FFECTS OF EXPERIMENTAL *Trypanosoma evansi* INFECTION ON PREGNANCY AND SOME PREGNANCY ASSOCIATED SERUM FACTORS INYANKASA EWES

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DECLARATION

I declare that the work in thisDissertationentitled "**Effects of Experimental *Trypanosoma evansi*Infection on Pregnancy andSome Pregnancy Associated Serum Factorsin Yankasa Ewes**" has beencarried out by me in the Department of Theriogenology and Production.The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this University or any other Institution.

Adewale AyodejiADEYEYE _____ _____
(Name of Student) Signature Date

CERTIFICATION

This dissertationentitled "**EFFECTS OF EXPERIMENTAL *Trypanosoma evansi* INFECTION ON PREGNANCY AND SOME PREGNANCY ASSOCIATED SERUM FACTORS IN YANKASA EWES**" by Adewale AyodejiADEYEYE meets the regulations governing the award of the degree ofDoctor of Philosophyin Theriogenology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the Almighty God, for sending Jesus Christ to die on the cross of Calvary for my sake. HE is the sustainer and preserver of my life and family.

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ABSTRACT

Twenty pregnantYankasa ewes which were oestrus synchronized and mated naturally were assigned into 3 groups according to their weights, to determine the effect of *Trypanosomaevansi*infection on pregnancy and some pregnancy associated serum factors. Groups A and B comprising7 ewes each were infected with approximately 1.0 $x10^6$ of *T. evansi* per ewe through venipunctureat the second and third trimesters of pregnancy, respectively. Group C comprising 6 ewes served as uninfected control. Parasitaemia was detected between days 11 and 25 post-infection (pi) and between days 13 and 22 pi in groups A and B, respectively and fluctuated thereafter, while ewes in group C remained aparasitaemic throughout the study. There was slight pyrexia in the infected groups (A and B) but absent in group C. The mean body weight changes, glucose concentration and packed cell volume (PCV) of ewes in group A were not significantly($p>0.05$) different from those in group C throughout the study. There was also no significant ($p>0.05$) difference in mean glucose concentration between group B and C. However, in group B, the mean body weight was significantly($p<0.05$) lower compared to group C at week 2 and from week 4 pi till the end of the study; the PCV also significantly($p<0.05$) decreased but at weeks 4 and 6 pi only. Leukopenia was observed in group A at week 1 pi, followed by leukocytosis at week 4 pi and then leukopenia at week 7 pi. Ewes in group B also showed leukopenia but at week 3 pi and thiscoincided with peak parasitaemia. The mean plasma protein (PPr) of ewes in group A was significantly($p<0.05$)increasedcompared to those of group C at weeks 7and 11 pi, thereafter till the end of the study. On the contrary, the PPr of ewes in group B decreased significantly($p<0.05$) compared to group C at weeks 2 and 6 pi.The mean progesterone (P_4) and pregnancy associated glycoprotein (PAG) levels of ewes

in group A were significantly ($p<0.05$) higher than ewes in group C at weeks 4 and 12 pi, respectively, while there were no significant ($p>0.05$) differences in the mean P_4 and PAG level between ewes in groups B and C. There was significant ($p<0.05$) decrease in mean estrone sulfate (E_1S) of ewes in group A compared with group C at weeks 2 and 11 pi, but no significant difference between groups B and C. The mean cortisol level of ewes in group A significantly($p<0.05$)decreased at week 3 pi compared with group C, while a significant ($p<0.05$)increased was observed in group B compared to group C at week 12 pi. There was no significant ($p>0.05$)correlation among the pregnancy associated serum factors of ewes in group A throughout the study.For ewes in group B, there was also nosignificant($p>0.05$) correlationamong the pregnancy associated serum factors determined, except between PAG and cortisolwhich weresignificantly correlated ($r = 0.77$, $p<0.05$). No gross lesion was observed in the infected and control ewes humanly sacrificed 10 days post-partum. There was also no gross lesion on the placenta. However, the infected groups had histopathological changes in the hypothalamus characterized by neuronal degeneration and microglial infiltration while in the pituitary gland, there was macrophageinfiltration. The thyroid gland wasinfiltrated by macrophages and loss its glandular secretions. All these histopathological changes were absent in group C.There were no significant ($p>0.05$) differences in the gestation length and lamb percentage body-weights between ewes in the infected groups (A and B) and the group C. Mouse inoculation withpost-partum blood from infected ewes, became parasitaemic 18-25 days pi, for group B, while none of the mice were parasitaemic in groups A and C. Lambs born from the infected groups (A and B) were aparasitaemic,for 40 days post-partum.It was therefore concluded that the*T. evansi*isolate usedin this study caused mild trypanosomosis when infected at third trimesterwhileewes

infected at second trimesterwere resistant. The infection was more severe onpregnancy associated serum factors at second trimester than third trimester.

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ABREVIATIONS

ABUCAUC	-	Ahmadu Bello University Committee on Animal use and care
ACTH	-	Adrenocorticotropic hormone
ANOVA	-	Analysis of variance
BUN	-	Blood urea nitrogen
CATT	-	Card agglutination test for trypanosomosis
CL	-	Corpus luteum
CRH	-	Cortisol releasing hormone
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylene diamine tetra-acetic acid
ELISA	-	Enzyme-linked immunosorbent assay
E_1	-	Estrone
E_1S	-	Estrone sulphate
E_2	-	Estradiol
E_3	-	Estriol
FAO	-	Food and agriculture organisation
FSH	-	Follicular stimulating hormone
GnRH	-	Gonadotropin releasing hormone
H & E	-	Haematoxylin and eosin
ILRAD	-	International laboratory for research on animal diseases
mAb	-	Monoclonal antibodies
NITR	-	Nigerian institute for trypanosomiasis research
NITOR	-	Nigerian institute for trypanosomiasis and onchocerciasis research
Ns	-	Not significant
OIE	-	Office of the international epizootic
PAG	-	Pregnancy associated glycoproteins
PCR	-	Polymerase chain reaction
PCV	-	Packed cell volume
Pi	-	Post-infection
PL	-	Placental lactogen
PP	-	Prepatent period
P_4	-	Progesterone
RIA	-	Radioimmunoassay
RIMS	-	Resource information management system
TMB	-	Tetramethylbenzidine
VSG	-	Variant surface glycoprotein
WAD	-	West African dwarf
WAITR	-	West African institute for trypanosomiasis research
WHO	-	World health organisation

CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Trypanosomosis is a diseaseofeconomic and zoonotic importance, recognized worldwide (WHO, 2005).It is awasting disease of man and animals caused by blood parasites of the family Trypanosomatidae and genus *Trypanosoma* (Steverding, 2008). It is characterized by intermittent pyrexia, anaemia, anorexia, increased respiratory and heart rates, depression, lacrimation, nasal discharge, subcutaneous oedema, enlargement of lymph nodes, central nervous system disorders, corneal opacity, genital lesions, diarrhoea or dark hard faeces and death (Ogwu *et al*., 1992; Bentivoglio*et al*, 1994; Prashant *et al*., 2005;Adamu*et al*., 2006).

The disease is endemic in tropical areas of the world particularly Africa and South America (Swallow, 2000), and remains one of the major diseases limiting the growth of livestock industry in Africa (Irungu *et al*., 2002). *Trypanosomacongolense*, *T. vivax*and *T. brucei*, have the widest host range among domestic animals in which they cause the disease, '*nagana'*, and are regarded as the most pathogenic trypanosomes (Desquesnes, 2004). *T. evansi* infection is known as '*Surra*' (Mbaya *et al*., 2010) affecting dogs, buffaloes, camels, horses, sheep, goats (Sekoni, 1994) and humans (Prashant *et al*., 2005). Other species include *T. equinum* in horses and dogs, *T. equiperdum* affecting horses alone in which it causes '*dourine*', *T. suis* and *T. simiae* in pigs (Sekoni, 1994). Human African Trypanosomosis (HAT) is caused by *T. brucei gambiense* in West Africa (Truc, 2003) and *T. bruceirhodesiense* in East Africa (Ugochukwu, 2008) while human trypanosomosis is caused by *T. cruzi* in South America (Desquesnes, 2004).

African trypanosomosis is transmitted biologically (cyclically) by tsetse fly (*Glossina*) and mechanically by haematophagus flies such as *Tabanus* (Steverding, 2008). However,*T. equiperdum* is sexually transmitted (Wanga and Munga, 2011). Transplacental transmission of trypanosomosis has been reported in natural (Ate *et al*., 2006) and experimental infections of calves (Ogwu *et al*., 1986). This has also been demonstratedexperimentally in sheep (Ikede and Loss, 1972) and mice (Ogwu and Nuru, 1981; Griffin, 1983).

Trypanosomosis causes pathological disorders in the reproductive system (Ikede *et al*., 1988; Sekoni, 1994). In the male, it is characterized by severe testicular degeneration, scrotal inflammation, penile protrusion, prepucial inflammation, testicular odema, epididymitis, abnormal spermatogenesis and deterioration of semen characteristics (Sekoni *et al*., 1990a; Sekoni *et al*., 2004; Adamu *et al*., 2006; Adamu *et al*., 2007; Victor *et al*., 2012; Kilekoung*et al*., 2014; Okubanjo *et al*., 2014b; Yasameen*et al*., 2015). In the female, there is abortion, irregular oestrus cycle, cystic degeneration of the ovary, follicular cyst, flaccidity of the uterine horn, decreased conception rate, low birth-weights and neonatal deaths (Paikne and Dhake, 1972; Ogwu *et al*., 1984; Elhassan *et al*., 1994; Elhassan *et al*., 1995; Faye *et al*., 2004; Bawa *et al*., 2005; Dalal *et al*., 2008; Leigh and Feyemi, 2013; Rodrigues *et al*., 2013; Allam*et al*., 2014). In addition, pregnant animals infected with trypanosomosis may die before or after parturition (Anene and Omamegbe, 1984; Luckins *et al*., 1986; Anene *et al*., 1991; Waitumbi and Conor, 1991; Bawa *et al*., 2005).

Trypanosomaevansi is an extracellular parasite, mechanically transmitted by haematophagous insects such as Tabanid flies (Luckins, 1999; Prashant *et al*., 2005). *T. evansi* infections (Surra) naturally affect camels, horses, donkeys and dogs (Al-Rawashdeh *et al*., 2000), but infections ranging from mild/chronic to acute forms have been reported in rats, rabbit, cats,

pigs, deer, water buffalos, mules, Ilamas, elephants, cattle, sheep and goats (Franke *et al*., 1994; Audu *et al*., 1999; Prashant *et al*., 2005; Shehu *et al*., 2006b; Jatau *et al*., 2010, Villareal *et al*., 2013; Yasameen*et al*., 2015). In pregnant ewes, the parasitehas been reported to cause abortion, stillbirth and birth of weak lambs that subsequently died. (Dalal *et al*., 2008).

Pregnancy associated serum factors arehormones and proteins secreted by the placenta and corpus luteum during pregnancy (Sousa *et al*., 2008), and then released into maternal circulation where their concentrations are detected and used to determine pregnancy and obstetric diseases (Karen *et al*., 2003). This includes progesterone (P_4),estrone sulfate (E_1S),pregnancy associated glycoprotein (PAG),placental lactogen (PL) and cortisol. All of these are produced by the placenta except P_4which is initially produced in the corpus luteum, but also produced by the placenta as pregnancy progresses in sheep (Al-Gubory *et al*., 1999; Sousa *et al*., 2008; Purohit *et al*., 2012). Their concentrations are used by both clinicians and researchers in studying the pathophysiology of ruminant pregnancy (Breukelman *et al*., 2005).

1.2 Statement of Research Problem

The occurrence of animal trypanosomosis in Nigeria is high,in spite ofseveral years of research (Adamu *et al*., 2007). The disease remains the single most important disease limiting livestock production (Sekoni 1994; Irungu *et al*., 2002) by causingsevere economic losses, due to livestock death, reproductive disorders and high cost of chemotherapeutic and prophylactic control (Swallow, 2000).

There is a changing status of the camel from being “the desert ship” to a “food security animal” (Abubakar *et al*., 2011),leading to significant increase in its production. The camel is a major host of *Trypanosomaevansi*. Consequently, the herding together of camels with other

domestic animals, pose a serious threat to these animals. As a result of this, *T. evansi*is assuming greater economic importance among other animals particularly sheep, where acute and chronic diseases have been reported (Audu *et al*., 1999; Shehu *et al*., 2006b). In addition to this, *T. evansi* has been reported to cause reproductive losses among ewes experimentally infected with the parasite in Egypt (Dalal *et al*., 2008).

Records on the study of trypanosomosis in pregnant Yankasa ewes in Nigeria are few (Bawa *et al*., 2005), while reports on similar studies in goats are contradictory (Sekoni, 1994). For instance, Luckins *et al*. (1986) reported increase in blood progesterone while Faye*et al*. (2004) reported decreased plasma progesterone.

The pathogenesis of *Trypanosome* infection-induced abortion is not well known. However, Sekoni (1994) suggested that chronic fluctuation in pyrexia or direct effect of the organism on embryo/foetus is responsible for reproductive failure. This is not well understood since theparasitecauses significant pathology on other parts of the female reproductive tract such as the ovaries (Ogwu and Njoku, 1991; Rodrigues *et al*., 2013;Leigh *et al*., 2015).The effect of trypanosome infection on pregnancy associated serum factorshave not been seriously investigated in spite of their roles as biomarkers of pregnancy, hence the need for this study.

