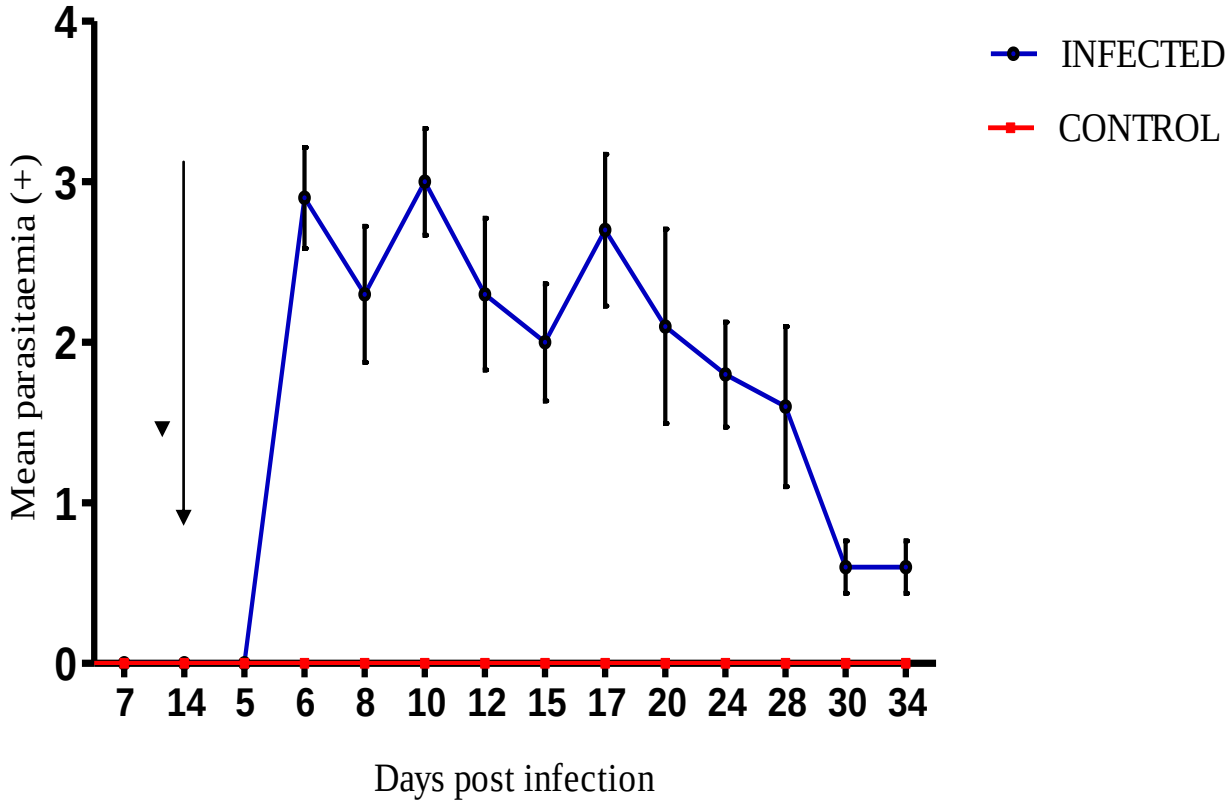
4.1 Parasitological Profile (parasitaemia) of the Infected Rabbits

There were fluctuations in the levels of parasitaemia with peak parasitaemia reached in 10 days post infection. The mean parasitaemia was 1.99 ± 0.25. This is illustrated in table 4.1.

TABLE 4.1 Mean (±SEM) Parasitological profile (parasitaemia) of infected and non infected rabbit bucks pre and post infection

	Time	Control (n=10)		Infected (n=10)	
Period	(Days)	Mean	SEM	Mean	SEM
Pre-infection	7	0.00	0.00	0.00	0.00
	14	0.00	0.00	0.00	0.00
Day of infection	5	0.00	0.00	0.00	0.00
Post infection	6	0.00	0.00	2.90	0.31
	8	0.00	0.00	2.30	0.42
	10	0.00	0.00	3.00	0.33
	12	0.00	0.00	2.30	0.47
	15	0.00	0.00	2.00	0.37
	17	0.00	0.00	2.70	0.47
	20	0.00	0.00	2.10	0.60
	24	0.00	0.00	1.80	0.33
	28	0.00	0.00	1.60	0.50
	30	0.00	0.00	0.60	0.16
	34	0.00	0.00	0.60	0.16

[ab] Means in the same row of each parameter with different superscript letters are statistically (p□ 0.05) different



Arrow indicates point of infection.

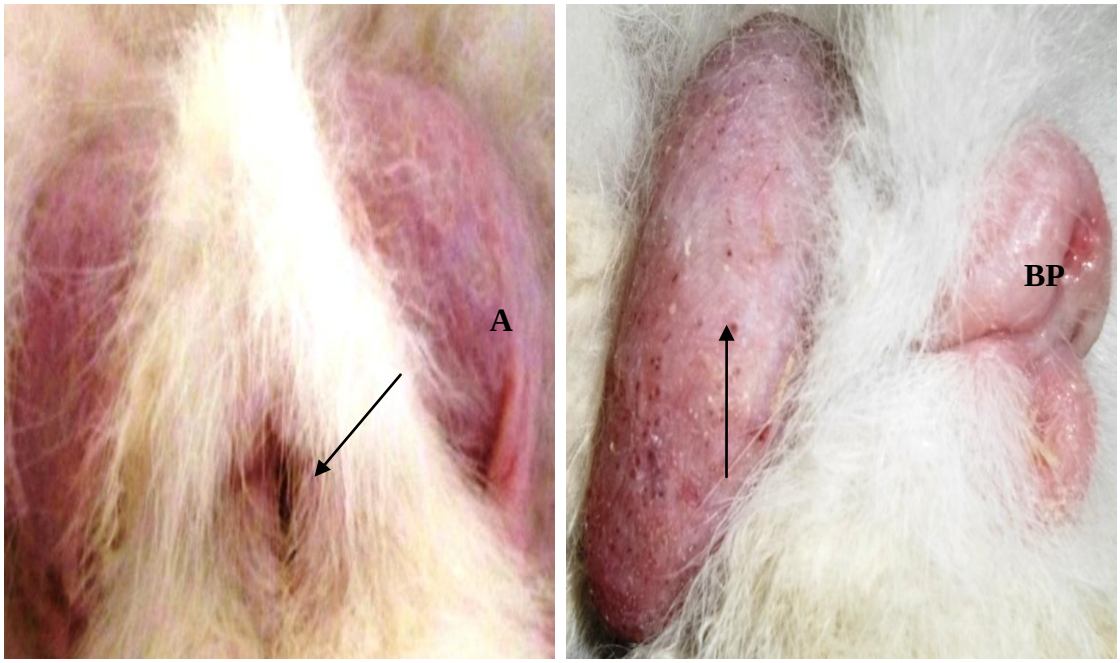
Figure 4.1: Mean (±SEM) Parasitological profile of infected rabbit bucks post infection.

Key:

+ 1-5 parasites/field
++ 1-10 parasites/field
+++ 1-15 parasites/field
++++ 1-20 parasites/field

4.2 Clinical Signs

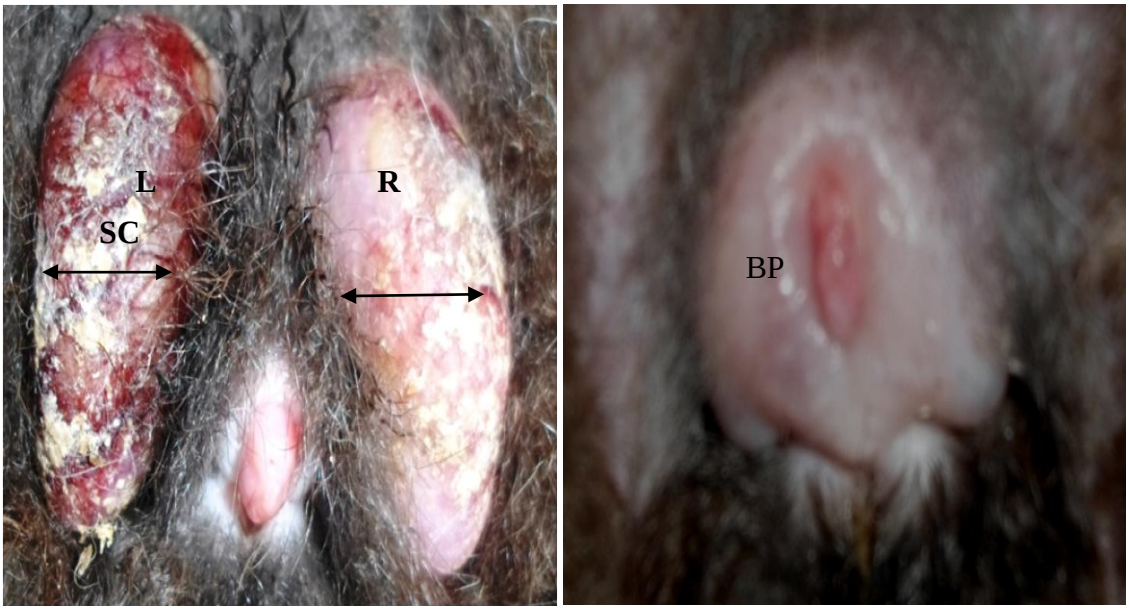
There were manifestations of some clinical signs 6-10 days post infection in the infected bucks. The signs were rough hair coat, generalized scab formation with alopecia, weakness, periorchitis, balanopostitis, scrotal inflammation, increased scrotal diameters, reduced libido and anorexia. Some of the signs observed are shown in plates I to III.



A B

Plate I: A. Photograph of the genitourinary tract of the control buck showing normal scrotal skin (A) and penile region (arrow).

B. Photograph of the genitourinary tract from the infected group. Note the scrotal dermatitis/orchitis (arrowed) and balanoposthitis (BP) at 13 days post infection.



A B

Plate II: A. Photograph of the genitourinary tract of a buck with increased scrotal diameter on both sides (arrow), scrotal necrosis and excessive thickening of the scrotal corneum (SC) of the left side 29 days post infection

B. Photograph of the genitourinary tract of a buck showing balanoposthitis (BP) 29 days post infection

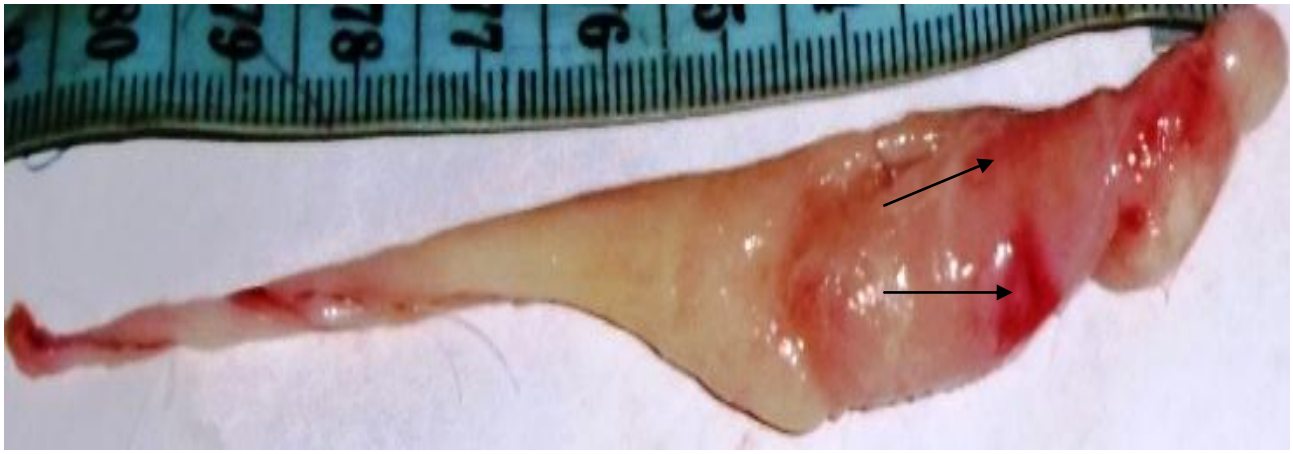


Plate III: Photograph of the testes and epididymis of a buck showing inflammation and haemorrhages (arrow) 29 days post infection

4.3 Haematological Values

Data on PCV values is presented in Figure 4.2. Statistically, there was significant difference in the PCV among the infected and control groups. The total post infection mean PCV values were 40.07 ± 0.49 % and 36.42 ± 1.15 % for the control and infected bucks, respectively. The infected rabbits showed decrease PCV 8 days post- infection (PI) with a mean value of 37.70 ±0.78 % and had the least PCV values at 7 weeks post infection with a mean value of 31.38±1.89 %. The control bucks had the highest PCV with a mean value of 42.83±2.82 %.

TABLE 4.2: Mean (±SEM) Packed Cell Volume (%) of infected and non infected rabbit bucks pre and post infection

	Time	Control (n=10)		Infected (n=10)	
Period	(Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	38.60	1.28	40.60	0.72
	2	39.11	1.16	41.30	1.25
Week of infection	3	39.11	1.16	37.70	0.78
Post infection	4	39.11	1.16	33.10	2.07
	5	38.71	1.45	34.50	1.45
	6	39.00	1.44[a]	32.50	1.44[b]
	7	39.29	1.44[a]	31.38	1.89[b]
	8	41.33	2.54	35.75	2.48
	9	42.83	2.82[a]	33.00	1.39[b]
	10	42.50	1.65	43.67	1.87
	11	42.50	1.65	39.00	2.02
	12	40.95	0.35[a]	38.86	0.26[b]

[ab] Means in the same row of each parameter with different superscript letters are statistically (p□ 0.05) different

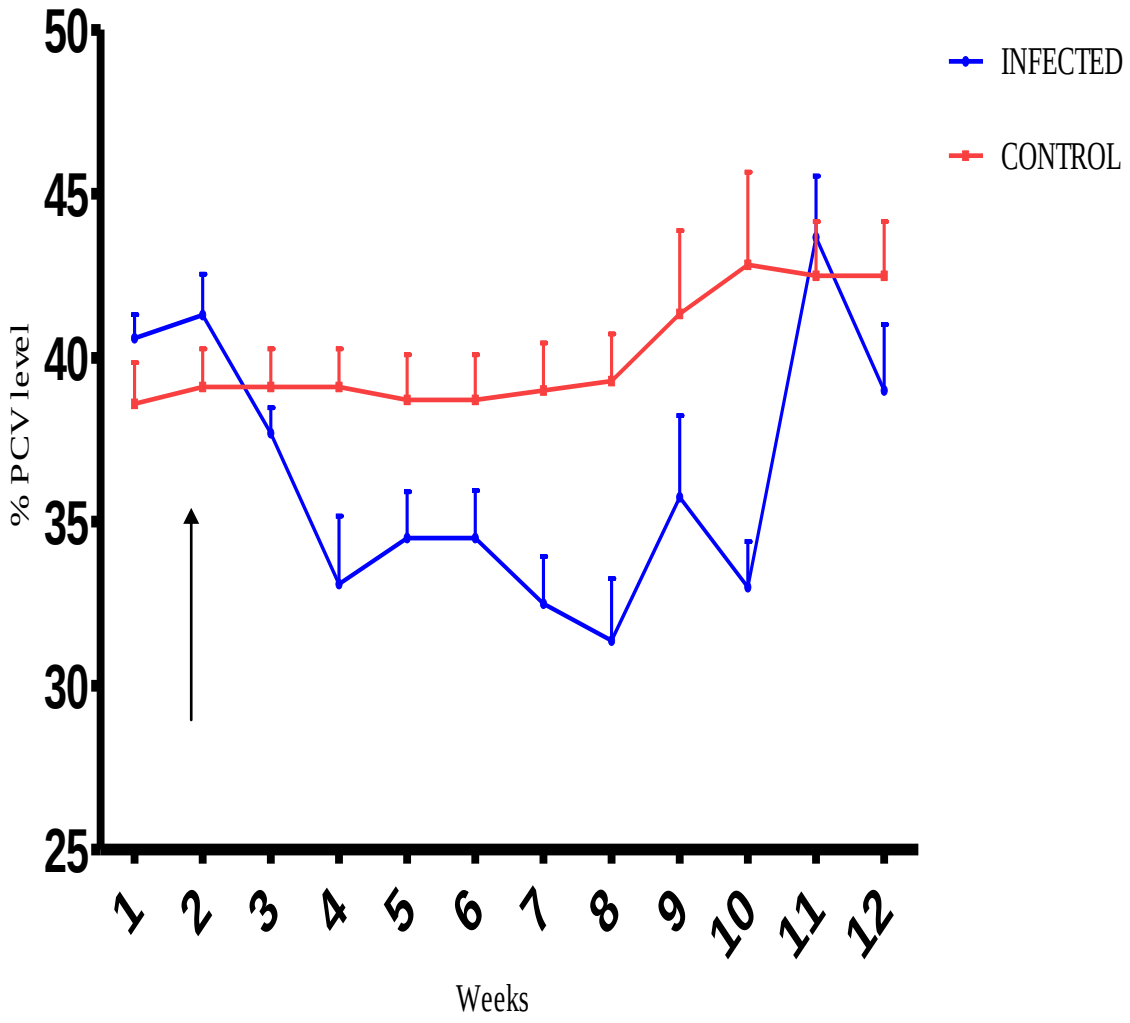


Figure 4.2: Mean (±SEM) Packed Cell Volume (%) of infected and non-infected rabbit bucks pre and post infection

Arrow indicates point of infection.