1.3 Justification of the Study

Previous studies on animal trypanosomosis tended to concentrate on cattle since the disease in small ruminants is viewed as less important especially in West Africa (Kalu *et al.,* 1986). Available evidences based on the epidemiology of the disease in Nigeria (Daniel *et al.,* 1994; Kalu and Lawani, 1996; Dadah *et al.,* 1997; Adeyeye *et al*., 2009) and other parts of Africa

(Ahmadu *et al.*, 2002), indicate that not only are infection rates high but alsosevere losses occur from the disease.

The effect of trypanosomosis on pregnancy has been studied in various domestic ruminants, but little attention has been paid to how pregnancy associated glycoprotein (PAG),estrone sulfate (E_1S)and cortisol levels are affected, despite the fact that their levels in obstetric diseasesare affected (Semambo *et al*., 1992; Karen *et al*., 2001; Sousa *et al*., 2008). Trypanosomosis is an obstetrics disease known to cause early embryonic death and foetal loss during pregnancy (Ikede *et al*, 1988; Sekoni, 1994). Apart from Faye *et al*. (2004), who studied the effect of experimental *T. congolense* infection on PAG in pregnant West African dwarf goats, there is dearth of information on similar study inNigerian indigenous domestic ruminants.Therefore, there is need to conduct this study in a different specie of animals with the view of re-assessing their findings or otherwise.

Studies onthe effect of*Trypanosomabrucei* and *T. vivax*infection at different stages of pregnancy in Nigerian indigenousewes have been carried out (Edeghere *et al*., 1992; Elhassan *et al*., 1995; Bawa *et al*., 2005). However, such information on *T. evansi*infection in the ewe is lacking. There is therefore need to investigate the effect of *T. evansi* infection on pregnancy in Yankasa ewes, which are the most numerous breed of sheep in the Guinea savanna region of Nigeria (RIMS, 1992). Information generated from the study could provide a better understanding of the pathophysiological mechanism of trypanosomeinfection-induced pregnancy disorder in ewes. Such information could be exploited in designing and developing control measures to reduce reproductive losses associated with the disease thereby increasing sheep productivity.

1.4 Aim and Objectives

1.4.1 Aim

This study was aimed at investigating the effect of *T. evansi*infection on the course of pregnancy and some pregnancy associated serum factors [progesterone (P_4), pregnancy associated glycoprotein (PAG), estrone sulfate (E_1S) and cortisol]in Yankasa ewes.

1.4.2 Objectives

The objectives were to:

a. determine the effect of *T. evansi* infection on pregnancy in Yankasa ewes
b. determinethe effect of *T. evansi* infection on some pregnancy associated serum factors in Yankasa ewe.
c. determine theassociation among the pregnancy associated serum factors in Yankasa ewe infected with*T. evansi*.
d. conduct gross and histological examinations on aborted foetus(es) (if available) and the reproductive tract and other organs of their dam.
e. comparethe effectof *T. evansi* infection at in the second and third trimester of pregnancy in Yankasa ewes.

1.5 Research Questions

1. Does *T. evansi* infection have any effect on pregnancy in Yankasa ewes?
2. Does *T. evansi* infection during pregnancy in Yankasa ewes have any effect on some pregnancy associated serum factors?

CHAPTER TWO
REVIEW OF LITERATURE

2.1 Significance of Sheep Production in Nigeria

The population of sheep in Nigeria was estimated to be 22.1 millionabout 2 decades ago (RIMS, 1992).These animals are found throughout the country, where they are kept for meat and milk (Blench 1999). They also playa socio-economic rolein the life of Nigerians, particularly in the northern partwhere they are keptand used during festive periods such as *eld-el kabir*, wedding andnaming ceremonies (Umaru *et al*., 2009).Small ruminants (sheep and goats) are part of almost all farming systems in Nigeria where they are associated with mixed farming and pastoralism (David-West, 1985). They are important in sustaining poor families particularlyin the rural areas (Yakubu *et al*.,2010).

2.2 Breeds of Sheep in Nigeria

Generally four breeds of sheep areindigenous to Nigeria, namely Balami, Udah, West African dwarf (WAD) and Yankasa (Adu and Ngere, 1979).

2.2.1 **Balami**

They are the largest breed of sheep in Nigeria (Wosu, 2002), found in the semi-arid north, extending up north from Maiduguri to Sokoto through Kano. It is a white and hairy breed with pendulous ears and long thin tails (Blench, 1999). Normally,rams are horned but ewes are polled. The Roman nose and the large bulbous nose of the Balami assist in distinguishing it from Yankasa breed(Wosu, 2002).

2.2.2 **Udah**

The Udah is identified by its characteristic coat colour pattern which has an entirely brown or black forequarters and white behind (Blench, 1999). It is a meat breed with distinctive markings(Mason, 1996) slightly smaller than the Balami even though their size range issimilar (Wosu, 2002). Udah breed is found in Niger Republic (Haumesser and Gerbaldi, 1980) extending into Nigeria from the far north to the middle belt region. They are also found in central Mali (Wilson and Light, 1986).

2.2.3 **West African Dwarf (WAD)**

It is a small-bodied breed with a wide range of coat colour which may be all white, black, brown or spotted black or brown on a white coat (Blench, 1999). It is found in the southern partof the country although isolated few are found on the Jos Plateau and close to the confluence of rivers Niger and Benue (Blench, 1999). WAD thrives well in areas infected with tsetse fly leading to the breed been clasified as a "trypanotolerant breed" (Adu and Ngere, 1979).

2.2.4 **Yankasa**

The Yankasais a meat breed (Mason, 1996)with white colour coated sheep with black patches around the eyes and sometimes on the feet (Blench, 1999). Their muzzle and ears are normally black. Just like the Balami, the rams are horned while the ewes are polled.Yankasa have been reported in all parts of Nigeria(Wosu, 2002), although their population reduces towards the northern border. They are the most numerous breed of sheep found in the Guinea savanna region of Nigeria (RIMS, 1992).

2.3 **Factors affecting Sheep Production in Nigeria**

There are lots of limitations to livestock production in sub-Saharan Africa such as diseases, poor feeding, substandard management practices and unwholesome breeding policies (Lughano and Kambarage, 1996; Oladele*et al*., 2013). Disease is the major singular entity limiting livestock production in Nigeria(Lamorde, 1996), leading to shortage in animal protein available for the populace. Trypanosomosis is one of such diseases. Ovine trypanosomosis along with caprine trypanosomosis were initially viewed as less important. However, following high prevalence of the disease in these animals (Kalu *et al*., 1986), the disease is now viewed seriously.

2.4**Trypanosomosis**

2.4.1 **Brief historical background of the disease**

Trypanosome was first described in Africa as a non-pathogenic agent in a frog and rat (Mulligan, 1970). However in 1880, it was attributed to a disease "Surra", occurring in camels, cattle, horses and mules (Röttcher*et al*., 1987).By 1894, the disease was detected in the blood of cow by Bruce in Zululand of South Africa, with signs that progressed graduallymaking the animal weak more and more; this led to the name "*nagana*" which means powerless/useless in Zulu language (Steverding, 2008). Bruce was also reported to have demonstrated that the pathogen was transmitted from game animals to cattle by *Glossina*.Between the 19thand 20thcentury, three severe outbreaks occurred. First from 1896 to 1906 in Uganda and Congo Basin, then in the 1920s which affected several African countries, and in 1970 which occurred in such epidemic as in Angola, Cameroon, Congo, Côte d'Ivoire, Equatorial Guinea, Gabon, Ghana, Guinea, Kenya, Malawi, Nigeria, Uganda, United Republic of Tanzania, Zambia and Zimbabwe(Klassen-Fischer *et al*., 2011).These epidemics led to

concerted efforts by governments on the African continent rolling out control measures to curtail the disease.Human trypanosomosis was documented as early as the 14th century in Sudan (Scaravilli, 1992).

The West African Institute for Trypanosomiasis Research(WAITR) was established in 1947 to curtail the disease in West Africa (Mulligan, 1970). It translated intothe Nigerian Institute for Trypanosomiasis Research (NITR) at independencein 1960 and now Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) (Mulligan, 1970).Infections caused by *Trypanosoma* were known as 'trypanosomiasis' but following the standardization of nomenclature foranimal parasitic diseases by the World Association for Advances in Veterinary Parasitology, the name was replaced by'trypanosomosis' (Kassai*et al*., 1988).

2.4.2 **Aetiology**

Trypanosomosisin sheep iscaused by*Trypanosomabrucei*(Adeyemo *et al*., 1990); *T.congolense*(Katunguka-Rwakishaya*et al*., 1997; Okubanjo *et al*., 2014b);*T. vivax*(Bawa*et al*., 2005; Rodrigues *et al*., 2013) and *T. evansi* (Audu *et al*., 1999, Dalal *et al*., 2008). *Trypanosomacruzi*have also been reported in sheep (Desquesnes, 2004).Trypanosomes areextracellular, elongated spindle-shaped protozoonmeasuring 8.0 to 39 micrometers in length (Prowse, 2005). It has a central nucleuscontaining the DNA and kinetoplast(Urquhart *et al*., 1996).*Trypanosomeevansi* is slender with free flagellum, visible undulating membrane and thin posterior end (Desquesnes *et al*., 2013).

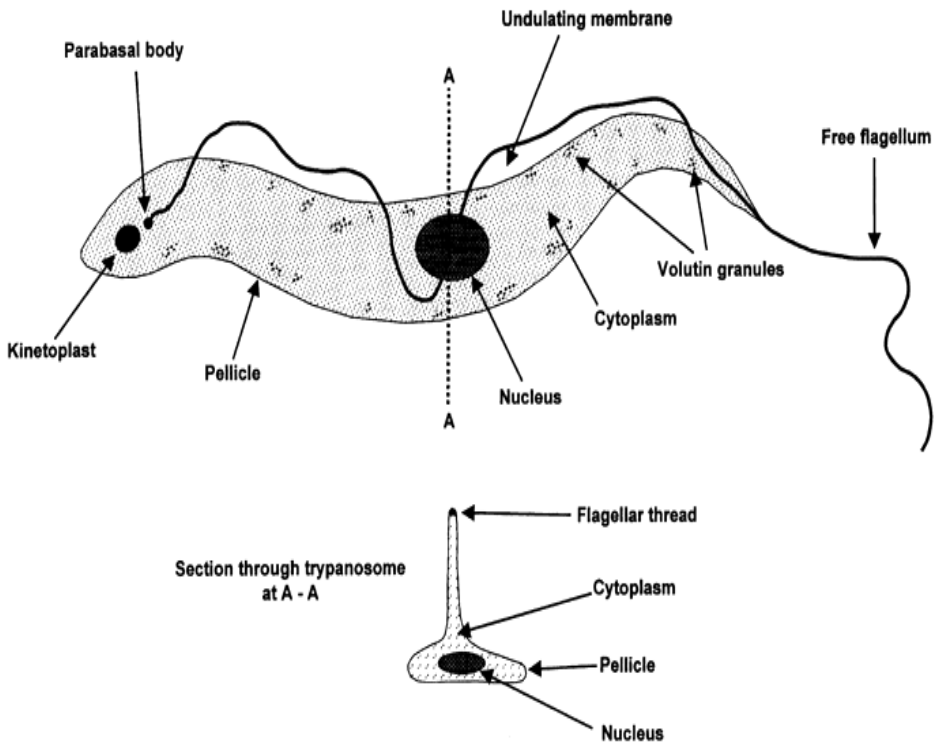

Figure 2.1: Diagrammatic illustration of a trypanosome (trypomastigote) as seen in a stained preparation made from the blood of an infected animal (Source - FAO, 2013).

2.4.3 **Classification**

Taxonomy of *Trypanosoma* (Desquesnes, 2004)

SubKingdom: Protozoa

Phylum: Sarcomastigophora

Sub Phylum: Mastigophora

Class: Zoomastigophora

Order: Kinetoplastida

SubOrder: Trypanostomatina

Family: Trypanosomatidae

Genus: *Trypanosoma*

The parasite is divided into twomajor sub-divisions of Salivaria and Stercoraria. The Salivariansub-divisionis further classified intofour sub-genus namely: Trypanozoon (*Trypanosomabrucei,T. evansi,T. equiperdum*);Duttonella (*T.vivax, T. uniforme*); Nannomanas (*T.congolense, T. simiae, T.godfreyi*)andPycnomonas (*T. suis*)(Desquesnes, 2004; OIE, 2013). *T. cruzi*the causative agent of Chagas disease in humansisthe most important member of the Stercorariasub-division (Desquesnes, 2004).

2.4.4 **Transmission**

Transmission is biologically (cyclically) by tsetse fly (*Glossina*) and mechanically by haematophagus flies (Prowse, 2005). Transplacental transmission of trypanosomes was reviewed by Ogwu and Nuru (1981). However, it has been reported by Ikede and Losos (1972),Ogwu*et al*. (1986) andAte *et al*.(2006). Oral transmission (Rabo *et al*., 2001) as well as sexualtransmission (Wanga and Munga, 2011)has also been reported. Trypanosomosis has been demonstrated in the prepucial washing of bucks experimentally infected with *T. vivax and T. brucei* (Osuagwuh, 2014), supportingthe possibility of coital transmission.In equine,*T. equiperdum* is sexually transmitted (Wanga and Munga, 2011).

In Africa, *Trypanosomacongolense, T. vivax and T. brucei* are predominantly transmitted cyclically by the Tsetse vector *Glossina*(Prowse, 2005). However, some reports suggest that the trio can be transmitted mechanically by haematophagous insects (Desquesnes and Dia, 2003; Desquesnes, 2004).*Trypanosomaevansi*is transmitted mechanicallyby haematophagus flies such as*Tabanus and Stomoxys*(OIE, 2012) while *Trypanosoma cruzi* is transmitted cyclically through triatomid bugs(Roberts *et al*., 1989; Desquesnes, 2004). However, transmission of *T. evansi* through milk has been reported (OIE, 2009). In the biological transmission of trypanosomosis, the vector feeds on a parasitaemic mammalian host where the

trypanosomeundergoes a series of morphological transformation and develops to a metatrypanosome which infects a susceptible mammalian host when feeding on it (FAO, 2013).

2.4.5 **Pathogenesis**

Following infection of a susceptible host with metatrypanosome, it multiplies at the site of infection leading to local inflammatory reaction on the skin site called‘chancre’ (FAO, 2013).Mature blood trypanosomes (trypomastigotes) enter the body system through the lymphatic system into the blood circulation, leading to parasitaemia (Morrison*et al*., 1981). The parasite is then distributed throughout the body,nourishing itself by feedingon the host (FAO, 2013).

Themechanismresponsible for reproductive damage in trypanosomosis is not well known. Nonetheless in the female,it has beensuggested that the chronic fluctuation in pyrexia as a result of the infection could partly be responsible for the infertility observed (Sekoni, 1994, Bawa *et al*., 2005). Ogwu *et al*. (1984) had demonstrated that fluctuation in pyrexia during trypanosomosis severelyaffected the ova, uterus, and embryo leading to conception failure, embryonic or foetal death. Recently, Allam *et al*. (2014) reported abortion due to pyrexia in gilts. It is also postulated that the direct effect of the organism on the embryo/foetus is responsible for the abortion and conception failure seen in infected females(Sekoni, 1994; Bawa, 2000). The parasite is known to invade the placenta (Ogwu *et al*., 1986; Ate *et al*., 2006) and cervix eliciting cervicitis (Bawa, 2000). Apart from this, earlier reports showed tissue reaction in cattle infected with *T. vivax* (Isoun and Esuruoso, 1972). It is therefore possible that, the embryonic and foetal death seen in pregnant animals is due to a direct invasion of the embryo as well as a result of tissue reaction (Sekoni, 1994).Foetal hypoxia as a

result of anaemia was reportedas a potential cause of abortion in *T. vivax* infected cattle (Ogwu, 1983). This was also observed in sheep infected with *T. vivax* (Silva *et al*., 2013), signifying a possible mechanism for reproductive loss.

In the male,the pathogenesis of reproductive trypanosomosis is similar to the female. It involves chronic intermittent fluctuations in pyrexia and a direct invasion of tissues by the parasite (Sekoni, 1994). Pyrexia raises testicular temperature above what is required for the process of spermatogenesis, leading to impairment in its physiological function (Anosa and Isoun, 1980). This is as a result of localization of the organism in the scrotal skin, provoking non-purulent inflammation that leads to degeneration of the seminiferous tubules (Ikede *et al*., 1988). Trypanosomes have been demonstrated to be present in the intertubular tissues of severely degenerated testes leading to cessation or decrease in gonadal sperm production due to impairment of spermatogenesis (Sekoni*et al*., 1990a).

2.4.6 **Clinical signs**

The onset of clinical signs is based on genetic and environment factors (Igbokwe and Mohammed, 1991). The genetic factors include specie, breed and individual factors while the environmental factors are tsetse and trypanosome characteristics, age and nutrition of the host as well as host exposure to stress (Okubanjo, 2001). However, anaemia is the most significant clinical feature in trypanosomosis (Anosa, 1988a).The diseasehas three phases namely: Acute (phase I), chronic (phase II) and recovery (phase III) (Anosa, 1988a). Others are intermittent pyrexia, loss of body condition, raised hair coat, droopiness, anorexia, nasal and ocular discharge, pneumonia, lacrimation, subcutaneous oedema, enlargement of lymph nodes, central nervous system disorders such as uncoordinated movement, corneal opacity, genital

lesions, abortion, diarrhoea or dark hard faeces and eventual death (Ogwu *et al*., 1992; Desquesnes, 2004; Bawa *et al*., 2005;Prashant *et al*., 2005; OIE, 2009; OIE, 2013).

Another consistent clinical feature is reproductive disorders (Ikede *et al*., 1988; Elhassan *et al*., 1994; Sekoni, 1994; Adamu *et al*., 2006; Allam *et al*., 2012). The disease in the female is associated withirregular oestrus, anoestrus, stillbirth, decreased conception rate, posterior paresis, low birth-weights or birth of anaemic offsprings, neonatal death, foetal resorption, abortion in various stages of pregnancy (Ogwu *et al*., 1984; Edeghere *et al*., 1992; Elhassan *et al*., 1994; Elhassan *et al*., 1995; Faye *et al*., 2004; Bawa *et al*., 2005; Ochiogu *et al*., 2008; Allam *et al*., 2012). Females infected during pregnancy may die before or after parturition (Anene and Omamegbe, 1984; Luckins *et al*., 1986; Anene *et al*., 1991; Waitumbi and Conor, 1991; Edeghere *et al*., 1992). There may be premature birth (Okech *et al*., 1996) or prolonged gestation (Ochiogu *et al*., 2008). In the male, orchitis and periorchitis have been reported in both humans and animals (Ikede, 1979).

2.4.7 **Pathology**

Trypanosomosis is an immuno-suppressive disease (Sulaiman and Adeyemi, 2010).The pathologicalchanges depend on the specie and strain of trypanosome involved (Anosa, 1988b; Ogunsanmi *et al*., 1994a), duration of infection and species and breed of the infected host (Igbokwe and Mohammed, 1991).

2.4.7.1 *Gross pathological changes*

The post-mortem lesions in animals infected with trypanosomes are not precise (OIE, 2009). However, the carcasses are generally emaciated and pale (Ikede and Losos, 1972; Shehu *et al*., 2006a; OIE. 2009; Okubanjo *et al*., 2014b). There is peri-orbital and scrotal oedema

(Kilekoung*et al*., 2014; Okubanjo *et al*., 2014b). Others are scrotal dermatitis (Ikede *et al*., 1988); sterility (Sekoni and Rekwot, 2003); epididymitis (Ikede *et al*., 1988) penile protrusionwith haemorrhages and posthitis (Ikede and Akpavie, 1982; Victor *et al*., 2012). Petechial and ecchymotic haemorrhages are common with congestionin most tissues and organs (Gardiner*et al*., 1989).There is enlargement of the lymph nodes (OIE, 2009); congestion and enlargement of the liver, lungs and kidney with petechial haemorrhages (Shehu *et al*., 2006a; OIE, 2013). Clots of fibrin have been reported in the ventricles of the brain (Gardiner*et al*., 1989); there is cattarhal enteritis suggestive of diarrhoea (Prashant *et al*., 2005; Shehu *et al*., 2006a). Oedema, hydroperitoneum and severemyocarditis are also common findings in *Trypanosoma* infected animals (Shehu *et al*., 2006a; OIE, 2013; Okubanjo *et al*., 2014b).

In the reproductive tract, there is atrophy of the ovaries in females (Adenowo *et al*., 2005) and cervicitis (Bawa*et al*, 2005), while inflammation of the scrotum may be present (Sekoni, 1994), absent (Sekoni *et al*., 1990a) or appear and disappear later in the male (Okubanjo *et al*., 2014b). In aborted animals, the cotyledons are enlarged, haemorrhagic and friable with focal areas of necrosis on the placenta (Leigh and Fayemi, 2013; Silva *et al*., 2013).

2.4.7.2 *Histological changes*

The histological changes associated with trypanosomosis involve severe tissue and organdamage particularly in the spleen, heart, CNS, liver, eyes, kidney and reproductive tract (Morrison *et al*., 1981; Shehu *et al*., 2006a).There is necrosis in the spleen with hyperplastic follicles (Damayanti *et al*. 1994; Shehu *et al*., 2006a). The myocardium is also necrotic with cellular infiltration (Morrison *et al*., 1981; Shehu *et al*., 2006a); interstitial pneumonia, interstitial myositis and hyperplastic bone marrow (Damayanti *et al*., 1994). An encephalitic

reaction characterized by lympho-plasma cellular infiltrates has been reported in the nervous system (Schmidt, 1983, Silva *et al*., 2013). Squamous metaplasia of follicular epithelium with fibroplasia and widened inter follicular connective tissue occur in the thyroid gland (Fatihu *et al*., 2007) while the pituitary gland is infiltrated by mononuclear cells (Adenowo *et al*., 2005). In the liver, there is necrosis, fatty degeneration and inflammatory reaction extending through the parenchyma (Alsaffar *et al*. 2007). The reproductive tract is characterized by testicular and epididymal degeneration in the male (Sekoni *et al*., 1990a; Okubanjo *et al*., 2014b), while follicular degeneration of the ovaries with cellular infiltration in the uterus and cervix (Ogwu and Njoku, 1991) are observed in the female.

2.4.7.3*Haematological changes*

The haematological changes in trypanosomosis are determined by the virulence of the parasite, the susceptibility of the host andstage of infection when samples are taken (Boid *et al*., 1981; Anosa, 1988a).The appearance of *Trypanosoma* in blood is a regular finding in all trypanosome infections. However, time of detection is dependent on the infectivity and pathogenicity of the infecting parasite (Jatau *et al*., 2013), as well as susceptibility of the animal host (Igbokwe and Mohammed, 1991).The level of parasitaemia is associated with anaemia and peak periods of pyrexia due to the destruction of red blood cells by the parasites (Mbaya *et al*., 2012).

Trypanosomosis causes decrease in packed cell volume, red blood cell mass and haemoglobin concentration leading to anaemia (Anosa, 1988a). This has consistently been seen in infections caused by *Trypanosomabrucei*(Abenga and Anosa, 2007; Mbaya *et al*., 2009), *T.congolense* (Banks, 1980; Goossens *et al*., 1998), *T.vivax* (Igbokwe and Anosa, 1989) and *T. evansi*(Audu *et al*., 1999; Shehu *et al*., 2006b).Decrease in erythrocytes values leads to

weakness and may progress to death if unattended to due to possibly due to reduced iron carry capacity of the blood.

Following infection with trypanosome, there is early leukopaeniadue to massive leukophagocytosis in the liver (Esievo and Saror, 1983; Igbokwe and Anosa, 1989) and plasma neuraminidase released (Esievo, 1979).The leukopaeniais characterized by lymphopaenia, neutropaenia, eosinopaenia and monocytosis (Anosa and Isoun, 1980; Anosa, 1988b) and corresponds with periods of peak parasitaemia (Esievo and Saror, 1983). Lymphopaenia is assumed to occur as a result of depletion in lymphocytes fromthe lymphoid nodules and the sequestration of manylymphocytes during the inflammatory reactions of trypamosome infection (Anosa and Isoun, 1983). Eosinopaeniais due to depression of the granulocyteprecursors in the bone marrow by trypanosome toxins(Igbokwe and Anosa, 1989) and splenic sequestration (Emeribe and Anosa, 1991) whileneutropaenia is suggested to be caused by the depression of granulocyteprecursors in the bone marrow together with trappingof neutrophils in the spleen due to hypersplenismsyndrome (Anosa, 1983). It is also attributed to the slow mechanisms of neutrophil response (Esievo and Saror, 1983).The leukopaenia is followed by leukocytosis(Esievo and Saror, 1983; Ogunsanmi *et al*., 1994a; Onah *et al*., 1996) particularly during the chronic phase of the infection when the parasites are less numerous in circulation (Goossens *et al*., 1998). Leukocytosis has been reported in*T. congolense*infected sheep (Katunguka-Rwakishaya *et al*., 1992) as well as rabbit infected with *T.brucei*(Emeribe and Anosa, 1991) and *T. evansi* (Jatau *et al*., 2013).It is associated with neutrophilia, eosinophilia, monocytosis and lymphocytosis (Emeribe and Anosa, 1991; Katunguka-Rwakishaya *et al*., 1992; Sulaiman and Adeyemi 2010). The neutrophilia is suggested to occur as a result of irritation of the bone marrow by weak trypanosomes, too

weak to cause hypoplasia of the marrow granulocyte (Anosa, 1988b).A consistent finding in trypanosome infection is eosinophilia (Igbokwe and Anosa, 1989; Emeribe and Anosa, 1991) and monocytosis which indicates depression of myeloid colony formation (Anosa, 1988b). Lymphocytosis is associated with recovery periods of trypanosomosis when parasitaemia is very mild. Basophils are seldomlypresent in the blood of mammals, and are seldommentioned in haematological studies of trypanosome-infected animals and man (Anosa, 1988b).