There was statistical significant difference in the haemoglobin values among the control and infected groups of the bucks at weeks 7, 8 and 10 as shown in figure 4.3. The control and infected bucks had a total mean post infection haemoglobin value of 14.31±1.01 g/dl and 12.21±0.39 g/dl respectively.

TABLE 4.3 Mean (±SEM) Haemoglobin Concentration (g/dl) of infected and non infected rabbit bucks pre and post infection

	Time	Control (n=10)		Infected (n=10)	
Period	(Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	12.86	0.42	13.54	0.24
	2	13.03	0.39	13.97	0.26
Week of infection	3	13.03	0.39	12.56	0.28
Post infection	4	25.26	12.19	11.04	0.69
	5	12.88	0.40	11.50	0.48
	6	12.90	0.46	11.50	0.49
	7	13.00	0.49[a]	10.85	0.48[b]
	8	13.09	0.49 [a]	10.45	0.63 [b]
	9	13.60	0.82	12.33	0.91
	10	13.77	1.14[a]	11.00	0.46[b]
	11	14.17	0.54	14.55	0.62
	12	14.17	0.54	13.18	0.79

[ab] Means in the same row of each parameter with different superscript letters are statistically (p□ 0.05) different

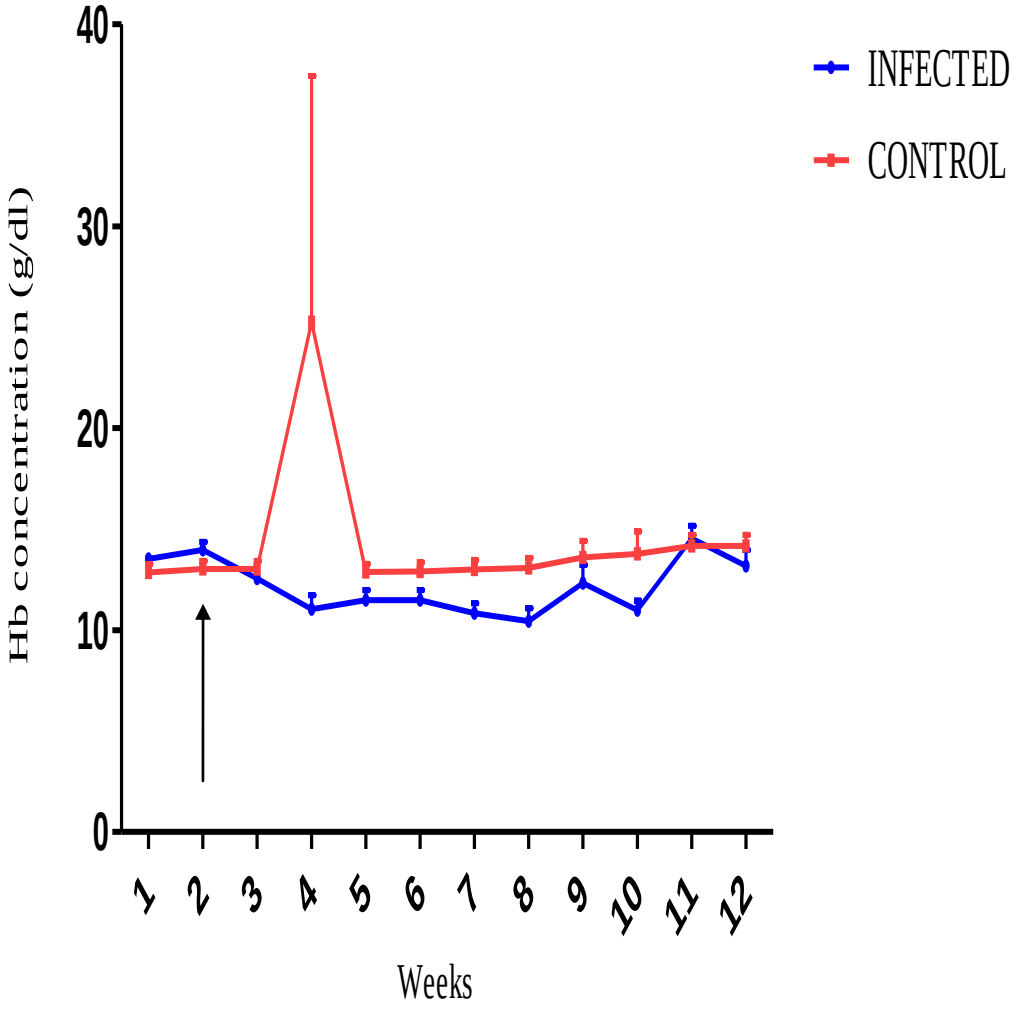


Figure 4.3: Mean (±SEM) haemoglobin concentration of infected and non infected rabbit bucks pre and post infection

Arrow indicates point of infection.

Statistically, there was significant difference in the mean plasma protein values at weeks 6 and 11 among the groups; this is illustrated in figure 4.4. The control and infected rabbit bucks had a total mean post infection plasma protein value of 6.48±0.08 gms/ml and 6.41±0.17 gms/ml.

TABLE 4.4 Mean (±SEM) Plasma Protein Concentration (gms/100ml) of infected and non infected rabbit bucks pre and post infection

	Time	Control (n=10)		Infected (n=10)	
Period	(Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	6.21	0.37	6.07	0.18
	2	6.35	0.31	6.00	0.12
Week of infection	3	6.35	0.31	6.26	0.17
Post infection	4	6.14	0.14	5.77	0.11
	5	6.50	0.22	5.88	0.08
	6	6.50	0.22[a]	5.80	0.18[b]
	7	6.50	0.22	6.48	0.25
	8	6.00	0.22	6.34	0.14
	9	6.93	0.19	6.90	0.39
	10	6.90	0.10	6.83	0.21
	11	6.67	0.21[a]	7.72	0.29[b]
	12	6.67	0.21	6.83	0.27

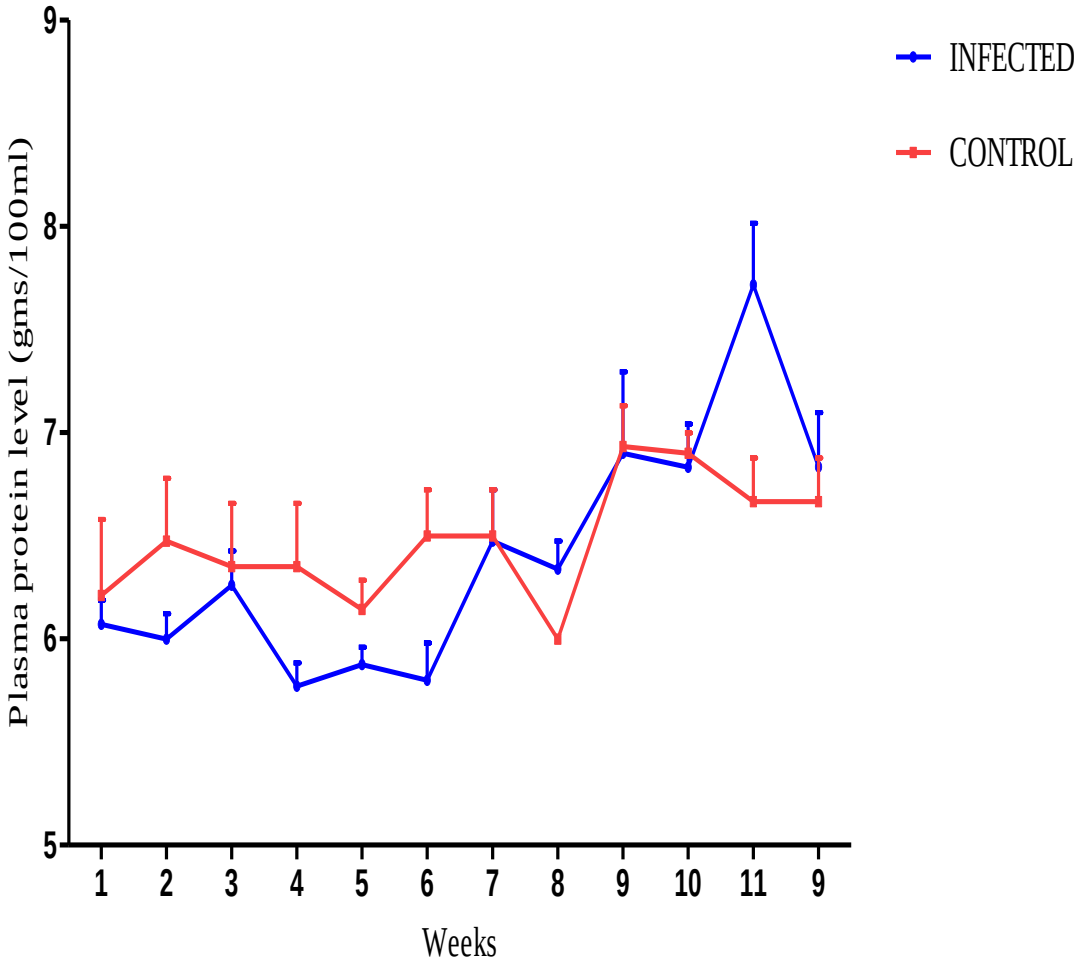


Figure 4.4: Mean (±SEM) plasma protein concentration values of infected and non infected rabbit bucks pre and post infection

Arrow indicates point of infection.

4.4 Semen Characteristics

Mean semen volume of infected and control rabbit bucks are shown in figure 4.5. There was statistical significant difference (P□ 0.05) in the volume of semen collected in the bucks at weeks 9 and 10. During the course of the experiment the control bucks had a total mean value of 0.52±0.05 mls, compared to the infected bucks with a total mean value of 0.43±0.08 mls. The semen volume progressively decreased from week four to week ten post infection, with a mean value of 0.41±0.02 mls and 0.26±0.10 mls for the control bucks and infected bucks respectively during the period of decrease.

TABLE 4.5 Mean (±SEM) Semen Volume (mls) of infected and non infected rabbit bucks pre and post infection

	Time	Control (n=10)		Infected (n=10)	
Period	(Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	0.48	0.18	0.44	0.19
	2	0.53	0.19	0.56	0.12
Week of infection	3	0.72	0.16	0.67	0.16
Post infection	4	0.76	0.17	0.63	0.14
	5	0.49	0.18	0.54	0.19
	6	0.44	0.07	0.54	0.15
	7	0.40	0.07	0.45	0.13
	8	0.35	0.08	0.17	0.06
	9	0.46	0.09^{a}	0.08	0.05^{b}
	10	0.42	0.12^{a}	0.06	0.05^{b}

[ab] Means in the same row of each parameter with different superscript letters are statistically ($p \square 0.05$) different

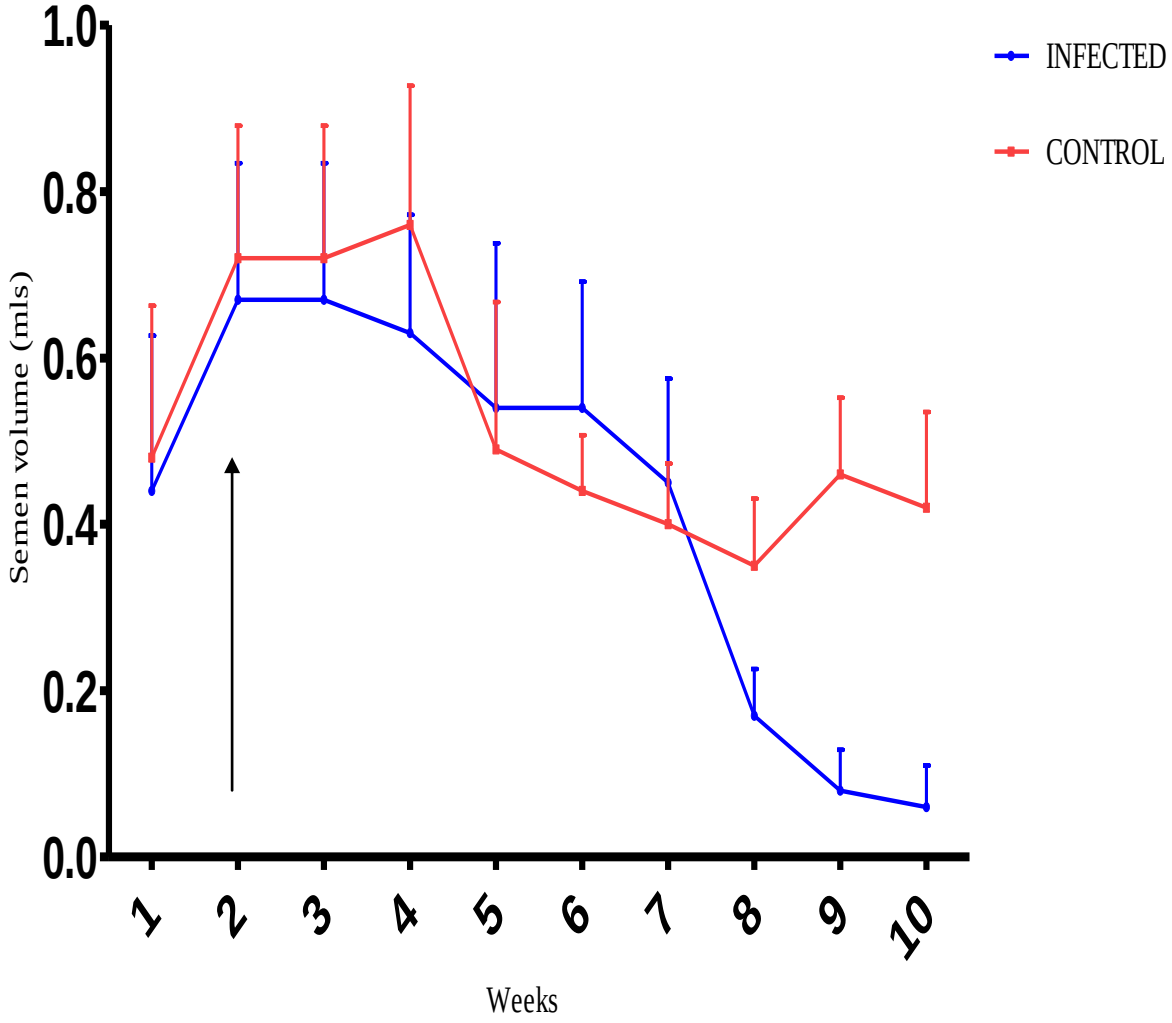


Figure 4.5: Mean (±SEM) semen volume of infected and non infected rabbit bucks pre and post infection

Arrow indicates point of infection.

There was a consistent decrease in the semen motility from week six post infection, with a significant difference of (P□ 0.05) and mean value of 64.67±4.83 % for control group and 45.20±5.79 % for infected group. The highest motility was at the beginning of the experiment giving mean values 85.30±4.55 % and 77.50±9.17 % for control group and infected group respectively, while the lowest motility was at the end of the experiment with mean values of 53.00±12.28 % for control group and 22.00±8.95 % for infected group as shown in figure 4.6.