2.4.7.4 *Biochemical changes*

Elevated blood urea nitrogen (BUN) levels have been observed in some trypanosomosis infection (Anosa, 1988b). This increase has been reported in *T. evansi* infected rats (El-Baky and Salem, 2011), camel (Gutierrez *et al*., 2006); *T. brucei rhodesiense* infected mice and monkeys (Anosa, 1988b); *T. brucei* infected rabbits (Oyewusi and Saba, 2013); dog (Omotainse *et al*., 1994); sheep (Ogunsanmi *et al*., 1994b) and goats (Adejimi and Akinboade, 2000); *T. brucei gambiense* infected monkeys (Abenga and Anosa, 2007) as well as *T. vivax*and *T. congolense* infected cattle, where it coincides with periods of high parasitaemia (Anosa, 1988b). Elevated BUN level is caused by fever and glomerulonephritis which are common features of trypanosomosis (Anosa, 1988b). However, Kadima *et al*. (2000) and Awobode (2006) reported decreased urea levels in experimental *T. vivax*infected cattle and natural human trypanosomosis, respectively, while El-Baky and Salem (2011) reported no significant difference in camels infected with *T. evansi*.

Trypanosomosis decreasesserum glucose level in peracute *T. simiae* infection of pigs (Anosa, 1988b); acute phase of *T. evansi* infection of buffalo calves (Singh and Gaur, 1983) and camel (Gutierrez *et al*., 2006) as well as in chronic*T. simiae* infections of sheep. Hypoglycemia has also been reported in *T. vivax* infected sheep (Silva *et al*., 2013) and cattle (Kadima *et al*.,

2000). The depressed levels of glucose often followed periods of high parasitaemia due to the utilization of glucose by the parasite for metabolism (Anosa, 1988b). Studies have shown that glucose levels were depressed in diabetes mellitus rats infected with *Trypanosomabrucei* (Ukwueze and Anene, 2011) and in cattle infused with lactose saline following *Trypanosomavivax* (Kadima *et al*., 2000).

Changes in lipid profile have been reported in *Trypanosoma* infected animals (Katunguka-Rwakishaya *et al*., 1992; Abenga and Anosa, 2007; Adamu *et al*., 2009). There is decrease in lipid profile of *T. congolense* infected sheep (Katunguka-Rwakishaya *et al*., 1992); *T. vivax*infected cattle (Anosa, 1988b) and *T. brucei* infected pigs (Adamu *et al*., 2009). However, there was increase in *T. brucei gambiense* infected monkeys (Abenga and Anosa, 2007), rabbit and rats as well as *T. brucei rhodesiense* infected rats (Anosa, 1988b).

Hyperferraemia has been reported in *T. congolense*infected cattle (Anosa, 1988b) and *T. brucei* infected sheep (Ogunsanmi *et al*., 1994b) and cattle (Anosa, 1988b). However, decreased levels were reported in *T. evansi* infected camels (Gutierrez *et al*., 2006). Serum copper levels decrease in *T. brucei* infected sheep (Ogunsanmi *et al*., 1994b) but fluctuated in *T. vivax* infected cattle (Saror, 1976). The level of serum calcium decreased in *T. evansi*infected camels (Anosa, 1988b) and *T. brucei* infected sheep (Ogunsanmi *et al*., 1994b) but were unchanged in *T. rhodesiense* infected rats (Anosa, 1988b). Phosphorus levels decreased in *T. evansi* infected camels and *T. congolense* infected cattle (Anosa, 1988b), but increased in *T. brucei* infected sheep (Ogunsanmi *et al*., 1994b). These sheep also had decreased levels of magnesium and potassium while their chloride and bicarbonate levels increased.

The effect of *Trypanosoma* infection on total protein varies. Anosa (1988b) described a study in *T. brucei* infected mice which became anaemic yet its total protein levels remained unchanged. However, in an *in-vitro* study of sheep using *T. congolense* and *T. brucei*, Taiwo *et al*. (2003) reported increased levels of total plasma protein. Sulaiman and Adeyemi(2010) and Oyewusi and Saba(2010) also reported elevated total protein levels in *T. brucei* infected rats and rabbits, respectively. The former attributed the increase to cell derived proteins due to haemolysis. However, decrease in total protein has been reported in *T. congolense* and *T. vivax* infected bulls (Sekoni *et al*., 1990b). Adeiza *et al*. (2008) also reported decreased total protein in Savannah brown goats experimentally infected with *T. brucei*and *T. vivax*. Nevertheless, the most consistent alterations in serum protein are decreased albumin and increased globulin levels (Anosa, 1988b; Ogunsanmi *et al*., 1994b).

Serum creatinine levels were initially unchanged but decreased 8 weeks post-infection in *T. congolense* infected cattle (Anosa, 1988b). However, it declined slightly in *T. brucei* infected rabbits (Oyewusi and Saba, 2013) but increased in monkeys and mice infected with *T. brucei*and *T. congolense,* respectively (Anosa, 1988b).

2.4.7.5*Hormonal changes*

The hypothalamus produces gonadotropic releasing hormone (GnRH), which stimulates the anterior pituitary gland to produce follicular stimulating hormone (FSH) and luteinizing hormone (LH) that causes the gonads to secrete gonadal hormones (Anon, 2010). Lesions associated with trypanosomosis have been seen at the anterior pituitary gland, hypothalamus and gonads (Sekoni *et al*., 1990a; Mutayoba *et al*., 1995b; Adenowo *et al*., 2005; Adamu *et al*., 2006; Shehu *et al*., 2006a; Leigh *et al*., 2015) leading to reproductive disorders. In the trypanosome-infected males, there is decrease in testosterone levels (Adeyemo *et al*., 1990;

Adamu *et al*., 2006), while in the female, there is decrease in plasma progesterone levels (Faye*et al*., 2004) leading to decline or stoppage of cyclicity (Sekoni, 1994). However, Luckins *et al*. (1986) reported increase in blood progesterone. Trypanosomosis is also associated withincrease in cortisol secretion induced by corticotrophin-releasing hormone (CRH) secreted from the anterior pituitary (Mutayoba *et al*., 1995a). This suggests that the *Trypanosoma* infection modulates adrenocortical activity. The decrease was attributed to malfunctioning of the hypothalamic-pituitary axis caused by infection with pathogenic trypanosomes (Ikede *et al*., 1988).

2.4.8 **Diagnosis**

A range of diagnostic procedures are available for the diagnosis of trypanosomosis (OIE, 2013), although there are variations in their sensitivity and specificity. Microscopic examination of blood indicates presence of trypanosome thereby permitting confirmatory diagnosis (Chappuis *et al*., 2005).

2.4.8.1 *Wet blood film*

This is a simple method that gives immediate results. It is made by placing a drop of blood on a clean microscopic slide and covering with a cover-slip (OIE, 2013). The blood is then examined microscopically at X400 magnification with phase-contrast (Chappuis *et al*., 2005). The trypanosomes can be recognized by their movement (OIE, 2013).

2.4.8.2 *Thin blood smear*

Thin smear is made by placing a drop of blood at one end of a clean microscopic slide and another slide placed at an angle of approximately 30^0 to the other slide (OIE, 2013). The second slide is drawn back to make contact with the blood and allowed to run along its edge

and then spreadto theend of the first slide in a rapid but smooth motion. The slide is quickly air driedbefore fixing with methanol for 3–5 minutes then stained with Giemsa for 25-30 minutes. It is washed thrice indistilled water and once in buffer working solution. The slide is observed at X100 oil immersion objective.Thin smear method allows for morphological identification of the trypanosome (Chappuis *et al*., 2005).

2.4.8.3 *Thick blood smear*

It is made by placing a drop of blood at one end of a clean microscopic slide and spreading it over an area of thickness using the edge of another slide (OIE, 2013). The slide is dried by waving in the air or allowed to dry for 24 hrs. It is dehaemoglobinised by immersion in distilled water for up to 10 minutes before staining in Giemsa after which it is washed with distilled water again and examined at X100 oil immersion objective (Chappuis *et al*., 2005). This method is cheap but delays result due to the staining process (OIE, 2013).

2.4.8.4 *Haematocrit centrifugation technique*

This is a widely used method and known as the Woo method. It was first described in 1969 (Woo, 1969) but modified in 1970 (Woo, 1970). This method is done by collecting blood into heparinized capillary tubes which aresealed at one-end and placed in a microhaematocrit centrifuge and centrifuged at 9000 g for 5 minutes. It is then mounted on the microscope and examined under low power magnification (x100) for mobile parasites. This technique is more sensitive than the direct method (wet, thin and thick film).

2.4.8.5 *Buffy coat technique*

This technique is also called the Murray method (Murray *et al*., 1977). It is a modification of the Woo method. This involves cutting the capillary tube after centrifuging, below the buffy coat to include the top layer of RBCs using a diamond pencil. The buffy coat is extruded on a clean microscopic slide and examined for the motile trypanosome with a dark-ground or phase-contrast microscope at X 40 objective lens.

2.4.8.6 *Animal inoculation*

It involves intraperitoneal inoculation of rodents (mice or rats) with freshly collected blood. A drop of blood is collected daily from the tip of the Laboratory animal`s tail and examined by wet film for at least 30 days (OIE, 2013). This method is more sensitive than other methods described above for all trypanosomes, except *T. vivax*. However, it raises animal welfare concerns.

2.4.8.7 *Serological test*

Numerous antibody tests for trypanosomosis have been developed. These include indirect fluorescent antibody test (Katende *et al*., 1987); antibody-detection and enzyme-linked immunosorbent assay (Luckins, 1977; Desquesenes, 2004).Serological tests are expensive and require expertise experience on sophisticated equipment, which are not always available thereby limiting their use (OIE, 2013). Apart from this, field evaluations of these tests give conflicting results, raising questions about their sensitivity and specificity (IAEA, 1993). However, card agglutination test for trypanosomosis (CATT) and LATEX/*T. b. gambiense*have been developed for human trypanosomosis with some level of improvement (Chappuis *et al*., 2005).

2.4.8.8*Polymerase chain reaction*

Polymerase chain reaction (PCR) is abetter diagnostictool to ascertain the presence of trypanosomes(Desquesnes and Tresse, 1996; Miyamoto*et al*., 2006; El-Metanaway *et al*., 2009) in man, animals and tsetse (OIE, 2013). Though expensive and relatively new tocertain parts of Africa, PCR is so sensitive and specific (Kirchhoff *et al*., 1996; Delespaux *et al*., 2003) that parasitaemia as low as10 parasites per milliliter of blood can be detected using PCR(Desquesnes and Davila, 2002). Another advantage is the ability of this technique to be used for large scale surveys (Takeet*et al*., 2012).

2.4.9 **Treatment**

Anti-trypanosomal drugs have been the choice for the control of trypanosomosis in animals since the 1950s (Kinabo, 1993). Treatment of *Trypanosomabrucei*, *T. congolense* and *T. vivax* depends primarily on homidium, isometamidium and diminazene (Peregrine and Mamman, 1993). However, there are incidences of resistance (Kinabo, 1993; Geerts and Holmes, 1998; Ezeh *et al*., 2009) and toxicity due to counterfeit and parasite mutation,stressing the need for better control drugs (Nok, 2005).Arsenic compound such as melarsenoxide cysteamine is effective against *T. evansi* but not *T. simiae*(Kinabo, 1993). Effective treatment is achieved by combined therapy which reduces dosage but produces synergistic effect (Nok, 2005).Supplementation of these drugs with antioxidantsis alsoeffective (Eghianruwa, 2012).Recently, Udensi and Fagbenro-Beyioku (2012) reported increased survival period of mice infected with *T. brucei* using a single 300 µg/ml/kg dose of ivermectin. There are medicinal plants withstrong trypanocidal effect ontrypanosomes (Nok, 2005). This includes*Allium sativa* (Nok *et al*., 1996),*Azadiracta indica* (Nok, 2005) as well as Aloin and its derivatives (Tewabe*et al*., 2014).

2.4.10 **Immunology**

The immune response in *Trypanosoma* infection is first innate immunity and secondarily specific immunity (Vincendeau and Bouteille, 2006).Nonetheless, Variant Surface Glycoprotein (VSG) is the predominant surface antigen produced in trypanosomosis, and it covers the membrane of the trypanosomes blocking the efficiency of the specific host antibodies.The parasites achieve this by changing their surface coat antigens continuously (Vincendeau and Bouteille, 2006) forming an important barrier against the host's immune system.Certain breeds of sheep such as the Djallonke (West Africa Dwarf) sheep are generally known to be trypanotolerant surviving in tsetse infested areas (Bengaly *et al*., 1993), while others such as Yankasa breed are highly susceptible to trypanosomosis (Audu *et al*., 1999, Adenowo *et al*., 2005). Trypanotolerance is believed to result from a complex of innate, non-specific and specific immune mechanism mediated by physiological, biochemical and immunological factors (Vickerman and Barry, 1982)

2.4.11 **Prevention and control**

There are no vaccines presently available for the prevention of trypanosomosis (OIE, 2013). Control is dependent on the use of drugs which are few and have been greatly used, leading to increased drug resistance (Geerts and Holmes, 1998).Other factors such as antigenic variation among trypanosomes, and the absence of coherent environmental friendly and sustainable vector control strategies such as sterile insect techniques(ILRAD, 1990), pose a serious challenge to theprevention and control of the disease, leading to high prevalence in endemic areas.

2.4.12 **Effect of trypanosomosis on reproduction in the sheep**

Trypanosomosis causes varying degrees of disease in male and female sheep (Sekoni, 1994). Abortion, stillbirth and endocrine depression, leading to progesterone depression have been reported in *T. vivax* infected ewes (Elhassan *et al*., 1994; Elhassan *et al*., 1995; Bawa *et al*., 2005; Silva *et al*., 2013). These observations were also seen in *T.brucei* infected ewes (Edeghere *et al*., 1992). Adenowo *et al*. (2005) suggested that the *T. vivax* induced lesions may be a consequence of endocrine disorder. Furthermore, abnormal oestrouscycle characterized by silent oestrus or irregular oestrus as well as temporaryor complete anestrus hasbeen observed in ovine trypanosomosis (Sekoni, 1994). Infertility associated with trypanosomosis has also been reported in trypanotolerant West Africa dwarf sheep (Reynolds and Ekwuruke, 1988; Abubakar, 1997).

There is decreased testosterone levels in rams infected with*T. brucei* and*T. congolense*(Adeyemo *et al*., 1990). Testicular and epididymal pathology characterized by degeneration and necrosis leading to loss of epididymal sperm reserve have also been reported (Okubanjo *et al*., 2014b). Okubanjo *et al*. (2008) also reported decrease in α-frucolidase in the semen of rams infected with *T. congolense.* Alpha-frucolidase is an enzyme present in the acrosomal membrane of the spermatozoa required for penetration of the ova (Sivestava *et al*., 1986). Poor quality semen characterized by high percentage of abnormal spermatozoa have been reported in rams infected with*T. brucei* (Akpavie *et al*., 1987),*T. congolense*(Okubanjo *et al*., 2014a) and*T. vivax* (Akpavie *et al*., 1987; Sekoni, 1992; Sekoni, 1993).

2.5 **Pregnancy Associated Serum Factors**

Pregnancy associated serum factors include progesterone (P_4), estrone sulfate (E_1S), pregnancy associated glycoprotein (PAG) and placental lactogen (PL).

2.5.1 **Progesterone**

Progesterone (P_4) is a steroid hormone initially known as "CL factor" and responsible for pregnancy maintenance (Corner and Allen, 1929). However, following its isolation in 1934, the name progesterone was selected as the scientific name for the hormone (Allen, 1974). The main physiological source of P_4 is the corpus luteum and placenta. It is synthesized in the corpus luteum of the ovary through the conversion of pregnenolone to progesterone. In the placenta, it is synthesized mainly by hydroxylation of low-density lipoprotein fraction of cholesterol to pregnenolone, then to progesterone. The hormone is also found in the adrenal glands of many mammals (de Cremoux*et al*., 2008).

Physiologically, P_4 plays an important role in pregnancy maintenance, expression of oestrus, normal cyclic function and in hypophyseal-gonadal interrelationship (Conneely*et al*., 2002). Progesterone is essential for uterine development, implantation, blastocyst development, maintenance of foetus and nullification of uterine tone during pregnancy. It maintains the endometrium and is important for the continuance of pregnancy. It may have effect on uterine tubule and uterine transport of sperm and ova (Al-Asmakh, 2007). Progesterone promotes mucus formation in the vagina and participates in the development of alveolar system in the mammary gland (Clarkand Sutherland, 1990; Graham and Clarke, 1997). Wilmut *et al*. (1985) reported that a normal period of progesterone exposure during the luteal phase was necessary to achieve normal pregnancy rates. P_4blocksthe effect of oxytocin on myometrium (Siiteri and Seron-Ferre, 1981).

In the sheep, corpus luteum (CL) is necessary to maintain pregnancy until at least day 60 (Purohit *et al*., 2012). After this day, the CL and the placenta together secrete sufficient quantities of progesterone required for pregnancy maintenance. Lutectomy at mid pregnancy has no apparent effect on gestation length, lamb birth-weights or lamb mortality (Al-Gubory *et al*., 1999). In pregnant ewes, the concentration of P_4 will increase steadily from the first week of breeding to the 7^{th} to 9^{th} week although with slight fluctuations (Tsang, 1978). Subsequently, it increases gradually then sharply reaching a peak at week 17 andlater decreasing reaching the lowest level few days to parturition (Alwan *et al*., 2010).

Concentration of progesterone is determined using enzyme immunoassay (EIA) and radioimmunoassay (RIA) (Karen *et al*., 2003). Both assays have high levels of accuracy in differentiating between pregnant from non-pregnant animals (Susmel and Piasentier 1992; Gvozdic and Ivkov, 1994). However, early embryonic death, uterine and ovarian pathology may be a source of false positive cases (Karen *et al*., 2003).

2.5.2 **Pregnancy associated glycoprotein**

Glycoproteins are proteins that contain oligosaccharide chains (glycans), covalently attached to polypeptide side-chains, that is, they are molecules that consist of a carbohydrate plus a protein(Murray *et al*., 2003). Pregnancy associated glycoprotein (PAG) constitute a large family of placental glycoproteins which belong to a group of proteolytic enzymes known as aspartic proteinases (Xie, *et al*., 1994). They were first discovered in ruminants although they are also expressed in other mammalian species (Sousa *et al*., 2008). PAG are synthesized in the superficial layer of the developing trophoblast and then released into maternal circulation where their concentrations can be detected and used to investigate placental function in ongoing pregnancy and in obstetric diseases(Sasser *et al*., 1986; Zoli *et al*., 1992; Breukelman

et al., 2005). These glycoproteins reflect the trophoplastic secretory function of the developing conceptus (Sousa *et al*., 1999).

Ovine PAG is known as ovine PAG (ovPAG) (Sousa, *et al*., 2006), ovine Pregnancy Specific Protein-B (oPSPB) (Willard *et al*., 1995) and SBU-3 antigen (Atkinson *et al*., 1993). PAGs are found in peripheral circulation of pregnant ewes and are immunologically related to bovine PAG (Ruder *et al*., 1988) although they are quite different from each other (Zoli *et al*., 1992). Recent studies have shown that by day 18 of gestation, PAG concentrations are detectable in pregnant ewes while they are low or undetectable in non-pregnant ewes (El-Amiri *et al*., 2003). In the pregnant ewes, PAG concentrations increase faster from 3rd to 4th week reaching highest level during the first month of pregnancy. Thereafter concentrations decrease until day 90 and increase again to remain elevated until parturition (Ranilla *et al*., 1994). Subsequently, no significant increase is observedtill parturition (Sousa, *et al*., 2006) like in the cow. During postpartum period, the rate of decline in PAG concentrations of the sheep is faster (28 days) than in cows (about 100 days) (Sousa, *et al*., 2008).

Concentrations of PAG are determined by using the RIA (Anghel *et al*., 2011) and ELISA test. The RIA measurements of PAG have an accuracy of 71% and 81% for detecting ewes carrying single and twin lambs between days 60 to 120 of gestation, respectively, (Willard *et al*. 1995). On the other hand, the ELISA test has an accuracy of 99 % of detecting pregnancies beyond day 30 of gestation (Redden and Passavant, 2013).

2.5.3 **Estrone sulfate**

Estrone (E_1) is one of the three common forms of oestrogen and the major estrone in foetal and maternal peripheral plasma (Anon, 2014). Others are estradiol (E_2), the hormone produced by the ovaries and estriol (E_3), a metabolic waste product from estradiol metabolism. Estrone sulfate (E_1S) is a conjugated product of estrone, produced by the fetoplacental unit (Worsfold*et al*., 1986) leading to increased plasma peripheral levels (Karen *et al*., 2001). E_1S is a steroid hormone synthesized in the foetal or cotyledonary portion of the placetomes(Hoffmann *et al*., 1979). E_1S ismore important in pigs where it can be detected at day 17 of gestation (Gaustad-Aas *et al*., 2002).

In the ewe, E_1S levels are first detected between days 30 to 35 of gestation (Illera *et al*., 2000). The concentration rises to the first peak at day 46 after conception but drops by day 55 (Carnegie and Robertson, 1978). These researchers also reported a gradual rise that peaksbetween days 100 and 120, although not in all ewes sampled.At about day 85 of gestation, significant difference in the levels of E_1S is observed in pregnant ewes compared to non-pregnant ewes (Karen *et al*., 2003). Following the second peak, there is a gradual decrease between day 130 and 140 but an upsurge is observed 2 to 3 days before parturition,(Tsang, 1978).Compared to E_1S, the plasma levels of other oestrogens are low and only become detected during the last days of pregnancy (Carnegie and Robertson, 1978; Tsang, 1978).Although the association between maternal concentration of E_1S and number of foetus has been established (Illera *et al*., 2000; Gaustad-Aas *et al*., 2002),it is not trustworthy because of the individual variations between foetuses.

The concentration of Estrone sulfate in pregnant ewes is detected by enzyme immune assay (EIA) (Karen *et al*., 2003) with an optimal accuracy for pregnancy detection between days 30 to 35 of gestation (Illera *et al*., 2000). However, due to considerable variation of the hormone levels between individual animal, the accuracy for detection in pregnant ewes was only 87.9 % while for detection of non-pregnant ewes was 44 % using a cut-off value of 0.1 ng/ml (Worsfold*et al*. 1986).

2.5.4 **Placentallactogen**

Placental lactogen (PL) is a hormoneproduced by the binucleate cells of the trophectoderm(Duello *et al*., 1986; Myers and Reimers, 1988; Byatt *et al*., 1992)and released into both the maternal and foetal circulations (Gootwine, 2004). Following secretion, it isstored in the secretory granules within thebinucleate cells before being released(Duello *et al*., 1986). PL was first reported in goats by Buttle *et al*.(1972). Shortly after,Kelly *et al*.(1974) discovered its existence in sheep. However, caprine and ovine PL arestructurally similar (Gootwine, 2004).

Ovine PL (oPL) is detectable in trophoblastic tissue at approximately day 16 in the ewe (Johnson *et al*., 1999; Stewart *et al*., 2000) and continues to be synthesized throughout pregnancy (Gootwine, 2004).The level of PLstarts to increase from day 70 to 90 of gestation and peaks during the last third of pregnancy (Butler *et al*., 1981; Bosc *et al*., 1982), between days 120 and 140 before declining (Gluckman *et al*., 1979; Kappes *et al*., 1992).Concentrationsof oPL in foetal plasma reaches maximum level by day 90 of pregnancy andare relatively low in the range of tens of nanograms per milliliter (Kappes *et al*., 1992).There is evidence of slight transfer of PL from the maternal circulation to the foetal compartment (Gootwine, 2004), the mechanism through which this occurs is presently

unknown. However, PL in foetal lamb circulation at midgestation decreasesby half, 1 to 2 weeks before parturition (Gluckman *et al*., 1979). The level ofPL during the last third of pregnancy is associated with the number offoetuses and placental mass (Hayden *et al*., 1980; Butler *et al*., 1981).

It has been suggested thatoPLplaysaroleinregulatingfoetalgrowth (Bauer*et al*. (1995), although the mechanism behind this remains unknown. However, resultsfrom earlier studiesinwhichoPLlevelswerealteredbyfeedingrestrictions affirms this (Butler*et al*.,1987). Further studies later showed that it stimulates voluntaryfood intakeinewes,which could affectfoetalgrowth and development(Min*et al*., 1996).Concentrations of oPL are determined using radioimmunoassay (RIA) with diagnostic accuracy of 97% and 100% in pregnant and non-pregnant ewes at day 64 of gestation, respectively (Robertson *et al*. 1980).

2.5.5 **Cortisol**

Cortisol is a catabolic hormone produced by the adrenal cortex in response to physical and psychological stress (Brownlee *et al*., 2005). Its primary functions are to increase blood sugar through gluconeogenesis, suppress the immune system, and aid the metabolism of fat, protein, and carbohydrate (Hoehn and Marieb, 2010). It has also been associated with decrease in bone formation (Chyun *et al*., 1984). The hormone is released following the secretion of corticotrophin-releasing hormone (CRH) from the hypothalamus, which stimulates the release of adrenocorticotropic hormone (ACTH) by the anterior pituitary gland, and subsequently releases cortisol from the adrenal cortex (Kivlighana *et al*., 2008). CRH has been identified in other areas of the brain with high quantities localizing in the central nucleus of the amygdala and the bed nucleus of the *Stria terminalis* (Schulkin, 1999; Avishai-Eliner *et al*., 2002).

In pregnancy, there is a gradual increase in cortisol concentration as pregnancy advances and this doubles towards the end of gestation (Bell *et al*. 1991). This increase has been attributed to maturation of the foetal adrenal response to corticotrophic stimulation (Carr *et al*., 1981; Challis *et al*., 1984). In addition, it has been reported that peripheral CRH of placental origin rises dramatically over non-pregnant levels, this corresponds with elevation in both ACTH and total cortisol levels (Mastorakos and Ilias, 2003; Levine *et al*., 2007).

The actual time of gestation when cortisol levels increase in pregnant ewes is unknown, probably because no study has been designed in this regard. However, Mescher *et al*. (1975) reported that cortisol levels in foetal lambs begin to increase from about day 130 to 135 of gestation, although later studies suggest that, majority of foetal cortisol is of maternal origin during the first 122 days of gestation (Hennessy *et al*., 1982). Magyar *et al*. (1980) showed that the time foetal cortisol level increases in sheep varies, but averages at about 12 days before parturition. This is triggered by the production of cortisol by the foetal adrenal.They further showed that this leads to a cortisol surge that triggers removal of the progesterone block on cervical dilation and myometrial contraction, thereby initiating parturition. More recently, El-Sherif and Assad (2001) demonstrated that plasma urea levels increase at about day 70 of pregnancy in ewes, reaching a peak at parturition. This in domestic ruminants is ascribed to the cortisol-stimulated catabolism of proteins in the body (Silanikove, 2000).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Location

The study was carried out from July, 2013 to February, 2014, at the experimental animal house, Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. Zaria is situated in the Northern Guinea Savannah of Nigeria, between 11^0 and 12^0 N and between 7^0 and 8^0 E at an elevation of 650m above sea level.