TABLE 4.6 Mean (±SEM) Spermatozoa Motility (%) of infected and non infected rabbit bucks pre and post infection

	Time	Control (n=10)		Infected (n=10)	
Period	(Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	86.60	3.51	44.25	15.25
Week of infection	2	73.50	2.24	40.00	9.78
Post infection	3	85.30	4.55	56.11	7.67
	4	71.90	8.37[a]	31.00	13.31[b]
	5	73.00	10.81	77.50	9.17
	6	60.00	8.27	37.50	10.81
	7	70.00	9.97[a]	26.00	9.66[b]
	8	62.50	8.51[a]	37.00	6.51[b]
	9	68.50	8.92	61.90	12.51
	10	53.00	12.28[a]	22.00	4.61[b]

[ab] Means in the same row of each parameter with different superscript letters are statistically ($p \leq 0.05$) different

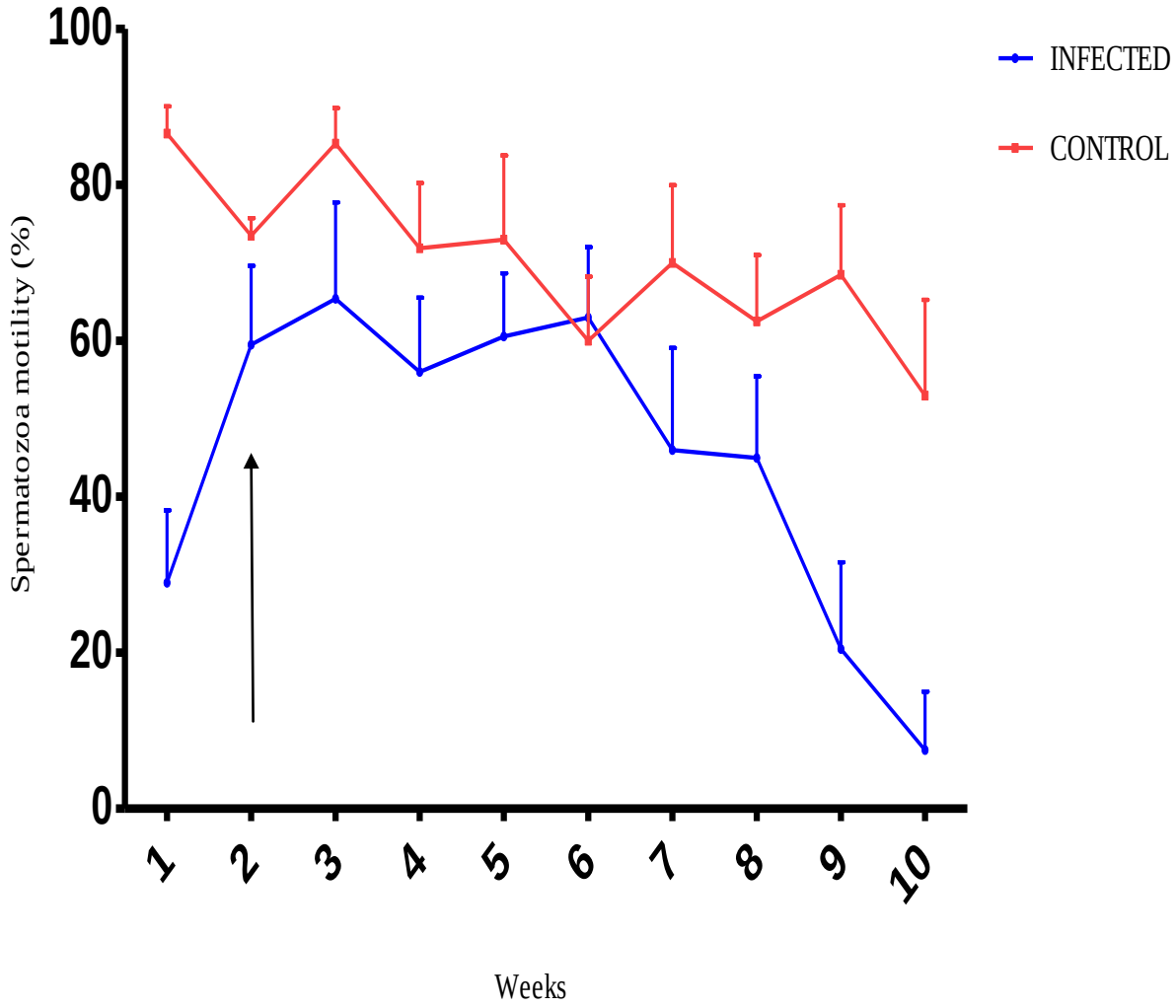


Figure 4.6: Mean (±SEM) spermatozoa motility (%) of infected and non infected rabbit bucks pre and post infection

Arrow indicates point of infection.

Figure 4.7 shows spermatozoa concentration which progressively decreased from five weeks post infection to ten weeks post infection, with significant difference (P□ 0.05). Control group had the highest spermatozoa concentration of 165.80±23.13 $X10^6$/ml at week five and lowest of 50.80±17.81 $X10^6$/ml at week one. Infected group had the highest sperm concentration of 87.80±25.26 $X10^6$/ml in the fifth week post-infection. Thereafter, there was a continual decreasing fluctuation with mean values of 45.40±19.54 $X10^6$/ml at week six till the end of the experiment giving mean values of 38.70±30.11 $X10^6$/ml at week ten post infection.

TABLE 4.7 Mean (±SEM) Spermatozoa Concentration (X 10^6/ml) of control and infected rabbit bucks pre and post infection

	Time	Control (n=10)		Infected (n=10)	
Period	(Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	50.80	17.81	45.70	17.64
	2	50.80	17.81	45.70	17.64
Week of infection	3	50.80	17.81	45.70	17.64
Post infection	4	109.00	8.46[a]	39.30	23.79[b]
	5	165.80	23.13[a]	87.80	25.26 [b]
	6	134.20	9.92[a]	45.40	19.54[b]
	7	118.70	13.51[a]	32.60	8.57[b]
	8	117.50	5.82[a]	42.80	23.45[b]
	9	113.80	19.40[a]	32.0	10.61[b]
	10	143.30	13.80[a]	38.70	30.11[b]

[ab] Means in the same row of each parameter with different superscript letters are statistically ($p \leq 0.05$) different

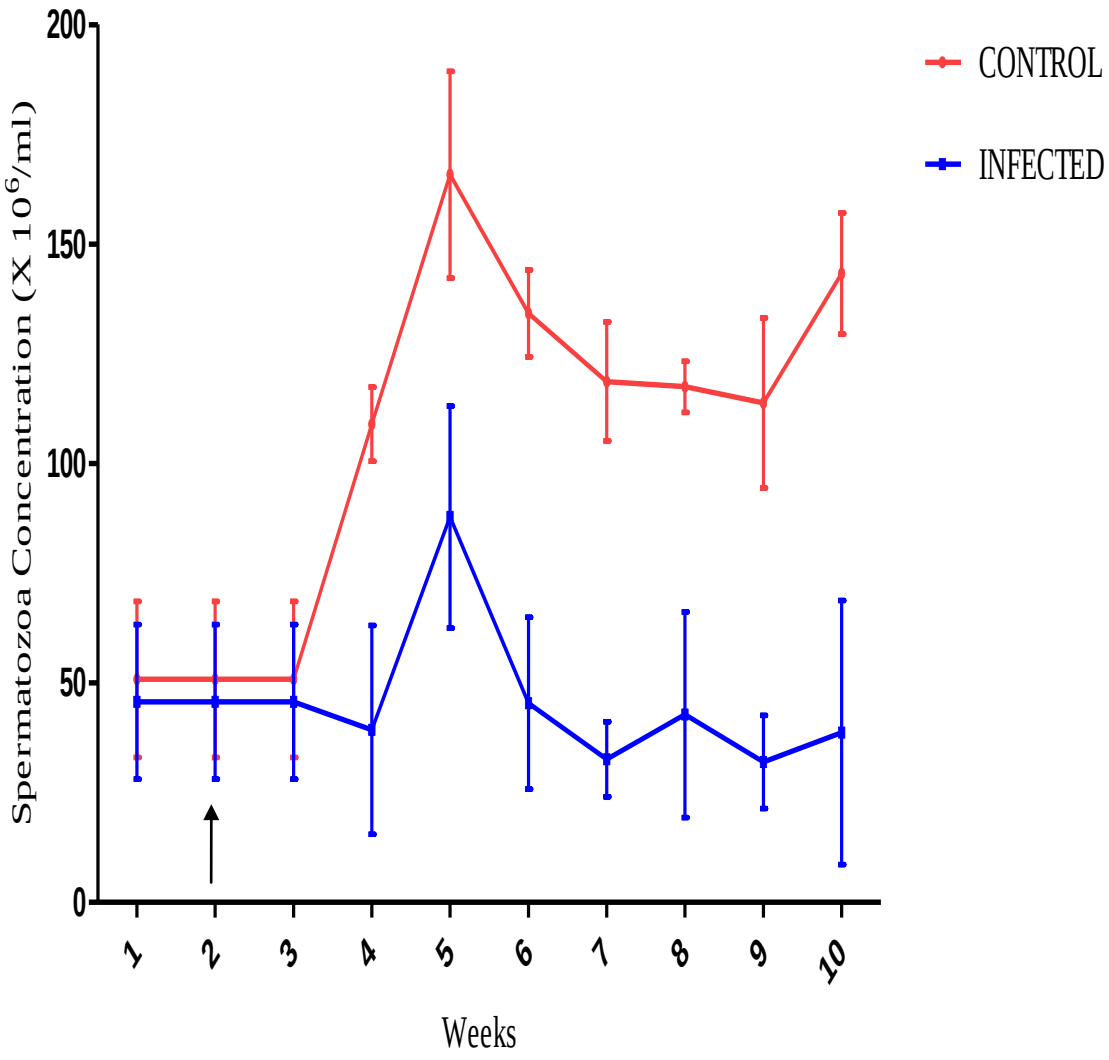


Figure 4.7 Mean (±SEM) Spermatozoa Concentration (X 10^6/ml) of infected and non infected rabbit bucks pre and post infection

Arrow indicates point of infection.

Experimental trypanosomosis also had effects on sperm morphology, with significant difference (P□ 0.05) andconsistent fluctuations. The control group had a mean value of 38.73±1.21 % for normal cells and 17.84±1.32 % for the infected group, detached heads had a mean value of 9.46 ±1.57 % and 3.85 ±0.40 % for control and infected groups respectively. Folded tails had mean values of 8.43±1.05 % for control group and 3.92 ± 0.54 % for infected group. The mean values for bent tails for the control group was 11.35± 0.90 % and 6.36 ± 1.18 % for infected group. This is illustrated in figure 4.8.

TABLE 4.8: Mean (±SEM) % values of Spermatozoa Morphological Abnormalities of infected and non-infected Rabbit bucks pre and post infection.

Variable	Control n=10		Infected n=10	
	Mean	SEM	Mean	SEM
Normal spermatozoa cells	38.73	1.21	17.84	1.32
Detached heads	9.46	1.57	3.85	0.40
Folded tails	8.43	1.05	3.92	0.54
Bent tails	11.35	0.90	6.36	1.18

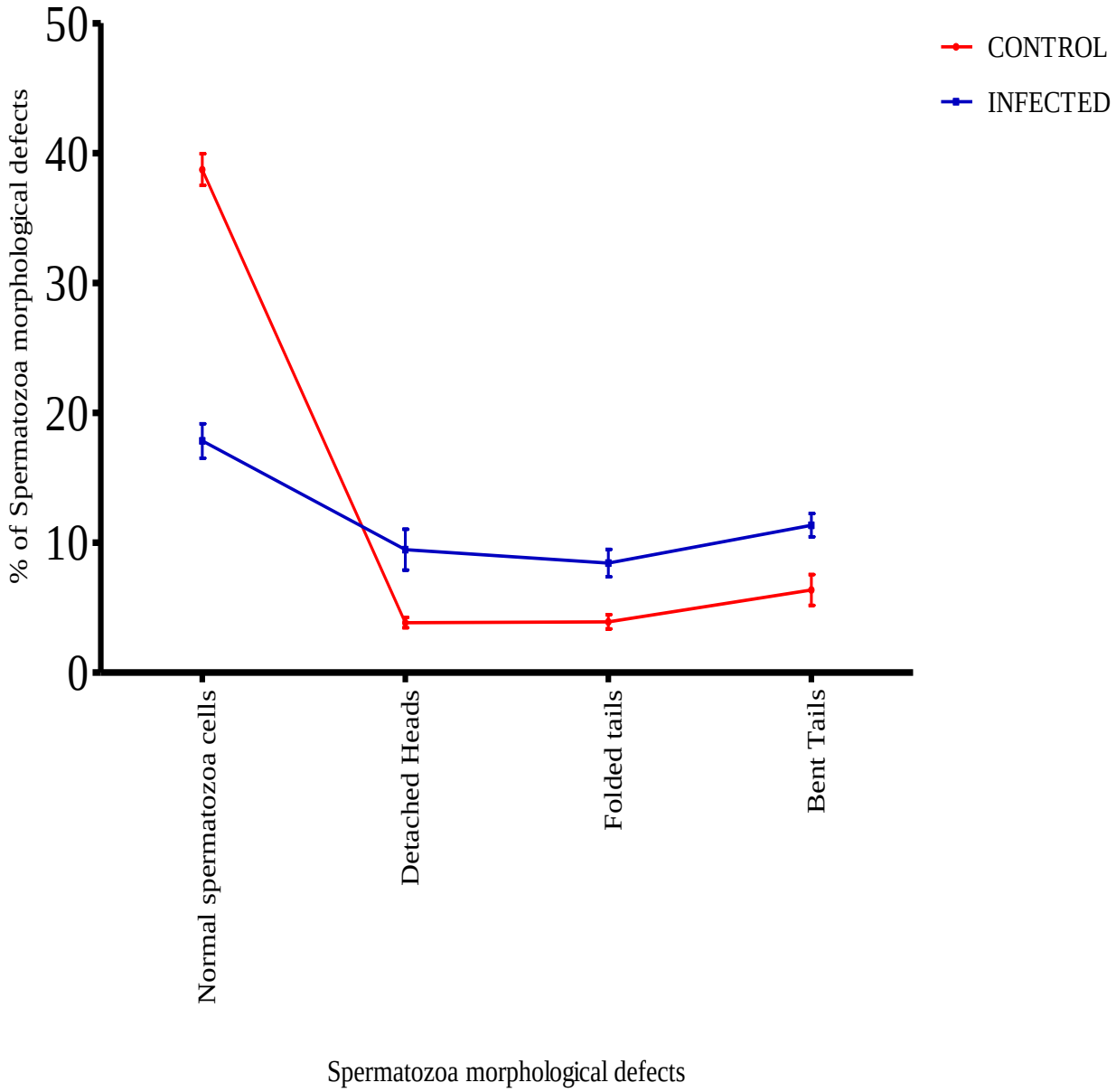


Figure 4.8: Mean (±SEM) spermatozoa morphological defects of infected and non infected rabbit bucks pre and post infection

There was statistical significant difference in the percentage live sperm cell ratio at weeks nine and ten. Control group had live mean values of 59.33±2.21 % compared to infected group that had live mean values of 48.11±5.86 % as presented in Figure 4.9.

TABLE 4.9 Mean (±SEM) Live Spermatozoa (%) of infected and non infected rabbit bucks pre and post infection

	Time	Control (n=10)		Infected (n=10)	
Period	(Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	58.00	8.67	58.00	8.67
	2	58.00	8.67	58.00	8.67
Week of infection	3	68.00	4.67	56.00	6.86
Post infection	4	67.00	7.75	66.00	2.21
	5	61.00	7.06	57.00	6.51
	6	66.00	7.77	56.00	7.18
	7	51.00	10.27	30.00	10.43
	8	51.00	10.27	41.90	9.00
	9	54.00	10.13[a]	11.00	7.06[b]
	10	67.00	7.75 [a]	11.00	7.06 [b]

[ab] Means in the same row of each parameter with different superscript letters are statistically (p□ 0.05) different

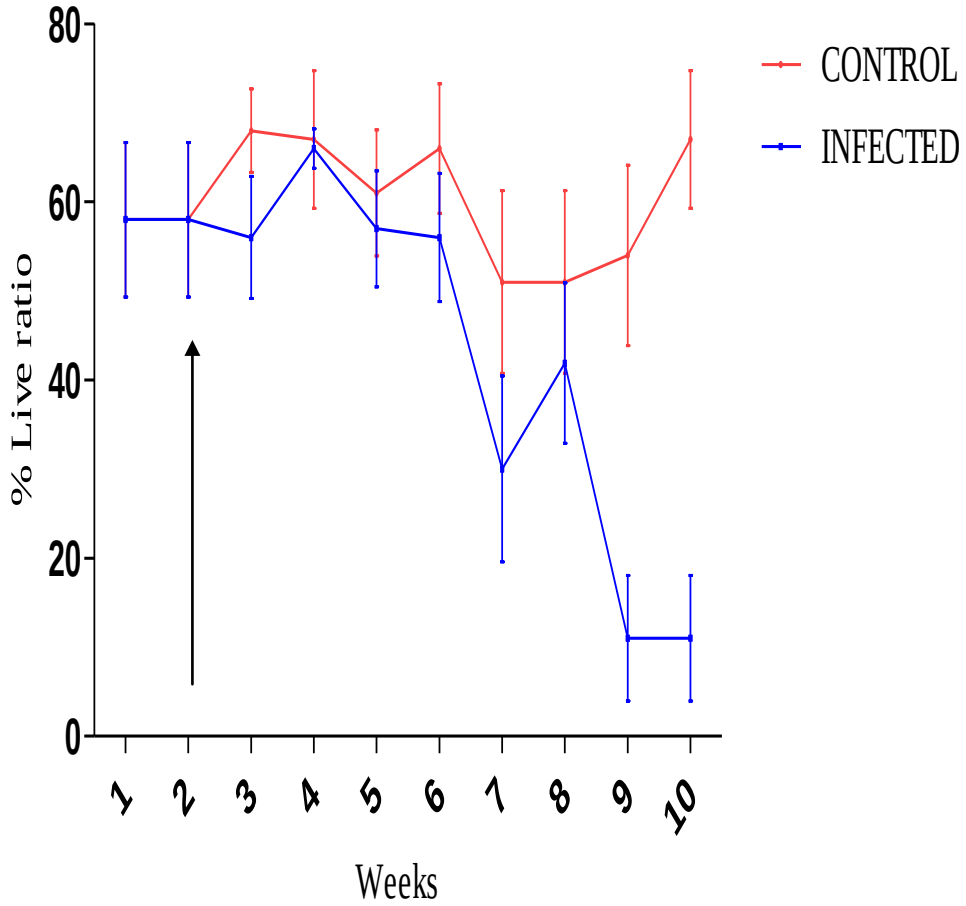


Figure 4.9 Mean (±SEM) Spermatozoa live ratio (%) of infected and non infected rabbit bucks pre and post infection

Arrow indicates point of infection.