3.2 Experimental Animals

Fifty post pubertal Yankasa ewes between 18-30 months old were purchased from local markets around Zaria for the study.Their age was estimated using the pattern of eruption of their dentition as described by Wosu (2002), while breeding history was obtained from the sellers where possible.The animals werekept in fly-proof pensand acclimatized for six weeks. During this period,they were screened for haemoparasites and helminths by collectingblood and faecal samples, respectively,for examination. Albendazole was used for dewormingwhile20% oxyteracycline was administered to treat nasal discharge. The animals were fed twice a day with hay and concentrate supplement while salt lick and water were given*ad libitum*.Ethical clearance was sought for and approval was obtained from the Ahmadu Bello University Committee on Animal use and care (ABUCAUC/02/2013/002).

3.3 Source of*Trypanosoma evansi*Parasite

The *T. evansi* parasite used in this study was obtained from the Protozoology Laboratory, Department of Parasitology and Entomology, Faculty of Veterinary Medicine, ABU-Zaria. It was initially isolated from a camel slaughtered at the Sokoto metropolitan abattoir and maintained in mice and rats until use.

3.4 Parasitological Characterization of *T. evansi*

Half mililitre of blood containing about 1 x10^6 of *T. evansi* was diluted in normal saline and inoculated subcutaneously into rodents (3 mice and 2 Albino rats). Wet mount was made daily from the rodents till parasitaemia was established. Prepatent periods of 3-4 days and 7-8 days were observed in the mice and rats, respectively. Thin blood smear was made at peak parasitaemia. However, the mice died 37-40 days post-infection (pi) while the rats died 42-47 pi.

3.5 Study Design

Fifty Yankasa ewes were synchronized by two intramuscular administration of Prostaglandin $F_2\alpha$ (estromate®) 11 days apart. Twenty-four ewes whichlater came tooestrus were bred with Yankasa rams showing good reproductive capacity, at the ratio of 1 ram to 8 ewes. The reproductive capacity of the rams was determined by evaluating their semen which gave good semen characteristics. Twenty-one days after breeding, the 24 ewes were confirmed pregnant using ELISA technique for determining progesterone (P_4) levels (Oyedipe *et al*., 1986) and absence of oestrus 17-18 days after breeding. However, 4 of these ewes had P_4values similar to those observed during the "standing heat" period 45 days post-breeding and were excluded from the study. The remaining 20 were assigned into 3 groups (A, B and C) based on their weights, consisting of 7 ewes per group except group C which had 6 ewes.

Each ewe in groups A and B were each inoculated with 0.5 ml of blood containing approximately 1.0 x10^6 of *Trypanosomaevansi*(*T. evansi*) through the jugular vein on days59 and 110 of pregnancy, respectively while group C served as the uninfected control. Clinical signs of trypanosomosis were monitored throughout the period of the study. However, parasitaemia was determined daily until patency established. Subsequently, itwasdetermined

twice a week until the termination of the study. Rectal temperature was also taken twice a week while PCV, body weight,glucose and plasma protein were measured once a week till the end of the study. Serum was obtained weekly for progesterone, PAG, estrone sulfate and cortisol assay throughout the study.These parameters were also determined for 3 weeks before infection to establish a pre-infection baseline data. One animal from each group was sacrificed10 days after parturition for gross and histological studies.

3.6 Monitoring of Clinical Signs

Following infection, all the ewes infected with *T. evansi* were closely monitored for clinical signs of trypanosomosis such as pyrexia, palor of the visible mucous membranes, anorexia, depression, lacrimation, nasal discharge, enlargement of lymph nodes.

3.7 Determination of Rectal Temperature

Temperature was taken twice weekly between 7.30 am and 8.30 am by inserting a thermometer into the rectum of the ewes for a minimum of one minute then withdrawing it to take the reading.

3.8 Determination of Body Weight

This was measured in the pen using a bathroom scale (Camry ®,made in China). It involved first, taking the weight of an individual who would then carry the ewe and stand on the scale and the total weight then recorded. The weight of the animals was obtained by deducting the weight of the individual from the weight of individual when carrying ewe as below:

Weight of individual whencarrying ewe- Weight of individual = Weight of ewe.

The lamb birth-weights were determined using a digital scale (Beurer GmbH ®, made in Germany). This involved placing a plastic bowl on the scale and setting the reading to

zerothen placingthe lamb on the bowl until it stabilizes andthen the reading was taken. Thepercentage lamb birth-weights were calculated as a percentage of their dam`s weight at parturition.

3.9 Determination of Gestation Length

This was determined by noting the days ofbreeding (standing oestrus)and parturition. The gestation lengthwas calculated as the difference between the day of breeding and parturition.

3.10Blood Collection

Seven millilitres of blood was collected from the jugular vein of the each ewe. Out of this, 2 mls was dispensed into sample bottle containingethylenediaminetetra-acetic acid (EDTA) as anticoagulant. The remaining 5 mls was dispensed into another sample bottle without anticoagulant.

3.11 Estimation of Parasitaemia

Parasitaemia wasdeterminedusing wet mount and haematocrit centrifugation techniques for the detection of trypanosome as described by Woo (1969) and Murray *et al*. (1977).

3.12Determination of Blood Glucose

This was determined using a glucometer (ACUU-CHEK®, made in Germany). A drop of freshly collected blood from the animal was placed on a test strip. The strip absorbedthe blood, determined the glucose (mg/dL) level and displayed the result which was then recorded.

3.13 Haematological Examination

The 2 mls of blood was used to estimate parasitaemia and determine glucose, packed cell volume, total plasma protein and total leukocyte count.

3.13.1 Determination of packed cell volume

This was determined using the standard microcapillary method as described by Coles(1986). Briefly, blood was drawn into heparinized microcapillary tubes up to two-thirds of their capacity. The tube wassealed at one end using plasticine and centrifuged at 9,000 g for 3minutes in a microhaematocrit centrifuge (Hawksley, England). The PCV was then read using microhaematocrit reader.

3.13.2 Determination oftotal leukocyte count

The total leukocytecounts weredetermined using the methods described by Schalm *et al*. (2010).

3.13.3 Determination of total plasma protein

This was determined by placing a drop of plasma from the spun microcapillary tube, after determining PCV, on the Goldberg hand refractometer and takingthe reading as described by Duncan *et al*. (1994).

3.14Assay for Pregnancy Associated Serum Factors

The 5 mls collected of blood collected was centrifuged at 9,000 g for 5 mins. Serum was then harvested to use for the assays.

3.14.1 Serum progesterone

Serum progesterone P_4 was determined using ACCU-BIND ® ELISA kits. The procedure was performed by following the manufacturer`s instruction. Twenty-five µL of test references and test samples were pipetted into antibody coated micro-plate. Fifty microliters of P_4 enzyme reagent was added to all the wells and swirled gently for 10-20 seconds to mix. P_4 biotin reagent (50 µL) was added to all the wells, swirled again andcovered with paraffin

paperandincubated for 60 mins. After, incubation, the contents of the microplate were decanted and dried with absorbent paper. Wash buffer (350 µL) was added, decanted and dried with absorbent paper,this was done 3 times repeatedly. One hundred microliters of substrate solution was added to all wells and incubated for 20 min at room temperature, after whichthe reaction was stopped by adding 50 µL of stop solution. The absorbance in each well was read within 30 mins of stopping the reaction using a multichannel ELISA plate reader at 450 mm. Results were calculated by plotting a graph ofthe optical density of test reference with its correspondingP$_4$ concentration and locating the P$_4$concentration for the test samples using its optical density. The assay has a sensitivity of 0.105 ng/ml and specificity of 100 %, with acorrelation coefficient is 98.9 %.

3.14.2 Serum pregnancy associated glycoprotein

Pregnancy associated glycoprotein was assayed using BIOPRYN ® sheep pregnancy ELISA kits, by following the manufacturer`s instruction. Briefly, 150 µL of pregnancy specific protein-B (PSPB) standards, control and sample serum were pipetted into PSPB antibodycoated micro-plates after appropriate dilution. The plates were incubated over night at room temperature. After incubation, the wells were washed with 300 µL of wash solution 4 times,decanting and blotting in each case. Two hundred microliters of detector solution was added to the wells, incubated for 1 hour and washed 2 times, decanted and blotted in each case. In the same way, 200 µL of enhancer solution was added and incubated for 1 hour but washed 4 times, decanting and blotting in each case. TMB (200 µL) was added and incubated for 15 mins. The reaction was stopped by adding 100µL of stop solution and absorbance read within 30 mins using a multi-channel ELISA plate reader at a wavelength of 450 mm. The values obtained were by inserting on a computer package (Excel) pre-programed bythe

manufacturers, from where actual results were obtained. The kits had a diagnostic sensitivity of 99.9 % and specificity range of 90 - 99 %.

3.14.3 Serum estrone sulfate

ABNOVA ® Sheep estrone sulfate ELISA kits were used to assay for E_1S following the manufacturer`s instruction. Fifty µL of reference standard and samples were pipetted into E_1S-coated micro titer plates. One hundred microliters of E_1S enzyme conjugate solution was added to all the wells except blanks and incubated at 37^{o}C for 2 hours. The contents of the wells were discarded and the plates washed 5 times with 300 µL per well of wash solution. One hundred microliters of TMB colour reagent was added into each well (including blanks) and incubated for 20 minutes at room temperature. The reaction was stopped by adding 50 µL of stop solution and gently mixed. The absorbance was read at 450 nm with an ELISAmicro well reader within 10 mins of stopping the reaction. The test kit had a sensitivity of 0.5 ng/ml and specificity of 99.6 %.

3.14.4 Serum cortisol

Cortisol levels were estimated using ABNOVA ® Sheep cortisol ELISA kits. The manufacturer`s instructions were followed in performing the test. Forty µL of cortisol standard and sera samples were pipetted into micro wells coated with cortisol mAb. Two hundred microliters of cortisol enzyme conjugate was added to all the wells and incubated for 60 mins at room temperature while shaking in the process. The contents of the wells were discarded after incubation and 300 µL of wash buffer was used to wash 3 times and blotting on absorbent paper towel each time. One hundred microliters of TMB substrate was added to each well and incubated for 15 mins at room temperature with shaking. Thereafter, 50 µL of

stop solution was added, gently shaked to mix and read within 20 mins on the ELISA reader at 450 mn. The ELISA kits had a sensitivity of 0.1 ng/ml and specificity of 100 %.

3.15 Gross and Histological Examinations

One ewe from each group was sacrificed10 days postpartum. Sections from the hypothalamus, thyroid gland, pituitary gland,ovary, oviduct, uterus and placenta were taken, fixed inBouin`s solution while the liver, lungs, heart, kidney and spleen were fixed in 10% buffered formalin for histological studies.

3.16 Data Analyses

Data obtainedwere analyzed statistically usingunpaired Student *t*-test (GraphPad, 2000). Data from pregnancy associated serum factors were further subjected to Pearson correlation. However, datagenerated from the gestation length and percentage lamb birth-weights were analyzed using one way ANOVA. Values of $p<0.05$ were considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Prepatent Period

The pre-patent period (PP) for infected ewes is presented in table 4.1. PP for ewes infectedat the second trimester of pregnancy(group A)ranged from 11-25 days with a mean of 21.71 day, while those infected at third trimester of pregnancy (group B)ranged from 13-22 days with a mean of 20.14 days.There was no significant ($p>0.05$) difference in the PP between ewes infected at the second and third trimesters. In group C, no parasite was seen throughout the study.

4.2 Clinical Observation

Many of the clinical signs suggestive of trypanosomosis were absent in all the groups throughout the study. However, slight pyrexia was observed in *T. evansi* infected groups (group A and B), compared with the control (group C).

4.3 Parasitaemia

The mean parasitaemia in group A is presented in figure 4.1.Parasitaemia was first detected on day 11pi with a mean of 0.40 x 10^6/ml. It peaked at day 25 to 2.79 x 10^6/ml, dropped to 0.40 x 10^6/ml on day 39 pi. It rose again to 1.60 x 10^6/ml on day 53 pi. It then dropped to 0.80 x 10^6/ml on day 67 pi before rising to 2.00 x 10^6/ml on day 81 pi and dropping to zero at day 89.

In group B (figure 4.2), parasitaemia was first detected on day 13 pi with a mean of 0.57 x 10^6/ml. All animals in this group were aparasitaemic on day 15 pi but a mean parasitaemia level of 1.14 x 10^6/ml was recorded on day16 pi, which dropped to 0.57 x 10^6/ml on day 17pi.

Parasitemia in this group peaked to 2.84 x 10^6/ml on day 22 pi. It then dropped to 1.13 x 10^6/ml on day 23 pi, and was zero subsequently, up till the end of the study.

Mice inoculated with blood from infected ewes in group B were parasitaemic within a pre-patent period of 18-25 days,and no mouse was parasitaemic following inoculation with blood from infected ewes in group A. Noneof the lambs bornby bothof the infectedgroups were parasitaemic when monitored upto 40 days postpartum. Neither ewes nor lambs in the control (group C) were parasitaemic throughout the study.

Table 4.1: Prepatent periods for *T. evansi* infected pregnant Yankasa ewes

Animal	Group A (n=7)	Group B (n=7)
1	25	22
2	25	20
3	20	13
4	25	22
5	25	22
6	11	22
7	21	20
Mean ± SEM	21.71 ± 1.96 days	20.14 ± 1.24 days
*Mouse	Aparasitaemic	Parasitaemic

Group A – infected at the second trimester of pregnancy
Group B– infected at the third trimester of pregnancy
*Mouse inoculation postpartum

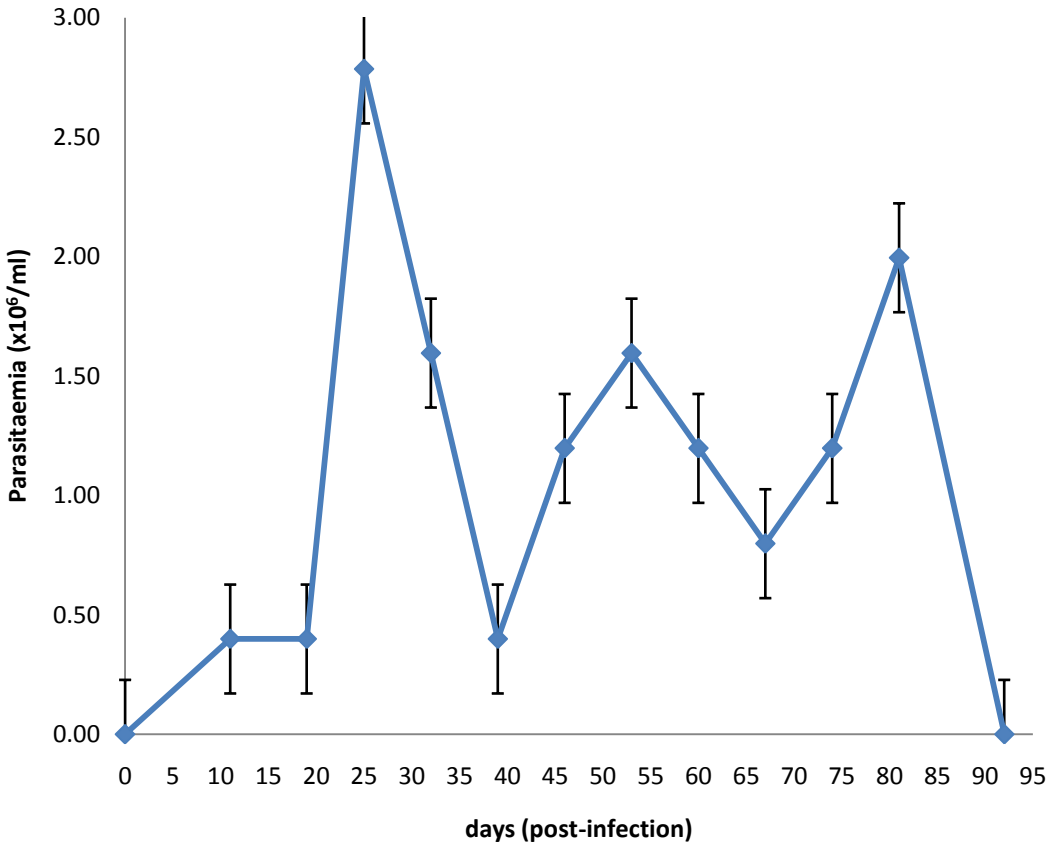

Figure 4.1: Mean ± SEM parasitaemia of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy

Day 0 = Day of infection (Day 59 of pregnancy)

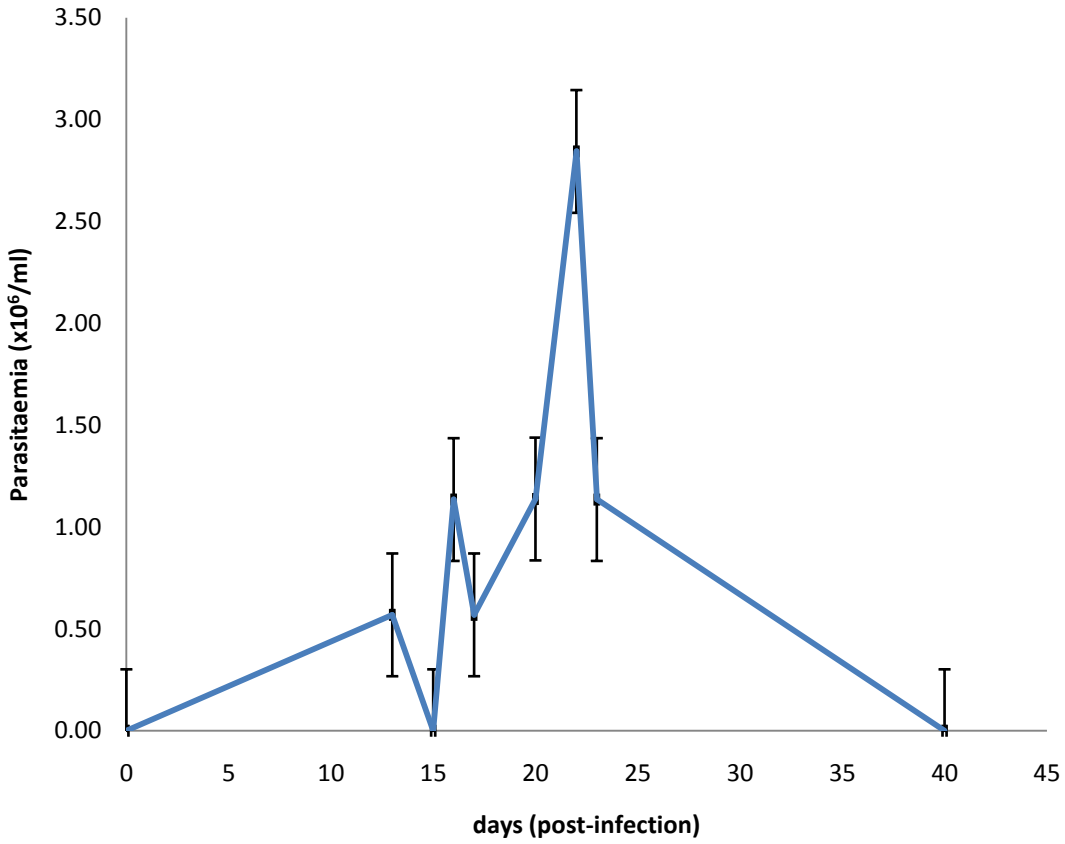

Figure 4.2: Mean ± SEM parasitaemia of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy

Day 0 = Day of infection (Day 110 of pregnancy)

4.4 Mean Rectal Temperature

The mean rectal temperature in pregnant Yankasa ewes infected at the second trimester of pregnancy is presented in figure 4.3. There was no significant ($p>0.05$) difference between infected and control groups at the onset of infection. However, significant ($p<0.05$)increase occurred at weeks 4, 6 and 12 pi with a significant ($p < 0.05$) decrease at week 11.In the group infected at the third trimester of pregnancy (figure 4.4), there was significant ($p<0.05$)increase between infected and control groups one week after infection. Subsequently, there was no significant ($p>0.05$) difference between them except at week 4pi (figure 4.4).

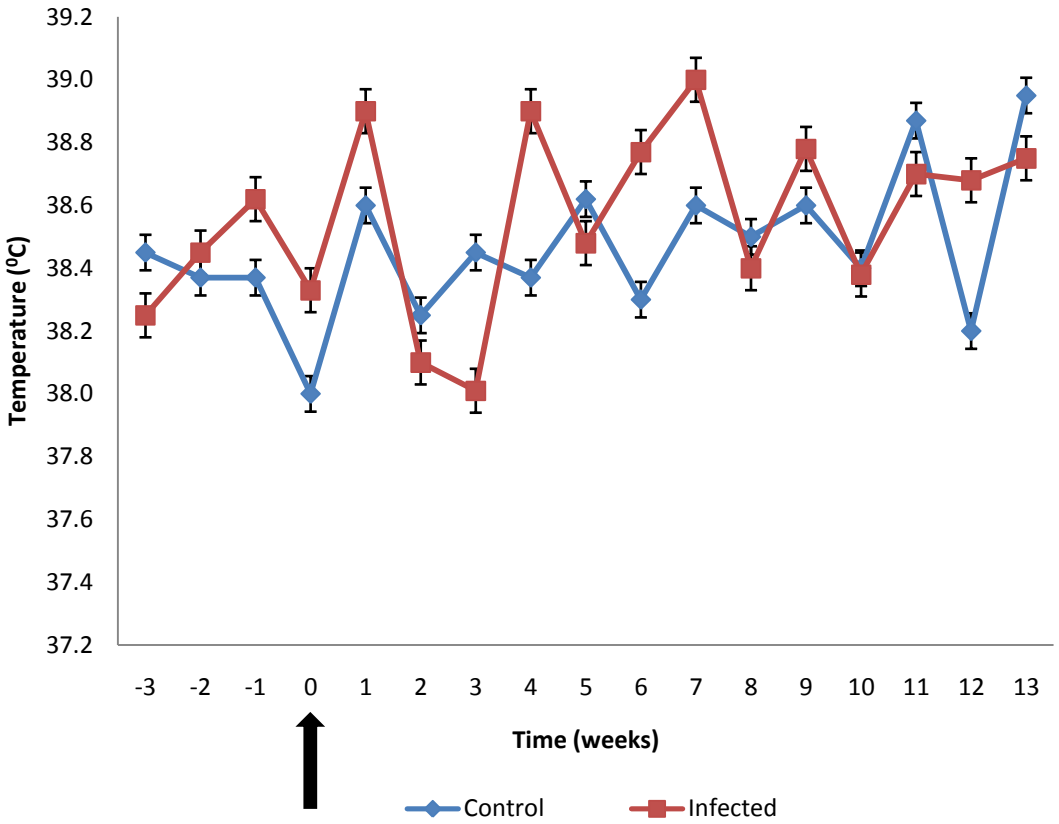

Figure 4.3: Mean ± SEMrectal temperature changes in pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

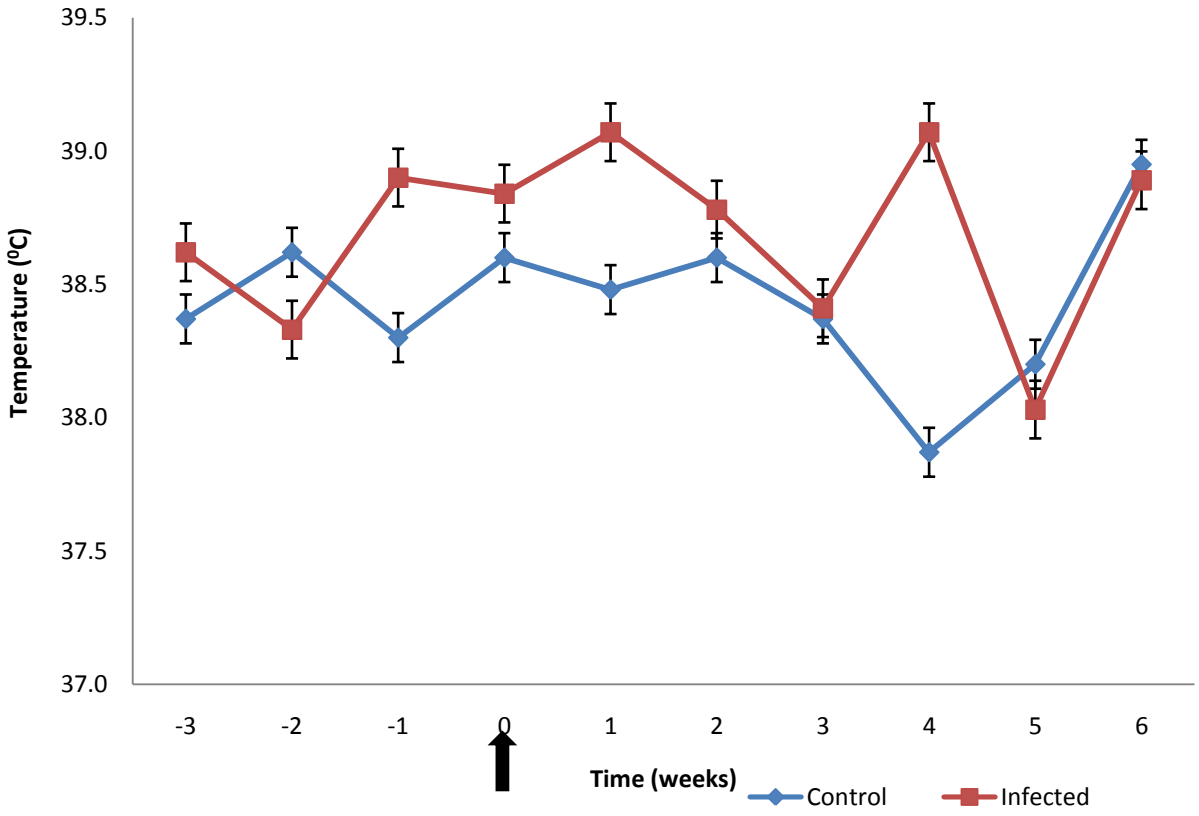

Figure 4.4: Mean ± SEMrectal temperature changes in pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.5 Mean Body Weight

The mean weekly body weight of Yankasa ewes infected at the second trimester of pregnancy compared with the control is presented in figure 4.5. There was no significant ($p>0.05$) difference between the mean body weight of the infectedand control groups throughout the study period. However, in the ewes infected at the third trimester of pregnancy, there was significant ($p<0.05$) decrease in the mean body weight of the infected groupcompared to the controlfrom week 2pi till the end of the study. However, 3 weekspi, there was no significant ($p>0.05$) difference between the infected and control groups (figure 4.6).