4.5 Gonadal/Epididymal Weight (G), Length (Cm) and Spermatozoa Reserves of control and infected rabbit bucks after infection with *T. brucei brucei*.

Mean weight and length are presented in Tables 4.10 and table 4.11 respectively. Rabbit bucks showed significant differences (P□ 0.05) between the left and right testes. The right and left epididymis of the testes were weighed and separated into caput, corpus and cauda respectively.

TABLE 4.10: Mean (±SEM) Testicular and Epididymal Weights of infected and non infected rabbit bucks post infection

Variables	Control n=4		Infected=4	
	Mean	SEM	Mean	SEM
Right Testis weight (g)	1.91	0.20	1.87	0.20
Left Testis Weight (g)	1.70	0.19	1.77	0.17
Right epididymal weight (g)	3.01	0.30	3.16	0.34
Left epididymal weight (g)	2.65	0.22	3.22	0.45
Right caput weight (g)	0.87	0.22	0.70	0.23
Left caput weight (g)	0.96	0.29	0.84	0.31
Right corpus weight (g)	0.12	0.05	0.14	0.32
Left corpus weight (g)	0.13	0.07	0.04	0.00
Right cauda weight (g)	0.99	0.21	0.95	0.23
Left cauda weight (g)	0.71	0.04	0.78	0.06

TABLE 4.11: Mean (±SEM) Testicular Length of infected and non infected rabbit bucks post infection

VARIABLE	Control (n=4)		Infected (n=4)	
	Mean	SEM	Mean	SEM
Right Testis length (cm)	7.08	0.53	7.53	0.41
Left Testis length (cm)	8.50	0.08	8.65	0.30

The gonadal spermatozoa reserve had no significant difference (P□ 0.05). Mean value for the control group was 247±65.13 $X10^6$/g testis while the infected group had 173.10±26.88 $X10^6$/g testis. The caput spermatozoa reserve had significant mean values of 106.90±10.63 $X10^6$/g, (P□ 0.05) compared to the infected group that had 46.00±7.25 $X10^6$/g. The corpus and cauda spermatozoa reserve had no significant difference, with mean values of 7.63±2.63 $X10^6$/g and 175.0±26.50 $X10^6$/g, compared to the infected group which had 4.13±0.38 $X10^6$/g and 42.63±37.13 $X10^6$/g respectively. Gonadal and epididymal spermatozoa reserve is presented in table 4.12.

TABLE 4.12: Mean (±SEM) Gonadal and extra gonadal Spermatozoa Reserve of infected and non infected rabbit bucks post infection

Variable	Control (n=4)		Infected (n=4)	
	Mean	SEM	Mean	SEM
Right GSR Testis (X 10^6gm)	182.50	84.38	146.25	95.39
Left GSR Testis (X 10^6gm)	312.75	107.52	200.00	114.25
MEAN	247.60	65.13	173.1	26.88
Right EGSR Caput (X 10^6gm)	117.50	34.02	53.25	28.44
Left EGSR Caput (X 10^6gm)	96.25	24.52	38.75	23.39
MEAN	106.9	10.63	46.00	7.25
Right EGSR Corpus (X 10^6gm)	5.00	1.73	4.50	1.44
Left EGSR Corpus (X 10^6gm)	10.25	1.89	3.75	2.46
MEAN	7.63	2.63	4.13	0.38
Right EGSR Cauda (X 10^6gm)	148.50	94.33	79.75	74.43
Left EGSR Cauda (X 10^6gm)	201.50	113.41	5.50	1.90
MEAN	175.00	26.50	42.63	37.13

4.5 Histopathological Findings

A total number of eight rabbit bucks (four for each group) were sacrificed at the end of the study for histological examination. Histopathology of the testis and epididymis of the infected rabbit bucks showed necrotized seminiferous tubules, empty seminiferous tubules in existing ones with cessation of spermatogenesis as no viable spermatozoa cell was seen. There was empty epididymal lumen with massive infiltration of inflammatory cells in the testis and epididymis indicative of orchitis and epididymitis.

The liver also showed perivascular infiltration of inflammatory (kupfer) cells with diffused areas of hepatic necrosis, the kidney showed necrotized cells of the renal tubules, renal edema in the cortex and nephritis.

The effects of trypanosomosis on the testis/epididymis, liver and kidney of rabbit bucks are illustrated on plates IV-VII.

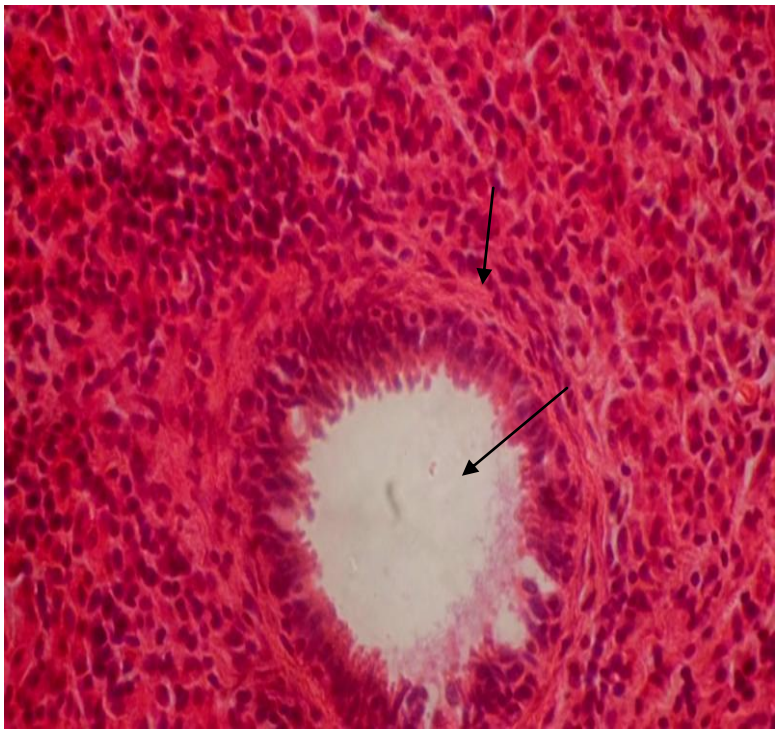


Plate IV: Photomicrograph of the epididymal lumen of an infected buck. Note the infiltration of inflammatory cells around the lumen and the empty epididymal lumen (arrow). H & E stains X40

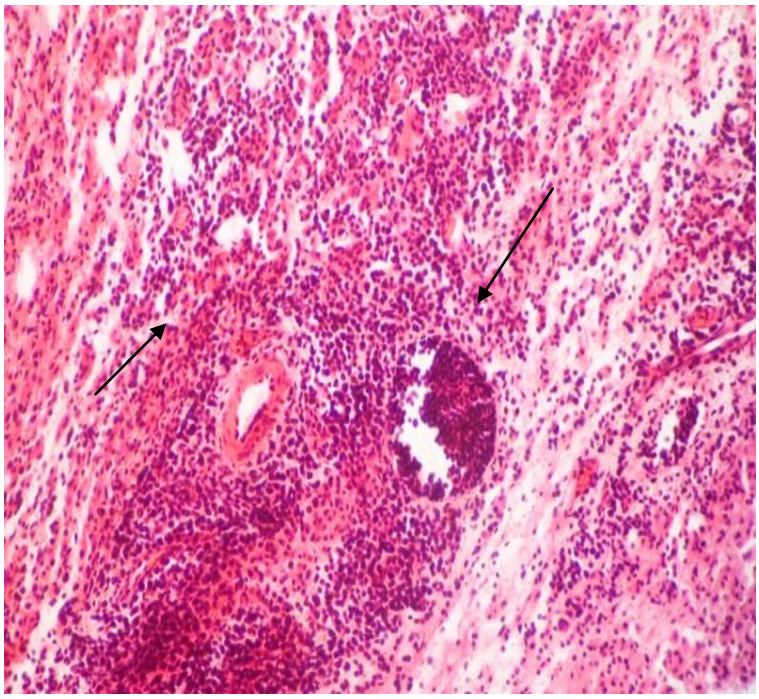
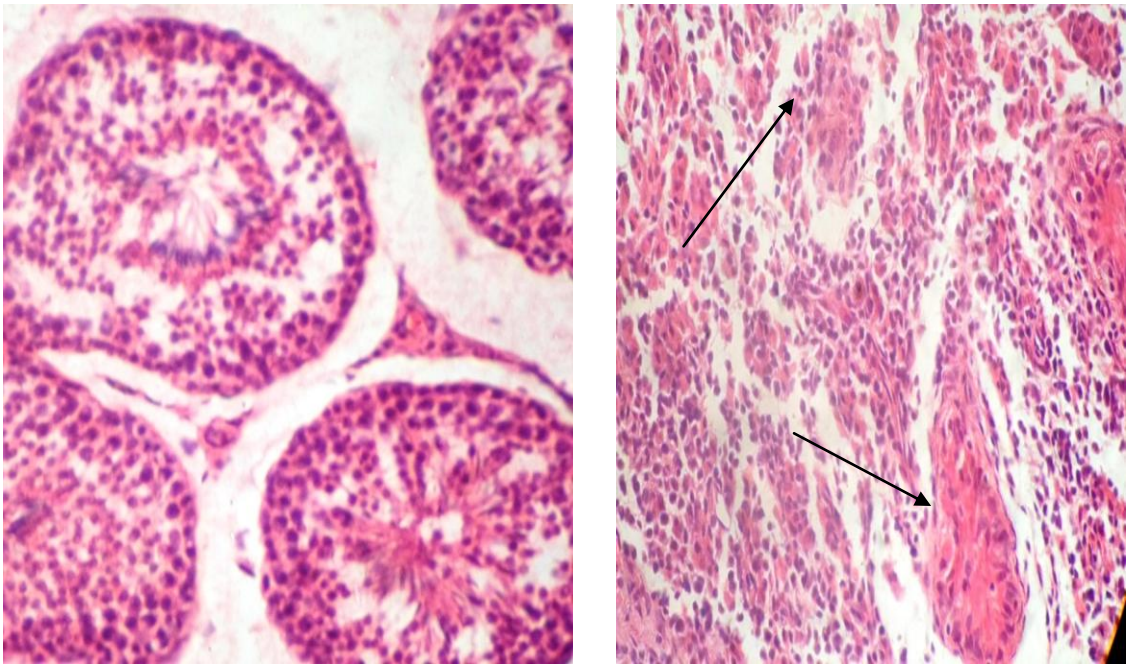


Plate V: Photomicrograph of the epididymis of an infected buck. Note areas of necrosis (Arrow) H & E stain X 40



A. B.

Plate VI: A. Photomicrograph of normal testis from the control group. Note the intact seminiferous tubules with active spermatogenic activity

B. Testis from an infected buck showing necrotized seminiferous tubules (arrow) H and E stains X 40.

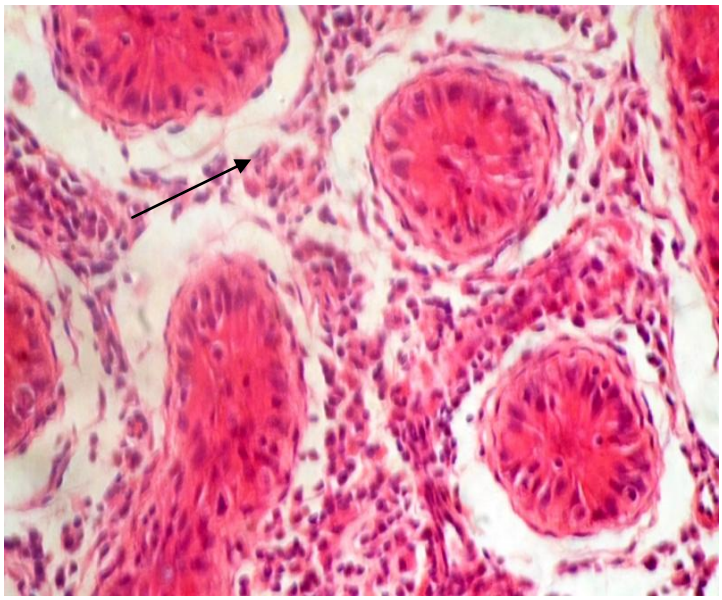


PLATE VII: Photomicrograph of testis from an infected buck showing infiltration of inflammatory cells surrounding existing seminiferous tubules (Arrow) H & E stains X40.

CHAPTER FIVE

DISCUSSION

The *Trypanosoma brucei brucei* used in this study caused clinical trypanosomosis in all the infected rabbits showing a marked pathogenicity in consistence with the findings in *Trypanosoma congolense* infected rabbits (Takeet and Fagbemi, 2009), *Trypanosoma congolense* infection in rats (Egbe-Nwiyi *et al.,* 2005) and *Trypanosoma brucei brucei* infection in rabbits (orhue *et al.,* 2005). The infection did not cause any death which is presumed to be due to the strain of the parasite used in this work.

The infected rabbit bucks had an average prepatent period of six days post infection which gradually rose to a peak by the 10^{th} day post infection thereby followed by a fluctuating parasitaemia and anorexia. Emaciation in the infected bucks was consistent with the findings of Ogunsanmi *et al.,* 1994, Omotainse *et al.,* 1994 which was reported to be as a result of the trypanolytic crisis which occurs in the peripheral blood of the infected host in the early stage of the disease (Seifert, 1996). Ogwu 1983, Agu and Bajeh, 1986 reported an eventual disappearance of parasites from peripheral circulation which was also observed during the course of this experiment 28 days post infection.

Anemia was prominent in this study shown by a reduced packed cell volume (PCV) and haemoglobin (Hb) in *Trypanosoma brucei* infections as indicated in infected rats by Eghianruwa and Anika, 2011, *Trypanosoma brucei brucei* infection in rabbits (orhue *et al.,* 2005), *Trypanosoma congolense* infected rabbits (Takeet and Fagbemi, 2009) and *Trypanosoma congolense* infection in rats (Egbe-Nwiyi *et al.,* 2005). Anemia in trypanosome infection has also been reported by several workers (Losos and Ikede, 1972, Murray and Dexter, 1988, Kaikabo and Salako, 2006, Toma *et al.,* 2008).

The drop in the PCV values observed during the course of the infection and the eventual increase in the PCV values noticed had been reported by Ogwu, (1983) and Seifert, (1996). The increase in PCV values usually follows the reduction and eventual disappearance of parasites from peripheral circulation, when this occurs, the direct effect of the parasites on the blood cells, the production of toxins and enzymes by the parasites which destroys the erythrocytes are removed leading to an increase in PCV.

There were fluctuations in the total plasma protein with a peak increase at 8 weeks post infection in consistence with the findings of Rajora *et al.,* (1968) who had an increase in total serum protein level to a peak at 21 days post infection but disagree with Sadique *et al.,* (2001) who reported decreased in total protein in cattle infected with *T. congolense*. The increase in total protein could be due to increase demand for the sub-fraction involved in the immune responses like immunoglobulin M (IgM) for the control of the infection (Takeet and Fagbemi, 2009)

The reduced weight gain and weight loss agree with findings associated with trypanosomosis infected animals (Losos 1986, Otesile 1992, Ogunsanmi 1994, Omotainse *et al.,* 1994, Seifert 1996). This could be attributed to the loss of appetite amongst the animals during the course of infection and also to the effects of cachectin, as animals were feeding and yet losing weight.

There are direct and indirect detrimental effects of trypanosomosis on reproduction in male and female animals. It is well established that most tissues and organs are damaged during the course of infection (Morrison *et al.*, 1981).

The reproductive system which is controlled by a well co-ordinated and efficient neuro-endocrine system (Nalbandov, 1976), has been reported in literature to be affected in both natural and experimental trypanosome infections (Apted; Isoun and Anosa, 1974, Ikede *et al.,* 1988). The hormones involved in reproduction originate in three principal structures which are the hypothalamus, the pituitary and the gonads. Any detrimental effect on any of the organs would result in detrimental effects on reproduction. It has been well established that severe degenerative changes of the interstitial cells of leydig within the testes takes place, and this cells are responsible for the production of testosterone, which is the hormone responsible for libido, anabolic effects and secondary male characteristics (Sekoni, 1991). Therefore, poor libido or lack of libido in all male species with trypanosomosis is a possibility, which can be supported by the observations of Mulligan *et al.* (1970) who reported that chronic *T. congolense* infections in cattle may lead to loss of libido, interference with reproduction and delayed puberty in calves. Waindi *et al.*, (1989) found low levels of plasma testosterone in goats infected with *T. congolense.*

Hoogenboezem and Swanepoel, (2001), reported that testicular degeneration might be due to nutritional deficiencies and management related factors. Degenerative changes in the seminiferous tubules, testicular degeneration, low semen output, low spermatozoa concentration, high percentage of dead spermatozoa and spermatozoa 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, 2001).

In this study, spermatozoa motility and concentration were significantly reduced, while morphological abnormalities of spermatozoa increased. These characteristics of the

ejaculate are not only vital to the physiological functions of the spermatozoa, which include spermatozoa migration and fertilization (Hunter, 1977) but are also considered in the evaluation of an animal for breeding soundness (Ott, 1986). This means that either or both natural and experimental *Trypanosoma brucei brucei* infection of bucks will result in reduced spermatozoa migration and fertilization and also the rejection of such bucks as potential breeders. Spermatozoon is produced from the seminiferous tubule of the testis in a complex process known as spermatogenesis (Roosen-Runge, 1977). The process of spermatogenesis is under the control and influence of both the endocrine and certain external factors such as photoperiod and presence of noxious agents (Ortavant *et al*., 1977, MacDonald and Pineda, 1989). Any effect such as invasion of the tissues involved in spermatogenesis by pathogens or other structures regulating the spermatogenic tissues will result in reduction of ejaculate qualities (White, 1933, Noakes *et al*., 2001). Since *Trypanosoma brucei brucei* is tissue invasive, the observed reduction in ejaculate characteristics can be linked to the effects of the infection.

Some pathogenic effects on the male reproductive systems have been reported. The reduced libido, increased scrotal diameters, scrotal inflammations, alopecia, periorchitis, severe testicular degenerations, balanitis, abnormal spermatogenesis in the infected rabbit bucks agrees with the findings of trypanosoma infected animals as reported by Ikede and Akpavie, 1982; Sekoni *et al.*, 1991; Sekoni 1992, which may be influenced by the effect of trypanosomes on the testis affecting the leydig cell steroidogenesis (Sekoni *et al.,* 1988) through the indirect effects of pyrexia and the accompanying waves of increasing parasitaemia during infection and increased scrotal temperatures (Mutayoba *et al.,* 1995b).

There were genital lesions within 13 - 29 days post infection such as alopecia, periorchitis, epididymitis, severe testicular degeneration and haemorhage and deteriorated semen characteristics which agrees with reports by Isoun and Anosa, 1974 and Anosa and Isoun, 1980.

Agu *et al.*, 1986 reported deteriorated semen characteristics in rams infected with *T. vivax* which was a consistent finding in this study. There was a consequent reduction in the volume of semen collected and also a reduction in the gross motility and concentration of the sperm cells with an increased number of morphological defective sperm cells such as the detached heads, free tails, bent tails and coiled tails which agrees with the findings of Akpavie *et al.,* (1986), Sekoni *et al.,* (1990) and Sekoni (1993) who reported aspermatogenesis and various sperm abnormalities in trypanosomosis infected animals.

Although the infection had no significant effects on the gonadal and epididymal sperm reserves, but numerical differences were observed between the groups, with an apparent superiority of the control group over the infected group in the parameters. These numerical differences may be related to the effect of the infectious agent on spermatogenesis, although this could vary with different species and strains of trypanosomes used. There was significant difference in the epididymal sperm reserve of the infected rabbits in the caput region of the epididymis.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

This study has indicated that *T. brucei brucei* is pathogenic to rabbit bucks. It reveals that *T. brucei brucei* may be a threat to the reproductive performance of rabbit bucks and could be detrimental to the efforts to increase animal protein, and the socio-economic wellbeing of the farmers and people in the tsetse endemic areas in the country where rabbit farming is important.

The study also showed a need of considering trypanosomosis as a differential diagnosis when previously productive rabbit bucks suddenly have a low libido and develop serious reproductive anomalies especially in tsetse endemic areas.

6.2 Recommendations

Based on the results of this study, it is recommended that further studies should be carried out in tsetse endemic areas on the role of nutrition in the pathogenicity, haematological and reproductive indices of *Trypanosoma brucei brucei* infection in rabbit bucks.

The study therefore also recommends that enlightenment be created where farmers are told of the devastating effect of this infection and how it could be controlled.

REFERENCES

Abdu, S.B. (2008). Effect of vitamins deficiencies on the histological structure of the testis of albino mice *Mus musculus*. *Saudi Journal of Biological Sciences,* 15, 269- 278.

Abdulmalik, M. E. (1994). Rabbit production. In Okaiyeto P. O, Ndubuisi A. H and Okoh A. E (editors). Advanced Animal Husbandry Practices for Subject Matter Specialists in the ADPs. Training Manual for FACU/NAPRI, Workshop, Zaria, 13-17 December 1994.

Acott, T.S. and Hoskins, D. (1989). Bovine spermatozoa forward motility protein binding to epididymal spermatozoa. *Biology Reproduction.* 24:2 34.

Adams, C. E. (1972). Induction of ovulation and A. I techniques in the rabbit. Veterinary Records. 91: 194 - 196.

Adams, C.E. (1981). Artificial insemination in the rabbit: The technique and application to practice. *Journal of Applied Rabbit Research.* 4: 10–13.

Adebayo, K. and Sorungbe, O.S. (2002). Farmers' perception of the epidemic of African Swine fever in Nigeria. *Nigerian journal of Animal Production,* 29(2):217-225.

Adebowale, O.A.L. (2012). Agriculture and Biological Sciences : "Livestock Production", book edited by Khalid Javed, ISBN 978 – 953.

Aduku, A.O. and Olukosi, J.O. (1990). Rabbit Management in the Tropics: Production, Processing, Utilization, Marketing, Economics, Practical training, Research and Future Prospects, Living Book Services, G.U. Publications, Abuja.

Afewerk, Y., Clausen, P.H., Abebe, G., Tilahun G. and Mehlitz, D. (2000). Multiple drug Resistant: *Trypanosoma congolense* populations in village cattle of metekel district, northwest Ethiopia. *Acta Tropica*, 76: 231-238.

Agu, W. T. and Bajeh, Z. T. (1986). An outbreak of *Trypanosoma brucei brucei* in pigs, in Benue State, Nigeria. *Tropical Veterinary* 4, 25-28.

Ahemen, I. and Bitto, I.I. (2007). Sperm production rate, gonadal and extra gonadal sperm resrves of West African Dwarf rams in Markurdi. *Proceedings 32nd Annual Conference of Nigerian Society of Animal Production,* Calabar, March18-22, pp: 99-101.

Ajala, A. A. (1990). A note on rabbit feeding in the traditional farming system of Nigeria (Oyo State) and implication for extension activities. *Journal of Applied Rabbit Research*. Vol. 12: 280 – 282.

Ajala, M.K. and Balogun, J.K. (2004): Economics of rabbit production in Zaria, Kaduna State. *Tropical Journal of Animal Science,* 7(1): 1-10

Akanji, M.A. and Ngaha, E. (1989). Effect of repeated administration of Berenil on urinary enzyme secretion with corresponding tissue patterns in rats. *Pharmacology and Toxicology*, 64: 272-275

Akpavie, S. O., Ikede, B. O. and Egbunike, G. N. (1986). Ejaculate characteristics of sheep infected with *Trypanosoma brucei* and *Trypanosoma vivax*: changes caused by treatment with diminazene aceturate. *Research of Veterinary Science*, 42(1): 1-6.

Akpavie, S.O., Ikede, B.O. and Egbunike, G.N. (1987). Ejaculate characteristics of sheep infected with *Trypanosoma brucei* and *Trypanosoma vivax* changes caused by treatment with diminazene aceturate. *Research of Veterinary Science*, 42(1):1-6.

Aksoy, Y., Aksoy, H., Altinkaynak, K., Aydin, H.R. and Ozkan, A. (2006) Sperm fatty acid composition in sub-fertile men. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 75, 75-79.

Alabi, J.F. (2005). Effects of energy supplementation on growth and reproductive function of Bunaji and Friesian X Bunaji bulls. PhD thesis, Ahmadu Bello University Zaria, Nigeria.

Allen, C.E. (1993). New horizons in animal agriculture: future challenges for animal scientists. *Journal of Animal Science* 57 supplement. (2): 16 http://jas.fass.org/cgi/reprint/57/supplement2/16.pdf

Al-Ghalban, A.M., M.J. Tabbaa and R.T. Kridli, (2004). Factors affecting semen characteristics and scrotal circumference in Damascus bucks. *Small Ruminant Research,* 53: 141-149.

Almeida, A.M., Schwalbach, L.M.J., Cardoso, L.A. and Greyling, J.P.C. (2007). Scrotal, testicular and semen characteristics of young Boer bucks fed winter veld hay: The effect of nutritional supplementation. *Small Ruminant Research*, 73, 216-220. *Journal of small ruminant research.*

Al-Qarawi, A.A., Omer, H.S., Abdel-Rahman, H.A, El-Mougy, S.A. and El-Belely, M.S. (2004). Trypanosomiasis induced infertility in dromedary (Camelus dromedaries) bulls: Changes in plasma steroids concentration and semen characteristics. *Animal Reproduction Science* 84:73-82.

Alvarino J.M.R. (2000). Reproductive performance of male rabbits. *In: Proceedings 7th World Rabbit Conference., Valencia Jul., 2000, vol. A, 13-35.*

Amaechi, N. (2001). Toxicity of antiprotozoan drug, diminazene aceturate in rats. Journal of Sust. Agriculture Environment, 3(2): 365-370.

Amann, R.P., Johnson, L. and Thompson, D.L. (1998). Daily spermatozoa production, epididymal spermatozoa reserves and transit time of spermatozoa through the epididymis of the rhesus monkey. *Biology of Reproduction,* 15:586.

Anosa, V. O. (1988a). Haematological and biochemical changes in human and animal trypanosomosis. Part II: *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux*, 4(1): 65-78.

Anosa, V. O. (1988b). Haematological and biochemical changes in human and animal trypanosomosis. Part II: *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux*, 4(2): 151-164.

Anosa, V.O. and Isoun, T.T. (1980). Further observations on the testicular pathology in Trypanosoma vivax infection of sheep and goats. Research in Veterinary Science. 28: 151-160.

Arienti, G., Carlini, E., De Cosmo, A.M., Di Profio, P.and Palmerini, C.A. (1998). Prostasome-like particles in stallion semen. *Biology and Reproduction*. 59: 309–13.

Baker,F.J. and Silverton, R.E. (1985). Introduction to Medical Laboratory Technology, 6h Edition, Butterworthand Co. Publishers Limited, U.K., 408.

Barth, A.D., Brito, L.F.C. and Kastelis, J.P. (2008). The effect of nutrition on sexual development of bulls. *Theriogenology*. 70:485-494.

Barth, A.D. and Oko, R.J. (1989). Abnormal morphology of Bovine Spermatozoa. Iowa State University Press. Ames.

Barrett, M.P., Burchmore, R.J. and Stich, A., (2003). "The Trypanosomiases" Lancet, 362(9394):1469-1480.

Battaglini, M., Constantini, F., Baldissera, C.N. and Castrovilli, C.F. (1982). Induction of ovulation and artificial insemination in the rabbit. *Coniglicoltura*. 19: 45–54.

Bawa, E.K., Oyedipe, E.O., Sanusi A., Eduvie, L.O., Ogwu D. and Esievo, K.A.N. (1990). Effects of *T. vivax* infections on pregnancy in Yankasa and West African Dwarf sheep. Comparative clinical observation between Yankasa and WAD sheep following infection at second and third trimester of pregnancy. *National Animal Production Research Institute –Ahmadu Bello University, Zaria Bulletin* Pp. 87-89.

Bawa, E.K., Sekoni, V.O., Ogwu, D., Esievo, K.A.N. and Uza, D.V. (2005). Results of Novidium (Homidium chloride) chemotherapy on clinical manifestation of *Trypanosoma vivax* infected pregnant Yankasa and West African Dwarf (WAD) Ewes. *Journal of Animal and Veterinary Advances,* 4(7): 637 – 641, 2005.

Bencheikh, N. (1995). Effet de la fréquence de collecte de la semence sur les caractéristiques du sperme. *Annals of Zoology. 44, 263-279.*

Benjamin, M.M. (1978). *Outline of Veterinary Clinical Pathology*, 3rd Ed., Iowa State Univ. Press, Ames, Iowa, USA.

Benjamin, M.M. (1985). Outline of veterinary Pathology, 3rd Edition, Kalyami Publishers, New Delhi, India.

Bhosrekar, M.R. and Rajdan, M.N. (1973*).Indian Journal of Animal Science.* 43: 196-200.

Bielli, A., Pedrana, G., Gastel, M.T., Castrillejo, A., Moraña, A., Lundeheim, N., Forsberg, M. and Rodriguez-Martinez, H. (1999). Influence of grazing management on the seasonal change in testicular morphology in Corriedale rams. *Animal Reproduction Science* 56, 93−105.

Biljani, R. L. (2012). Reproductive system. Fundamenta;s of physiology. Jaypee brothers publishers. Pp 240.

Biobaku, W. O. (1993). Effect of feeding raw and cooked belonix regia seed bean on the performance of rabbits. *Journal of Agricultural Technology*. Maiden Edition. 56-60.

Biobaku, W.O. and Oguntona, E.B. (1997). The effects of feeding multi nutrient mini blocks and pelleted diet on the growth of rabbits. *Nigeria Journal of Animal Production,* 24(2): 147-149.

Biryomumaisho, S. and Katunguka-Rwakishaya, E. (2007). The pathogenesis of anaemia in goats experimentally infected with *Trypanosoma congolense* or *Trypanosoma brucei*: Use of myeloid: erythroid ratio. *Veterinary Parasitology*, 143: 354-357.

Bleau, G. and Vandenheuvel, W.J. (1974). Desmosteryl sulfate and desmosterol in hamster epididymal spermatozoa. *Steroids.* 24: 549–55.

Blood, D.C and Radostits, O.M. (1989). Veterinary Medicine, a textbook of diseases of cattle, sheep, pigs, goats and horses. 7th edition. English language book society/Bailliere. Tindal:766.

Boiti, C., Castellini, C., Theau-Clément, M., Besenfelder, U., Liguori, L., Renieri , T. and Pizzi, F. (2005). Guidelines for the handling of rabbit bucks and semen. *International Rabbit Reproduction Group. World Rabbit Science.* 2005, 13: 71 – 91.

Bombeke, A., Okerman, F. and Moermans, R J. (1975). Etude comparative de differentes races de lapins de chair. Rev. Agric., 28 (2), 421-438.

Boukhliq, R., Adams, N.R.and Martin, G.B. (1996). Effect of nutrition on the balance of production of ovarian and pituitary hormones in ewes. *Animal Reproduction Science* 45, 59−70.

Brackett B.C., Bousquet D. and Dressel M.A. (1982). In Vitro sperm capacitation and in vitro fertilization with normal development in the rabbit. *Journal of Andrology. 3, 402-411.*

Brito, L.F.C., Silva A.E.D.F., Rodrigues L.H., Vieira F.V., Deragon L.A.G. and Kastelic, J.P. (2002). Effects of environmental factors, age and genotype on sperm production and semen quality in Bos indicus and Bos Taurus AI bulls in Brazil. *Animal Reproduction Science.* 70 (3-4), 181-190.

Brun, J.M., Theau-Clément, M. and Bolet, G. (2002). The relationship between rabbit semen characteristics and reproductive performance after artificial insemination. *Animal Reproduction Science, 70: 139-149.*

Burns, B.M., Fordyce, G. and Holroyd, R.G. (2010). A review of factors that impact on the capacity of beef cattle females to conceive, maintain a pregnancy and wean a calf-Implications for reproductive efficiency in northern Australia. *Animal Reproduction Science* 122, 1−22.

Burrows, W.H. and Quinn, J.P. (1937). The collection of spermatozoa from the domestic fowl and turkey. Poultry science, 26: 19 – 24.

Camier, B., Gagneur, O., Arlot, S., Thépot, F. and Vitse, M. (1989). Preliminary study of the use of intranasal Buserelin in the long-term protocol of ovarian stimulation with the view toward fertilization in vitro. *Gynecology Obstetrics.* 84: 659–661.

Cardinali, R., Dal Bosco, A., Mourvaki, E., Del Vecchio, M.T. and Sartini, B. (2007). Rabbit semen particles: secretion pattern and main effect on the sperm functions. *Journal of Submicroscell Cytolology Pathology.* 39: 3–10.

Carter, P.D., Hamilton, P.A. and Duffy, J.H. (1990). Electroejaculation in Goats. *Australian veterinary Journal,* 67:91-93.

Casady, R B. (1975): Advisory leaflet on rabbit meat production. Value and use of rabbit manure. Paper presentation Conference on Rabbit Meat Production, Malta.

Castellini, C., Mourvaki, E., Cardinali, R. and Del Vecchio, M.T. (2008). Rabbit seminal granules: secretion site and main role. In: Collodel G, Moretti E, editors. Sperm Morphology and Pathology. Kerala: *Research Signpost.* Pp 45–59.

Castellini, C., Cardinali, R., Dal Bosco, A., Minelli, A. and Camici, O. (2006). Lipid composition of the main fractions of rabbit semen. *Theriogenology.* 65: 703–12.

Castellini C., Lattaioli P., Cardinali R., Dal Bosco A. (2006). Effect of collection rhythm on spermatozoa and droplet concentration of rabbit semen. *World Rabbit Science 14: 101-106.*

Charlton, H. (2008). "Hypothalamic control of anterior pituitary function: a history". *Journal of Neuroendocrinology.* 20 (6): 641–6.

Cheeke, P.R., Patton, N.M., Luketahr, S.D. and McNitt, J.I. (1987). Rabbit production. 6th edition. The interstate printers and publishers Inc. U.S.A.

Cheon, Y.M., Yang, H.K.M., Yi, Y.J and Park, C.S. (2002). Effect of season influencing semen characteristics, frozen thawed spermatozoa viability and testosterone concentration in Duroc Boars. Asian –Australian Journal of Animal Science., 15:500-503.

Chinoy, N.J. and Ahmedabad, A.S. (1998). Amelioration of fluoride toxicity by vitamins E and D in reproductive functions of male mice. *Fluoride*, 31, 203-216.

Chung, S.S.W., Wang, X. and Wolgemuth, D.J. (2009). Expression of retinoic acid receptor alpha in the germline is essential for proper cellular association and spermiogenesis during spermatogenesis. *Development*, 136, 2091- 2100.

Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C. (1994). Infectious diseases of livestock. With special reference to Southern Africa. Oxford University Press, South Africa. 1605 pp. (ISBN 0-19-570506-8)

Coffin, D.L. (1953). Enumeration of mammalian spermatozoa. Manual of Veterinary Clinical Pathology, 3rd Ed. Comstock publication company, Ithaca, New York.

Connor, W.E., Lin, D.S., Wolf, D.P. and Alexander M. (1998). Uneven distribution of desmosterol and docosahexaenoic acid in the heads and tails of monkey sperm. *Journal of Lipid Research.* 39: 1404–11.

Courtin, D., Berthier, D., Thevenon, S., Dayo, G.K., Garcia, A. and Bucheton, B. (2008). "Host genetics in African trypanosomiasis". *Infection Genetics Evolution.* 8 (3): 229–38.

Daniel-Carlier, N., Harscoet, E., Thepot, D,, Auguste, A, Pailhoux, E. and Genevieve J. (2013). Gonad Differentiation in the Rabbit: Evidence of Species – Specific Features. *PLOS ONE.* 8(4): e60451.

de Blas C. and Wiseman J. (1998). The nutrition of the rabbit. *CABI Oxon, UK*.

Donnez, J., Nisolle-Pochet, M., Cleckx-Braun, F., Sandow, J. and Casanas-Roux, F. (1989). Administration of nasal Buserelin as compared with subcutaneous Buserelin implant for endometriosis. *Fertility and Sterility.* 52: 27–30.

Dorji, T. (2012). Semen collection. Animal reproduction. Pp. 1- 5.

Downs, J.L. and Wise, P.M. (2009). "The role of the brain in female reproductive aging". *Molecular Cell Endocrinology.* 299 (1): 32–8.

Ebisch, I.M.W., Thomas, C.M.G., Peters, W.H.M., Braat, D.D.M. and Steegers-Theunissen, R.P.M. (2007) The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. *Human Reproduction Update*, 13, 163-174.

Edmond, J.R.; Korsak, A.; Morrow, J.W.; Torok-Both, G. and Catlin, T.H. (1990). The origin of cholesterol in brain of developing rat. *FASEB Journal.* 4: A532.

Edmondson, M.A., Roberts, J.F., Baird, A.N., Bychawski, S. and Pugh, D.G. (2012). *Theriogenology of Sheep and Goats.* Pp. 150 – 174.

Egbe-Nwiyi, T. N., Igbokwe, I.O. and Onyeyili, P. A. (2005). Pathogenicity of *Trypanosoma congolense* infection following oral calcium chloride administration in rats. *African Journal of Biomedical Research,* 8: 197-201.

Egbunike, G.N. (1997): What is Animal Science? And how can Nigeria get out of malnutrition. In: Livestock Products. Ologhobo, A.D., Iyayi E.A., Adesehinwa A.O.K and Bamgbose A.M.(eds). Proceedings 2nd Annual Conference of Animal Science Association of Nigeria held at Airport Hotel, Ikeja - Lagos on the 16th -17th September, 1997: 1-12.

Egbunike, G.N., Holtz W. and Smidt, D. (1976). Reproductive capacity of German Landrace boars. II: Sperm production rates as determined by quantitative testicular histology and from gonadal sperm reserves. Zuchthygiene, 11: 35-37.

Eghianruwa, K. I. and Anika, S. M. (2011). Diminazene, tocopherol and selenium on *T. brucei* induced anemia. *Veterinary arhiv 81* (5), 647-656, 2011

Emanuele, M.A. and Emanuele, N.V. (1998) Alcohol's effects on male reproduction. *Alcohol Health and Research World*, 22, 195-201.

Engle, E.T. (1926). Journal of mammalogy, 7:119.

Esteso M.C, Ferna´ndez-Santos M.R, Soler A.J, Montoro V., Quintero-Moreno A, Garde J.J. (2006). The effects of cryopreservation on the morphometric dimensions of Iberian red deer (Cervus elaphus ispanicus) epididymal sperm heads. *Reproduction of Domestic Animals.* 2006;41:241–246.

Federal Department of Livestock (FDL) (1992). Nigerian Livestock Resources. Vol. II, National Synthesis.

Finzi, A., Macchioni, P. and Kuzminsky, G. (1994). Circadian cycle of rabbit body temperature in the hot season. In 1st international conference on "Rabbit production in Hot Climate".Cairo, Egypt.

Flipse, R.J. and Almaquist, J.O. (1961). *Journal of Dairy Science.* 40: 1612.

Foley, G.L. (2001) Overview of male reproductive pathology. *Toxicologic Pathology,* 29, 49-63.

Foote, R.H., Hafs, H.D., Staples, R.E., Gregoire, A.T. and Bratton, R.W. (1963). Ovulation rates and litter sizes in sexually receptive and nonreceptive artificially inseminated rabbits given varying dosages of luteinizing hormone. *Journal of Reproduction and Fertility.* 5: 59–66.

Foote, R.H. (1978). Journal of *Animal Science.* 47 (Suppl. 2): 1-11.

Food and Agriculture Organization (FAO) (1986). African Agriculture the Next 25 years. FAO of the United Nations Rome, Italy.

Gasser, H. S. (1963). The bane of Tropical Africa. *Lancet, 1*:1091.

Gbadamosi A.J. and Egbunike G.N. (1999). Studies on cock semen (1) Effects of frequency of ejaculation and breed on physical characteristics. *Tropical Journal of Animal Science.* 1999; 1: 157-164.

Geerts, S. and Holmes, P.H. (1997). Drug management and parasite resistance in animal trypanosomiasis in Africa. Presented at the 24th Meeting of the International Scientific Council for Trypanosomosis Research and Control (ISCTRC), Maputo, Mozambique.

Giojalas L., Rovasio R., Fabro G., Gakamsky A. and Eisenbach M. (2004). Timing of sperm capacitation appears to be programmed according to egg availability in the female genital tract. *Fertility and Sterility 82; 247-249.*

Gogol P., Bochenek M. and Smorag Z. (2002). Effect of rabbit age on spermatozoa chromatin structure. *Reproduction of Domestic Animals. 37: 92-95.*

Gow, A. G., Simpson, J. W. and Picozzi, K. (2007). First report of canine Africa trypanosomosis in the UK. *Journal of Small Animal Practice,* 48; 658-661.

Giwercman, A. and Giwercman, Y.L. (2011). Environmental factors and testicular function. *Best Practice & Research Clinical Endocrinology & Metabolism* 25, 391−402.

Graya, A. R. and Luckins, A. G. (2011). Centre for Tropical Veterinary Medicine, University of Edinburgh, Easter Bush, Roslin, Midlothian, Scotland. Published online: 19 September 2011.

Go, K.J. and Wolf, D.P. (1983). The role of sterols in sperm capacitation. *Advanced Lipid Research.* 20: 317–30.

Goldman, B., Gwinner, E., Karsch, F.J., Saunders, D., Zucker, I. and Ball, G.F. (2004). Circannual rhythms and photoperiodism. In : (Eds : Dunlap J.D., Loros J.J., DeCoursey P.J.), Chronobiology: biological time keeping. Sunderland, MA: Sinauer Associates. pp. 107–144.

Ghaoui Rel, H., Thomson, P.C., Evans, G. and Maxwell W.M. (2004). Characterization and localization of membrane vesicles in ejaculate fractions from the ram, boar and stallion. *Reproduction of Domestic Animals.* 39: 173–80.

Hafez, E.S.E. (1993). Reproducción, hormonas y factores de crecimiento. In: Reproducción e Inseminación Artificial en Animales. 6th edition. Hafez ESE (Ed), Mexico, DF: Interamericana- McGraw-Hill.

Haim, A., Shanas, U., Zubidad, A.S. and Scantelbury, M. (2005). Seasonality and seasons out of time—the thermoregulatory effects of light interference. *Chronobiology International* 22, 59−66.

Hassan, W. A. and Owolabi, R. O. (1996). Production performance of domestic rabbits in semi-arid zone of Nigeria. Proceedings of the 6th World Rabbit Congress, Toulouse, France 3: 359-363

Hawkes, W.C. and Turek, P.J. (2001) Effects of dietary selenium on sperm motility in healthy men. *Journal of Andrology*, 22, 764-772.

Herbert, W.J. and Lumsden, W.H.R (1976). Trypanosoma brucei: A rapid "matching" method for estimating the host's parasitaemia. *Experimental Parasitology,* 40: 427–431.

Hide, G. and Mottram, J.C. (1996). Trypanosomiasis and leishmaniasis: biology and control. CAB International, UK. 384 pp.

Hidiroglou, M. and Knipfel, J.E. (1984). Zinc in mammalian sperm: A review. *Journal of Dairy Science*, 67, 1147-1156.

Hoffman, R.S. and Smith, A.T. (2005). "Order Lagomorpha". In Wilson, Don E., and Reeder, DeeAnn M.,Mammal Species of the World: A Taxonomic and Geographic Reference (3rd Ed.). Baltimore: Johns Hopkins University Press, 2 vols. pp. 185-211.

Holman, P.J., Spencer, A.M., Telford, S.R., 3rd, Goethert, H.K., Allen, A.J., Knowles, D.P., Goff, W.L. (2005). Comparative infectivity of *Babesia divergens* and a zoonotic *Babesia divergens* – like parasite in cattle. *American Journal of Tropical Medicine and Hygiene.*73:865-870.

Hoogenboezeom and Swanepoel, (2001). Zootech, aspect of bull fertility. Animal Genetic Resource. Iowa state University press.

Hughes, P. and Varley, K. (1980). Fertility in the male. In: Reproduction in the pig. Pp: 185 – 195.

Hunter, R.H. (1977). Physiological factors influencing ovulation, fertilization, early embryonic development and establishment of pregnancy in pigs. *British Veterinary Journal.* 133:461-70.

Ibe, S.N. (1999). Livestock production in the South East in the New Millenium. *In: Proceedings of the 19th Annual South East Zone Farming System Research and Extension Worksop*. Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

Igboeli, G. and Rahka, A.M. (1971). Gonadal and extragonadal sperm reserves in Indigenous Central African Bulls. *Journal of Reproduction and Fertility,* 25: 107-109.

Ikede, B.O. (1979). Genital lesions in experimental chronic *Trypanosoma brucei* infection in rams. *Research in Veterinary Science,* 26: 145-151.

Ikede, B. O. (1989). Control of animal trypanosomiasis in Nigeria as a strategy for increased livestock production. *Proceedings of a Preparatory Workshop,* held at Vom, Plateau State, Nigeria, June 5-9. Hunter, R. H. (1977). Physiological factors influencing ovulation, fertilization, early embryonic development and establishment of pregnancy in pigs. *British Veterinary Journal. 133*:461-70, 1977.

Ikede, B.O., and Akpavie, S.O. (1982). Delay in resolution of trypanosoma induced genital lesions in male rabbits infected with *T. brucei* and infected with Diminazene aceturate. *Research in Veterinary Science,* 32; 374-376.

Ikede, B. O.; Elhassan, E. and Akpavie, S. O. (1988). Reproductive disorders in African Trypanosomiasis: a review. *Acta Tropica. 45(1)*:5-10.

Ilemobade, A. A. (1981). Research in field of animal trypanosomiasis in Nigeria: An overview: at the 1st National Conference on Tsetse and Trypanosomiasis Research in Kaduna.

ILRAD (1990). Annual Report of the International Laboratory for Research on Animal Diseases: Trypanosomiasis pp 28 – 42.

Ironkwe, M. O. and Amaefule, K. U. (2007). Economics of rabbit production in Rivers State, Nigeria. In Agiang E A, Agwunobi L N and Olawoyin O O (editors). Susutainability of the Livestock Industry in an Oil Economy, Proceedings of the 32nd Annual Conference of the Nigerian Society for Animal Production (NSAP), University of Calabar, Cross River State, Nigeria, 18-21 March 2007

Isoun, T. T. and Anosa, V. O. (1974). Lesions in the reproductive organs of sheep and goats infected with *Trypanosoma vivax. Z. Tropenmed. Parasitology, 25*:469-76.

Jeffcoate, I.A and Holmes P.H. (1997). *The Effects of Trypanosomosis on Reproduction in Domestic Ruminants in Trypanosomosis and Leishmaniasis*, eds Htdo, H., Mottram, J.S, Coombs, G.H., and Holmes., P.H. CAB, International, Pp 335-346.

Jibril, A., Ate, I.U., Rekwot, P.I. and Osuhor, C.U. (2010). Effect of varying levels and source of protein on scrotal circumference and semen profile of yankasa rams. *Paper presented at the 4th Annual Congress of the National Veterinary Medical Association*, held at the Benue State University, Makurdi, 4th-8th October, 2010.

Jones, E.F., Bain J.B., Odell, W.D. (1976). Post coital luteinizing hormone release in male and female rabbits as determined by radioimmunoassay. *Fertility and Sterility., 27, 848-852.*

Jones, R.C. (1999). To store or mature spermatozoa? The primary role of the Epididymis. *International Journal of Andrology*, 22, 57-67.

Johnson, H D., Ragsdale, A C and Chang, C S. (1957). Environmental physiology and shelter engineering with special reference to domestic animals. Comparison of the effects of environmental temperatures on rabbits and cattle. Influence of constant environmental temperatures (50° and 80°F) on the growth responses and physiological reactions of rabbits and cattle Research Bulletin Mo. Agric. Exp. Stn. No. 646.

Johnson, I. (1994). Spermatogenesis in Bulls: In Proceedings of the 15th Technical Conference on Artificial Insemination and Reproduction. Columbia, Missouri. Pp. 9 – 27.

Kaikabo, A. A. and Salako, M. A. (2006). Effects of vitamin E supplementation on anemia and tissue pathology in rats infected with *Trypanosoma brucei* (Federe strain). *Tropical Veterinary*. 24, 46-51.

Kamuanga, M. (2003). Socio-economic and cultural factors in the research and control of Trypanosomosis. *Information Division FAO Rome,* Pp 1-10.

Kann, M.L. and Serres, C. (1980). Development and Ini-tiation of sperm motility in the hamster epididymis. *Re-production Nutrition Development*, 20, 1739-1749.

Katja, H. and Marieb, E. N. (2007). *Human anatomy and physiology*. San Francisco: Pearson Benjamin Cummings. pp. 1090–1110.

Kaufmann, B.A. (2005). Reproductive performance of camels (*Camelus dromedaries*) under pastoral management and its influence on herd development. *Livestock Production Science,* 92: 17-29.

Kelly, W.R. (1984). The blood and blood forming organs. In: Kelly, W.R. (Ed.). Veterinary Clinical Diagnosis. 3rd Edition, Bailliere Tindal, London, pp. 312-317.

Kemppainen, J. K. (2007). "Kanit keskuudessamme (*The rabbits among us*)" (in Finnish). *Suomen Kuvalehti* volume 3:76–83.

Khalifa, R.M., El-Alamy, M.A. and Beshir, M.A. (2000). Vasectomized buck gave better reproductive results in artificial insemination techniques in rabbit than GnRH or HCG. 7th World Rabbit Congress, Valencia, Spain.

Khalifa, R.M. (1994). Mating frequency and artificial insemination in rabbit using vasectomized bucks. *World Rabbit Science*. 2: 1–5.

Knapp H.R. (1990). Prostaglandins in human semen during fish oil ingestion: evidence for in vivo cyclooxygenase inhibition and appearance of novel trienoic compounds. *Prostaglandins, 39: 407-423.*

Koonjaenak, S. (2006). Semen and spermatozoa characteristics of swamp buffalo (Bulbalus bubalis) bulls for Artificial Insemination in Thailand in relation to season. Doctoral thesis. Faculty of Veterinary Medicine and Animal Science. Sweedish University of Agric Science. Acta Universitatis Agruculturae

Suecieae, Vol. 114. Uppsala, Sweeden. ISSN 1652 - 6880 ISBN 91-576-7263-6. Pp 55.

Kothari, S., Thompson, A., Agarwalm, A. and du Plessis, S.S. (2010). Free radicals: Their beneficial and detrimental effects on sperm function. *Indian Journal of Experimental Biology*, 48, 425-435.

Kravets, F.G., Lee, J., Singh, B., Trocchia, A. and Pentyala, S.N. (2000). Prostasomes: current concepts. Prostate. 43: 169–74

Kumi-Diaka, J., Nagerathman, V. and Rwuaan, J.S. (1981). Seasonal age related changes in semen quality and testicular morphology in bulls in the tropics. *Veterinary Records.* 108:13-15.

Kwari, H.D. and Waziri, M.A. (2001). Body weight, withers height, scrotal circumference, penis size and spermatozoa reserve of Balami rams. Sokoto *Journal of veterinary Science.* 3L81-86.

Lalumiere, G., Bleau, G., Chapdelaine, A. and Roberts, K.D. (1976). Cholesteryl sulfate and sterol sulfatase in the human reproductive tract. *Steroids.* 27: 247–59.

Lee, B., Pine, M., Johnson, L., Rettori, V., Hiney, J.K. and Dees, W.L. (2006). Manganese acts centrally to acti-vate reproductive hormone secretion and pubertal development in male rats. *Reproductive Toxicology*, 22, 580- 585.

Lin, D.S., Connor, W.E., Wolf, D.P., Neuringer, M. and Hachey, D.L. (1993). Unique lipids of primate spermatozoa: desmosterol and docosahexaenoic acid. *Journal of Lipid Research.* 34: 491–99.

Llewelyn, C. A., Lunkins, A.G., Monro, C.D. and Perrie, J. (1987). The effect of *Trypanosoma congolense* infection on the estrus cycle of the goat. *British Veterinary Journal,* 143: 423-431.

Lombardi, L. and Fernandez, N. (2003). "Habitat-related Differences in Rabbit (Oryctolagus cuniculus) Abundance, Distribution, and Activity." *Journal of Mammology*.

Losos, G. J. and Ikede, B. O. (1972). Review of pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense*, *T. vivax*, *T. brucei, T. rhodesiense* and *T. gambiense*. *Journal of Veterinary Pathology.* 9, 1-17.

Losos, G.J. (1986). 'Trypanosomosis in Infectious Tropical Disease of domestic animals. Longman scientific and technical. Pp 183-318.

Luckins, A.G. (1988). *Trypanosoma evansi* in Asia. Parasitology. Volume 4: 137-142. *PubMed*.

Luzi F., Maertens L., Mijen P. and Pizzi F. (1996). Effect of feeding level and dietary protein on libido and semen characteristics of bucks. *In: Proceedings of the 6th World Rabbit Congress. Toulouse, vol. 2, 87-92.*

Mamabolo, M.J. (1999). Dietary, seasonally and environmental influence on semen quality and fertility status of indigenous goats. Mpumalonga province, South Africa. PhD Thesis. University of Pretoria, South Africa.

Marai, I.F.M., Ayyat, M.S. and Abdel-Monem, U.M. (2001). Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *Tropical Animal Health and Production,* 33: 451-460.

Martin, G.B. and Walkden-Brown, S.W. (1995). Nutritional influences on reproduction in mature male sheep and goats. *Journal of Reproduction and Fertility*, *Suppl*. 49, 437−449.

Martin, G.B., Roger, J. and Blache, D. (2004). Nutritional and environmental effects on reproduction in small ruminants. *Reproduction and Fertility Developement.* 16(4):491-501. [PubMed - indexed for MEDLINE]

Maser, P., Luscher, A. and Kaminsky, R. (2003). Drug transport and drug resistance in African trypanosomes. Drug Resistance Updates 6(5): 281 - 290.

Mason, K.E., Burns, W.A. and Smith Jr., J.C. (1982) Testicular damage associated with zinc deficiency in pre- and postpubertal rats: Response to zinc repletion. *The Journal of Nutrition*, 112, 1019-1028.

McDonald, L. E and Pineda, M. H. (1989). *Veterinary Endocrinology and Reproduction*. Philadelphia, Lea and Febiger.

Mcnitt, J.I., Pattton, N.M, Lukefahr, S.D. and Cheeke, P.R. (2000). Rabbit production. Interstate Publishers (seventh edition) Danville, IL pp: 493.

Meethal, S.V., Liu, T., Chan, H.W.; Ginsburg, E.; Wilson, A.C.; Gray, D.N.; Bowen, R.L.; Vonderhaar, B.K. and Atwood, C.S. (2009). "Identification of a regulatory loop for the synthesis of neurosteroids: a steroidogenic acute regulatory protein-dependent mechanism involving hypothalamic-pituitary-gonadal axis receptors". *Journal of Neurochemistry.* 110 (3): 1014–27.

Merck Veterinary Manual. (1998): A handbook of diagnosis and therapy for veterinarian. 8th edition. Merck and Co. Rahway, Ed. D.H. Siegmund.

Mercks veterinary manual. New viral disease. (2006). Retrieved 2007-06-17. *Clostidium piliforme*. Available from: http://merckvetmanual.com/mvm index.jsp?

Michelmann, H.W. (1973). Paufler SAI. Results following the use of LH-releasing hormone in rabbits. *Zuchthygiene,* 8: 178.

Mickelsen, W.D., Paisley., L.G. and Dahmen, J.J. (1991). The effect of season on the scrotal circumference and spermatozoa motility and morphology in rams. *Theriogenology.* 16:45-51.

Millar, R.P., Lu, Z.L., Pawson, A.J., Flanagan, C.A., Morgan, K. and Maudsley, S.R. (2004). "Gonadotropin-releasing hormone receptors". *Endocrinology Rev.* 25 (2): 235–275.

Mocé E., Lavara R. and Vicente J.S. (2005). Influence of donor male on the fertility of frozen-thawed rabbit semen after artificial insemination of females from different genotypes. *Reproduction of Domestic Animals 40, 516-521.*

Moce, E., Lavara, R, Lavara, F. and Vicente, J.S. (2000). Effect of reproductive rhythm on seminal parameters from a rabbit line selected with high growth rate. *In: Proceedings of 7th World Rabbit Congress. Valencia. July 2000. Vol A: 197-201.*

Morrell, J. M. (1995). Artificial insemination in rabbits. *British Veterinary Journal.* 151:477 - 488.

Morrell, J.M. (2011). Artificial Insemination: Current and future trends. In: Manafi, M. (ed). Artificial Insemination in farm animals. In Tech Janeza Tidine 9.51000. Rijeka, Croatia. Pp 1-12.

Morrison, I., Murray, M. and Mcintyre, I. (1981). Bovine trypanosomiasis, FAO/SIDA follow up seminar on veterinary pathology, Faculty of Veterinary Medicine, University of Nairobi, 7-27, September.

Mourvaki, E., Cardinal, R., Dal Bosco, A., Corazzi, L. and Castellini, C. (2010). Effects of flaxseed dietary supplementation on sperm quality and on lipid composition of sperm subfractions and prostatic granules in rabbit. *Theriogenology* 2010; 73: 629–37.

Mulligan, H.W., Potts, W.H. and Kershaw, W.E. (1970). The African Trypanosomiasis, London, George Allenand Unwin Ltd., pp. 661-794.

Mutayoba, B.M., Eckersall, P.D, Cestnik, V, Jeffcoate, I.A, Gray, C.E and Holmes P.H. (1995) effects of *T. congolense* on pituitary and adrenocortical function in sheep. Changes in the adrenal gland and cortisol secretion. *Research in Veterinary Science*. 58: 174-179.

Mutayoba, B.M., Meyer, H.H.D., Osaso, J. and Gombe, S. (1989). Trypanosoma induced increase in prostaglandin PF2-alpha and its relationship with corpus luteum function in the goat. *Theriogenology*, 32: 545- 555.

Mutayoba, B.M., Gome, S., Waindi, E.N. and Kaaya, G.P. (1988). Depression of ovarian function and plasma progesterone and estradiol-17 in female goats, chronically inoculated with *Trypanosoma congolense*. *Acta Tropica.* 45(39): 225-237.

Murray, M. and Dexter, T.M. (1988). Anemia in bovine animal trypanosomiasis. A review. Acta Tropica (Basel). 45, 389-432.

Nalbandov, A.V. (1976). Reproductive Physiology of Mammals and Birds. 3rd Edition. W.H. Freeman and Company, San Francisco. Pp. 334.

Nantulya, V.M., (1990). Trypanosomosis in domestic animals: The problems of diagnosis. *Revelational Science Technology.* 9: 357-367. PubMed

Nantulya, A. V., Musoka, A. J., Ruringwara, F. R., Sarbet, A. F., Ngaira, J. M. and Katende, J. M. (1982). Immunosuppression in African trypanosomosis: The role of antigenic competition. *Clinical Experimental Immunology,* 47: 234-240.

Nfon, C. K., Oyewunmi, O. B., Nottidge, H. O. and Taiwo, V. O. (2000). Experimental *T. brucei* and *T. congolense* infection in cats: Clinicopathological study. *Tropical Veterinarian,* 18: 220-227.

Ng, C.M., Blackman, M.R., Wang, C. and Swerdloff, R.S. (2004). The role of carnitine in the male reproductive system. *Annals New York Academy of Sciences,* 1033, 177-188. doi:10.1196/annals.1320.017

Nikolopoulou, M., Soucek, D.A. and Vary, J.C. (1986). Lipid composition of the membrane released after *in vitro* acrosome reaction of epididymal boar sperm. *Lipids.* 21: 566–9.

Noakes, D. E, Parkinson, T. J and England, G. C. W. (2001). *Arthur"s Veterinary Reproduction and Obstetrics.* New York, Saunders.

Nuti, L.C. (2012). Goat semen collection and processing. www.goatsemennuti.pdf.accessed 06/07/2012.

Nworgu, F.C. (2007). Economic importance and growth rate of broiler chicken served fluted pumpkin (Telfaria occidentalis) *African Journal of Biotechnology,* 2:6 34-39.

Obioha, F.C. (1992). *A Guide to Poultry Production in the Tropics*. Enugu: Acena Publishers: 1 – 5.

O'Bryan M.K., Schlatt S., Phillips D.J., DE Kretser D.M. and Hedger M.P. (2000). Bacterial lipopolysaccharide-induced inflammation compromises testicular function at multiple levels in vivo. *Endocrinology, 141: 238-246.*

Odubote, I.K., Ohiosimuan, O.O. and Oseni, S.O. (1995). The potentials of rabbit as a component of peri-urban livestock production in Nigeria. In: *Livestock Prod. and Diseases in the Tropics,* Proceedings VIII International Conference Association Insttitute of Veterinary Medicine Berlin, Germany, KH Zessin (Ed). Vol, 1: 233-237.

Office of International Epizootics, OIE. (1997). Manual of standards for diagnostic tests and vaccines. Third edition, 1996. Office International des Epizooties (World Organization for Animal Health), Paris. 723 pp. (ISBN 92-9044-423-1).

Ogunlade J.T., Ewuola, E.O., Gbore, F.A., Bandyopadhyay, R., Niezen J. and Egbunike G.N. (2006). Testicular and epididymal sperm reserves of rabbits fed fumonisin contaminated diets. *World Applied Sciences Journal* 1 35–38.

Ogunsanmi, A.O., Akpavie, S.O. and Anosa, V.O. (1994). Serum biochemical changes in WAD sheep experimentally infected with Trypanosoma brucei brucei. d'Elevage et de Medecine in dogs. Israel Journal of Veterinary Medicine. 49(1): 36 – 39.

Ogwu, D. (1983). Effects of Trypanosoma vivax infection on Female Reproduction in Cattle. Ph.D thesis in the Department of Surgery and Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Ogwu, D. and Njoku, C.O. (1987). Effects of pregnancy on the clinical manifestation of bovine trypanosomosis. *Veterinary Parasitology*, 24: 25-33.

Ogwu, D. and Nuru, S. (1981). Transplacental transmission of trypanosomosis in animals and man. A review. *Veterinary Bulletin* 51: 381-383.

Ogwu, D., Njoku, C.O. and Osori, D.I.K. (1986). Effects of experimental *Trypanosoma vivax* infection on first, second and third trimester's pregnancy in heifers. *Theriogenology*, 25: 383-398.

Ogwu, D., Njoku, C.O., Osori, D.I.K., Ezeokoli, C.D. and Kumi-Diaka. J. (1984). Effects of experimental *Trypanosoma vivax* infection on fertility of heifers. *Theriogenology*, 22: 625-633.

Omer, O.H, Magzoub, M, Haroun, E.M., Mahmoud, O.M and Hamid, Y.M.A. (1998). Diagnosis of *Trypanosoma evansi* in Saudi Arabian camels (*Camelus dromedaries*) by the passive haemagglutination test and Ag-ELISA. *Journal of Veterinary Medicine.* Series B Infectious Disease. Veterinary Public Health, 45: 627-633.PubMed Direct Link.

Omole, A.J., Omueti, O. and Ogunleke, O.J. (2005). Performance characteristics of weaned rabbits fed graded levels of dry cassava peel fortified with soycorn residue basal diet.

Omotainse, S.O., Anosa, V. O and Falaye, C. (1994). Clinical and biochemical changes in experimental *Trypanosoma brucei* infection in dogs. *Israel Journal of Veterinary Medicine*, 49 (1): 36-39.

Omotainse, S.O., Edegbhere, H., Omoogum, G.A., Thompson, G., Igweh, C.A., Ukah, J.A.C., Ikenga, M.A. and Halid, I. (2000). The prevalence of animal trypanosomosis in Konshisha Local Government Area of Benue State Nigeria. *Israel Veterinary Journal,* 55 (4).

Onifade A.A., Abu O.A., Obiyan R.J. and Abanikannda, O.T.F., (1999). Rabbit Production in Nigeria: Some aspects of current status and promotional strategies. *World Rabbit Science,* Vol. 7 (2), 51 – 58.

Onyekwelu, N.A. (1999). Toxicity of existing trypanocides. A review: *West African Journal of Pharmacology and Drug Research*, 15: 1-6

Orhue, N. E. J., Nwanze, E. A. C. and Okafor, A. (2005). Serum total protein, albumin and globulin levels in *Trypanosoma brucei* infected rabbits: Effects of orally administered *Scoparia dulcis*. *African Journal of Biotechnology*. 4 (10):1152-1155.

Ortavant, R., Courot, M. and de Reviers, H. (1977). *Spermatogenesis in domestic mammals. In: Reproduction in domestic animals.* London, Academic Press, 1977. pp. 223- 4.

Osmar, A. S., Jenning, F. W. and Holmes P. H. (1992). Rapid development of drug resistance by *Trypanosoma evansi* in immunosuppressed mice. *Acta Tropica.* 50: 249-255.

Ott, R. S. (1986). Breeding soundness examination of bulls. In: *Current Therapy in Theriogenology*, Morrow, D.A. (ed.). Philadelphia, W.B. Saunders Co. pp. 125-36.

Otesile, E. B., Fagbemi, B. O. and Adeyemo, O. (1991). The effect of *Trypanosoma brucei* infection on serum biochemical parameters in boars on different planes of dietary energy. Veterinary Parasitology. 40, 207-216.

Owen, O.J., Alawa, J.P., Wekhe S.N., Isirimah N.O., Chukuigwe E.C., Aniebo, A.O., Ngodigha, E.M. and Amakiri A.O. (2008). Incorporating poultry litter in animal feed: a solid waste management strategy. *Egyptian Journal of Animal Production.*

Ozor, N. and M. C. Madukwe (2001). Adoption of improved rabbit technologies by farmers in Nsukka L.G.A.: Implication for livestock extension. *Meeting the Challenges of Animal Production in the New Millennium*. Proceedings of the 6th Annual National Conference of Animal Science Association of Nigeria. September 17th – 19th University of Maiduguri: 199 – 202.

Pepin J. and Meda, H.A. (2001).The epidemiology and control of human African trypanosomiasis. *Advances in Parasitology,* 49: 71-132.

Presidential Task Force (1992). Alternative Formulation of Livestock Feeds. Report and Recommendation. Vol. 1: 15 pp.

Rai, R.B. (1988). A note on the efficacy of Ivermectin against ectoparasites in rabbits. *Journal of Applied Rabbit Research.* 11(2): 78-79.

Rajora, V. S., Raina, A. K., Sharma, P.D. and Singh, B. (1968). Serum protein changes in buffalo calve experimentally infected with *Trypanosoma evansi*. *Indian Journal of Veterinary Medicine*. 6:65-73.

Rebollar, P.G., Pérez-Cbal, M.A., Pereda, N., Lorenzo, P.L., Arias-Álvarez, M. and García-Rebollar, P. (2009). Effects of parity order and reproductive management on the efficiency of rabbit productive system. *Livestock Science,* 121: 227-233

Reincke, M., Arlt, W., Heppner, C., Petzke, F., Chrousus, G. P. and Allolio, B. (1998). Neuroendocrine dysfunction in African trypanosomiasis. The role of cytokines. *Ann. N Y Academic Science. 840*: 809-21.

Rekwot, P. I., Oyedipe, E. O., Akerejola, O. O and Kumi-Diaka, J. (1988). The effect of protein intake on body weight, scrotal circumference and semen production of Bunaji bulls and their Friesian crosses in Nigeria. *Animal Reproduction Science*. 16:l.

Rekwot, P.I., Oyedipe, E.O., Dawuda, P.M. and Sekoni, V.O. (1997). Age and hourly related changes of serum testosterone and spermiogram of prepubertal bulls fed two levels of nutrition. *The veterinary Journal*. 153:341 – 347.

Rekwot P. I., Oyedipe E. O. and Ehoche, O. W. (1994). The effects of feed restriction and realimentation on the growth and reproductive function of Bokoloji Bulls. *Theriogenology*, 42: 287-295.

Rekwot, P.I., Voh, A.A.$_{JR.}$, Oyedipe, E.O., Opaluwa, G.I., Sekoni, V.O. and Dawuda, P.M (1987). Influence of Season on characteristics of the ejaculate from bulls in an Artificial Insemination centre in Nigeria. *Reproductive Science,* 14: 187-194.

Rivers, T.M. (1930). Infectious myxomatosis of rabbits. Observations on the pathological changes induced by virus myxomatosum (sanarelli). *Journal of experimental Medicine.* 51(6):965 – 976.

Roosen-Runge, E. C. (1977) *The process of spermatogenesis in animals.* London, Cambridge University Press. pp.1- 12.

Rodel, H. G., Bora, A. (2004). "Density-dependent reproduction in the European rabbit: a consequence of individual response and age-dependent reproductive performance." *Oikos*

Sadique, N. A., Adejinmi, J.O. and Ariri, H. (2001). Haematological and plasma protein values of Zebu cattle in trypanosome-endemic zone. *Tropical Animal Production Investment*, 4: 219-223.

Sadleir, R.M. (1968). Reproductive responses to the environment in mammals. *Journal of Psychosomatic Research* 12, 3−9.

Salisbury, G.W, Van Demark, N.L. and Lodge, J.R. (1978). Physiology of Reproduction and Artificial Insemination of Cattle. 2nd ed., W.H. Freeman and Company, San Fransisco.

Seifert, H.S.H. (1996). Tropical Animal Health Kluwer Academic Publishers. Dorrech, Bonston, London, pp: 53-260. ISBN: 0-7923-3821-9.

Sekoni, V. O. (1990). Effects of Novidium (Homidium chloride) chemotherapy on genital lesions induced by *Trypanosoma vivax* and *Trypanosoma congolense* infections in the zebu bull. *British Veterinary Journal*, 146: 181-185.

Sekoni, V.O. (1991). Animal Trypanosomiasis and Reproductive Disorders. Paper presented at a symposium in National Animal Production Research Institute, on the 25th April.

Sekoni, V. O. (1992). Effects of *Trypanosoma vivax* infection of sperm characteristics of yankasa rams. *British Veterinary Journal*, 148: 501-506.

Sekoni, V. O. (1993). Elevated sperm morphological abnormalities of yankasa rams consequent to trypanosomosis. *Animal Reproductive Science,* 31: 243-248.

Sekoni, V. O. (1994). Reproductive disorders caused by animal trypanosomiasis: a review. *Theriogenology, 42(4)*:557- 570.

Sekoni, V. O., Kumi-Diaka, J., Saror, D. and Njoku, C. (1988a). The effects of *Trypanosoma vivax and Trypanosoma congolense* infection on the reaction time and semen characteristics in the zebu bull. *British Veterinary Journal*, 144: 388-394.

Sekoni, V.O., Kumi-Diaka, J., Saror, D.I., Njoku, C.O. and Olorunju, S.A.S. (1988b). seasonal and monthly variations in the incidence of morphological abnormalities in bovine spermatozoa in Shika, Zaria, Northern Nigeria. *Animal Reproduction Science*, 17:61-67. Elsevier Science Publishers, Amsterdam, Netherlands.

Sekoni, V. O., Njoku, C.O., Kumi-Diaka, J. and Saror, D.I. (1990a). Pathological changes in male genitalia of cattle infected with *Trypanosoma vivax* and *Trypanosoma congolense*. *British Veterinary Journal*, 146: 175-180.

Sekoni, V.O., Saror, D.I., Njoku, C.O., Kumi-Diaka, J. and Opaluwa, G.I. (1990b). comparative haematological changes following *Trypanosoma vivax* and *Trypanosoma congolense* infections in the Zebu bull. *Veterinary Parasitology*, 35;11-19. Elsevier Science Publishers, Amsterdam, Netherlands.

Sekoni, V. O., Saror, D.I., Njoku, C.O. and Kumi-Diaka, J. (1991). Effects of Novidium (Homidium chloride) chemotherapy on elevated spermatozoa morphological abnormalities in the semen of zebu bulls infected with *Trypanosoma vivax* and *Trypanosoma congolense*. *Animal Reproduction Science,* 24:249-258.

Siciliano, L., Marcianò, V. and Carpino, A. (2008). Prostasome-like vesicles stimulate acrosome reaction of pig spermatozoa. *Reproduction Biology and Endocrinology.* 6: 5.

Sion, B., Grizard, G. and Boucher, D. (2001). Quantitative analysis of desmosterol, cholesterol and cholesterol sulfate in semen by high-performance liquid chromatography. *Journal of Chromatography.* 935: 259–65.

Smith, A. T. and Boyer, A. F. (2008). *Oryctolagus cuniculus*. *IUCN Red List of Threatened Species* Version 2010.4.

Somade, B. and Adesina, A. (1990). Influence of the breed of doe and season on growth rate of rabbits. *Journal Beittrop Landwirtsch* Veterinary Medicine, 28: 175-183

Stewart, C. (1974). Prevention is essential as cures are difficult and expensive. *Commercial Rabbit*, 3 (2), 10-12.

Swierstra, E. E. and Foote, R. H. (1965). Duration of spermatogenesis and spermatozoan transport in the rabbit based on cytological changes, DNA synthesis and labeling with tritiated thymidine. *American Journal of Anatomy.* 116: 401–411. Volume 116, Issue 2, pages 401–411.

Taberlet, P., Coissac, E., Pansu, J. and Pompanon, F. (2011). Conservation genetics of cattle, sheep, and goats. *Comptes rendus biologies* 334, 247−254.

Taiwo, B.B.A., Ogundipe. I.I. and Ogunsiji O. (1999). Reproductive and growth performance of rabbits raised on forage crops. In: Proceedings of the 4th Annual Conference of the Animal Association of Nigeria held in Ibadan, Nigeria, pp 108-109.

Takeet, M.I. and Fagbemi, B.O. (2009). Haematological, Pathological and Plasma Biochemical changes in rabbits experimentally infected with Trypanosoma congolense. *Science World Journal* Volume 4 (No. 2). www.scienceworldjournal.org. ISSN 1597-6343.

Taylor, K. A. (1998). Immune response of cattle to African trypanosomosis: protective or pathogenic? *International Journal of Parasitology*, 28 (2): 219-240.

Taylor, K. and Authie E.M.L. (2004). From pathogenesis of animal trypanosomiasis. In *the Trypanosomiases*. Edited by Maudlin I, Holmes PH, Miles MA. Wallingford: CABI Publishing.

Theau-Clément, M., Bolet, G., Roustan, A. and Mercier, P. (1990). Comparaison de différents modes d'induction de l'ovulation chez les lapines multipares en relation avec leur stade physiologique et la réceptivité au moment de la mise à la reproduction. 5es Journées de la Recherche Cunicole, 12–13 Décembre, Paris, Tome I, Communication 6.

Theau-Clément M., Michel N., Esparbié J. and Bolet G. (1994). Effect of artificial photoperiods on sexual behaviour and sperm output in the rabbit. *Animal Science. 60, 143-149.*

Theau-Clément M., Brun J.M., Sabbioni E., Castellini C., Renieri T., Besenfelder U., Falières J., Esparbié J. and Saleil G. (2003). Comparison de la production spermatique de trois souches de lapins: moyennes et variabilités. *In Proceedings 10èmes Journées Recherche Cunicole, November 2003, Paris, France, 81-84.*

Tibary, A., Anouassi, A. and Sghiri, S. (2005). Factors Affecting Reproductive Performance of Camels at the Herd and Individual Level. In: Desertification Combat and Food Safety, Faye, B. and P. Esemov (Eds.). *Life and Behavioural Sciences,* IOS Press, Amsterdam, pp: 97-114.

Togun, V.A. and Egbunike, G.N. (2006). Seasonal variations in the sperm production characteristics of Zebu (white Fulani) cattle genitalia in the humid tropical environment. *Middle-East Journal of Science Research.* 1:87-95.

Toma, I., Shinggu, D. Y., Ezekiel, W. and Barminas, J.T. (2008). Effect of intraperitoneal administration of vitamin c (ascorbic acid) on anemia in experimental *Trypanosoma congolense* infected rabbits. *African Journal of Pure and Applied Chemistry.* 2, 37-40.

Triolo N. (1990). A case of coma due to Arsobal in a newborn affected with congenital *Trypanosoma gambiense Therapy Infectious Diseases.* 5: 165-166

Tuormaa, T.E. (2000). Chromium, selenium and copper and other trace minerals in health and reproduction. *Journal of Orthomolecular Medicine*, 15, 145-157.

Quinn, P.J. and White, I.G. (1967). Phospholipid and cholesterol content of epididymal and ejaculated ram spermatozoa and seminal plasma in relation to cold shock. *Australian Journal of Biological Sciences.* 20: 1205–15.

Ugwu , D.S. (2007). The role of small ruminants in the household economy of South-East Zone, *Nigeria. Research Journal of Applied Science.* 2(6):726-732.

Uilenberg, G. (1998). A field guide for the diagnosis treatment and prevention of African animal trypanosomosis. FAO, Rome, Italy, pp. 158.

United States Department of Agriculture (USDA) (1972): Selecting and Raising Rabbits, Agricultural Information Bulletin No. 358.

Vadakkadath, M. S. and Atwood, C.S. (2005). "The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain". *Cell. Molecular Life Science* 62 (3): 257–70.

Valge, L.A. (1970). *Veterinariya Mosk.,* 47(10): 101-104 (Press). Cited in *Animal Breeding Abstract,* 39: 1841.

Veldhuis, J.D., Keenan, D.M., Liu, P.Y., Iranmanesh, A., Takahashi, P.Y. and Nehra, A.X. (2009). "The aging male hypothalamic-pituitary-gonadal axis: pulsatility and feedback". *Molecular Cell Endocrinology.* 299 (1): 14–22.

Verstegen, J., Iguer-Ouada, M. and Onclin, K. (2002). Computer assisted semen analysers in andrology research and veterinary practice. *Theriogenology.* 57:149-179.

Vietmeyer, N.D. (1985). Potentials for microlivestock in developing countries. *Journal of Applied Rabbit Research.* 8 (10).

Viudes de Castro M.P., Marco-Jiménez F., Vicente J.S., Navarro E., Lavara R. and Mocé E. (2004). Sperm kinetic parameters and differences in seminal plasma composition among two rabbit lines. *In Proc. 8th Annual Conf. European Society of Domestic Animal Reproduction. Reproduction in Domestic Animals 394. 266 Abstract (P13). Warsaw Agricultural University, Poland.*

Voh, A. A. and Otchere, E. O. (1989). Reproductive performance of zebu cattle under traditional agropastoral management in northen Nigeria. Animal Reproduction Science 19: 191-203.

Waddad, S.A. and Gaill, E.S.E. (1985). *Acta. Veterinaria, Beograd,* 35: 299-304. World Health Organisation (WHO), *(*2001). *Tropical Diseases Database.*

Waindi, E.N., Gombe, S. and Odour-Okelo, D. (1986). Plasma testosterone in Toggerburg goats. Archives of Andrology. 17:9-17.

White, W. E. (1933). Duration of fertility and the histological changes in the reproductive organs after ligation of the vasa efferentia in the rat. *Proceedings Royal Society. (London), B113*:544- 550, 1933.

World Health Organisation. *Tropical diseases database. (*2001). Available in: http:// www-nt.who.int./imagelib.pl

Wong, W.Y., Flik, G., Groenena, P.M., Swinkels, D.W., Thomas, C.M., Copius-Peereboom, J.H.J., Merkus, H.M.W.M. and Steegers-Theunissen, R.P.M. (2001) The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. *Reproductive Toxicology*, 15, 131-136.

Wu, G., Bazer, F.W., Davis, T.A., Kim, S.W., Li, P., Marc Rhoads, J., Satterfield, M.C., Smith, S.B., Spencer, T.E. and Yin, Y. (2009) Arginine metabolism and nutrition in growth, health and disease. *Amino Acids*, 37, 153-168.

Yabsley, M.J. (2006). Detection of Babesia and Anaplasma Spps. In Texas and Georgia, U.S.A.

Yokoi, K., Uthus, E.O. and Nielsen, F.H. (2003) Nickel deficiency diminishes sperm quantity and movement in rats, *Biological Trace Element Research*, 93, 141-153.

Yue, D., Yan, L., Luo, H., Xu, X. and Jin, X. (2010). Effect of Vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxi-dant abilities in Aohan fine-wool sheep. *Animal Repro-duction Science*, 118, 217-222.

Zambrano, E., Rodríguez-González, G.L., Guzman, C., García-Becerra, R., Boeck, L., Díaz, L., Menjivar, M., Larrea, F. and Nathanielsz, P.W. (2005) A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. *Journal of Physiology*, 563, 275-284.

Zarazaga, L.A., Guzmán, J.L., Domínguez, C., Pérez, M.C. and Prieto, R. (2005). Effect of plane of nutrition on seasonality of reproduction in Spanish Payoya goats. *Animal Reproduction Science* 87, 253−267.

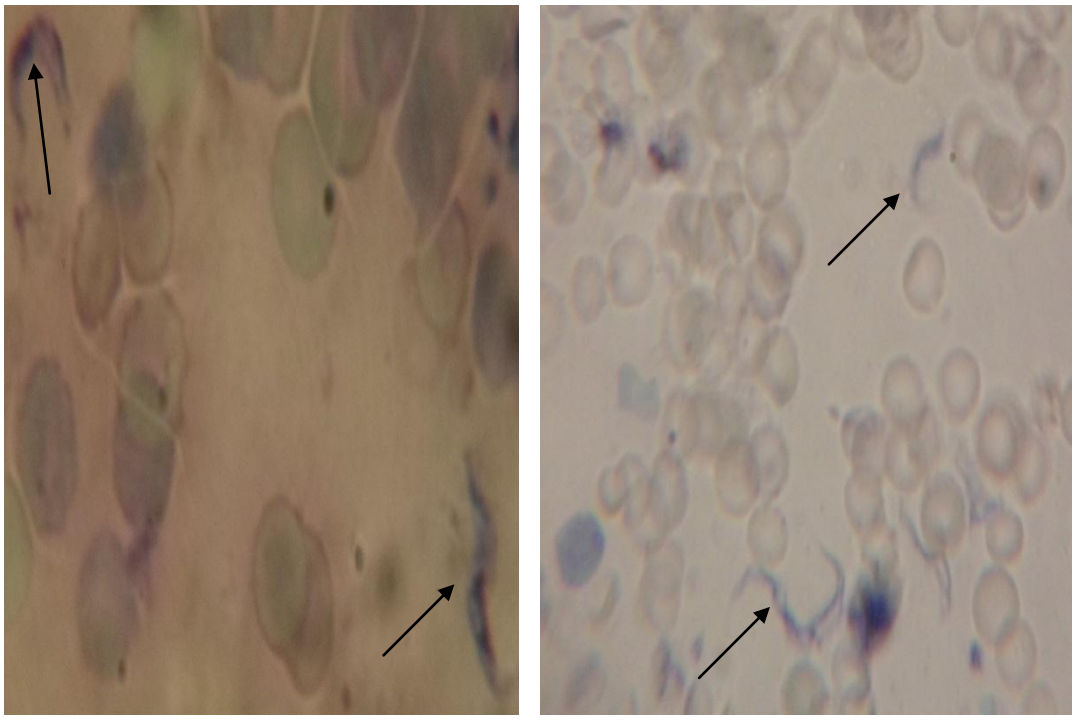
Zemjanis, R. (1970). *Diagnostic and Therapeutic Techniques in Animal Reproduction.* editor Baltimore, Williams and Wilkins.

APPENDICES

Appendix I: Photograph showing adapted Artificial vagina for rabbit bucks



Appendix II: Blood picture showing *T. brucei* during infection



a. b.