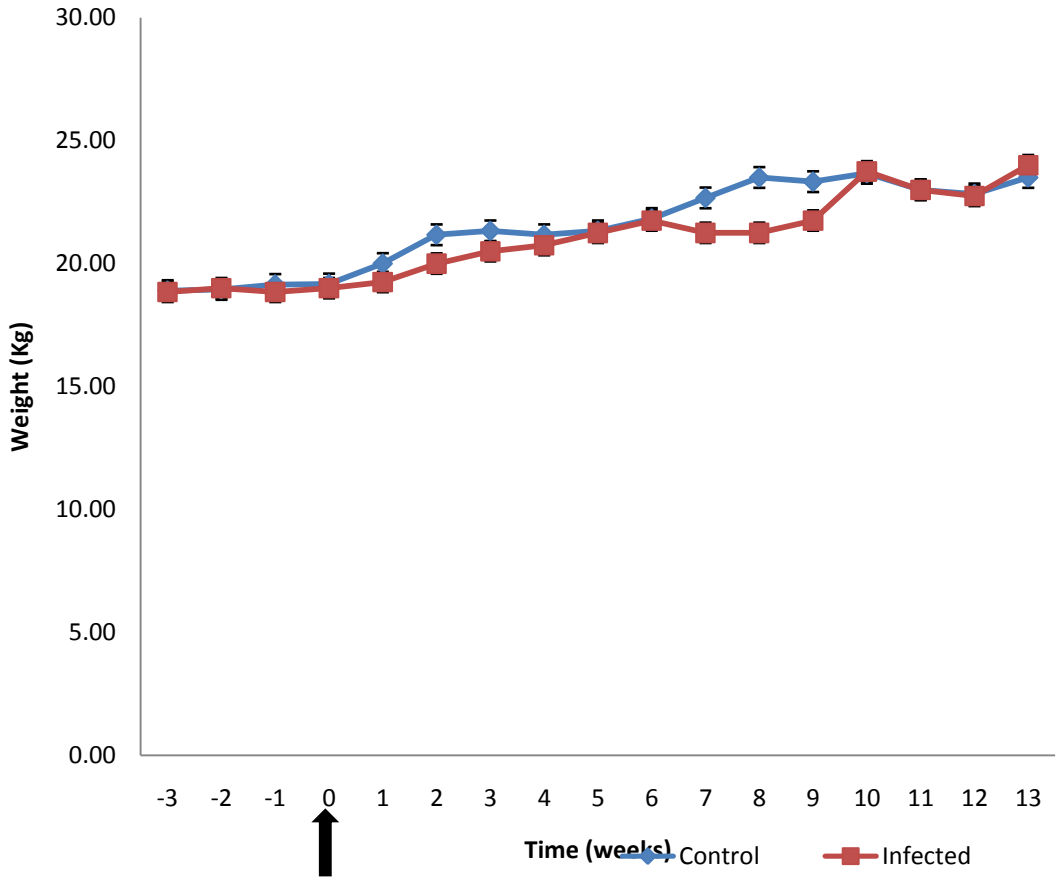

Figure 4.5:Mean ± SEM weight changes in pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

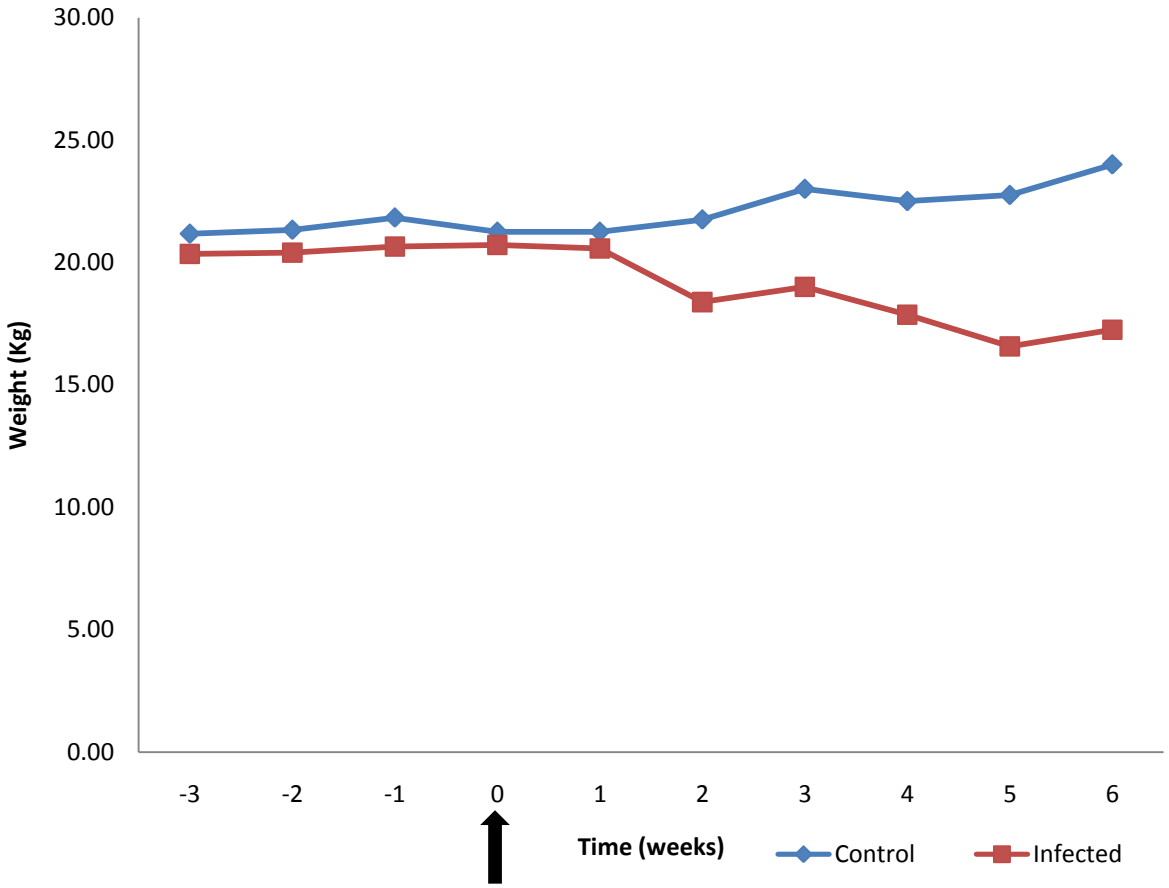

Figure 4.6: Mean ± SEM weight changes in pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.6 Mean Blood Glucose

The mean blood glucose of ewes infected at the second trimester of pregnancy is presentedin figure 4.7. There was no significant ($p>0.05$) difference between the mean blood glucose of infected and control groups from the time of infection till the end of the study. There was also no significant ($p>0.05$) difference between the mean blood glucose values ewes infected at the third trimester of pregnancy and the control ewes(figure 4.8).

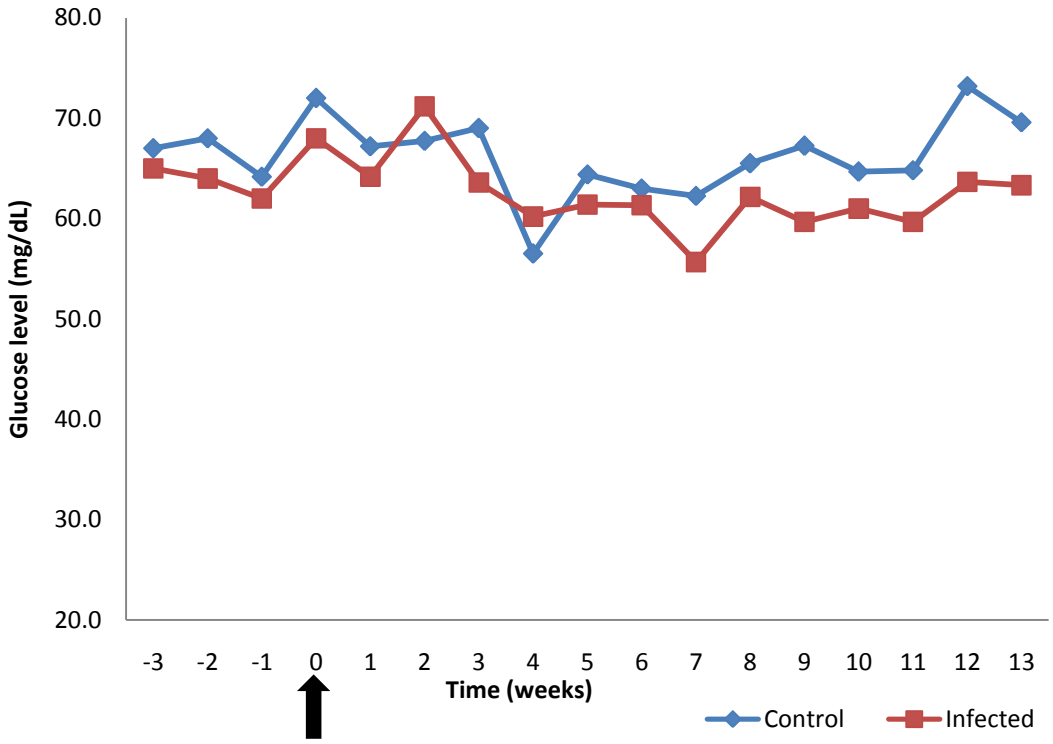

Figure 4.7: Mean ± SEM blood glucose level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

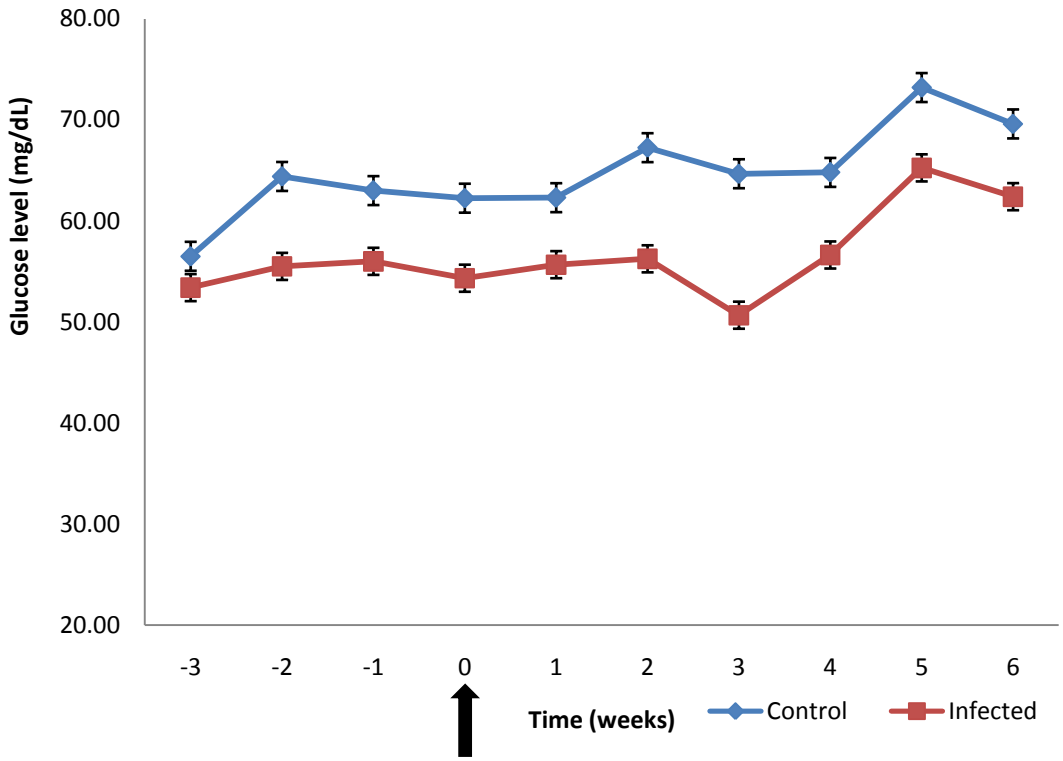

Figure 4.8: Mean ± SEM blood glucose level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.7 Mean Packed Cell Volume

The mean packed cell volume (PCV) of ewes infected at the second trimester of pregnancy is presented on figure 4.9. Although there was decrease in PCV of the infected group beginning from week 6 pi, the mean PCVwas not significantly($p>0.05$) differentfromthat of the control group throughout the study.

In the group infected at the third trimester of pregnancy (figure 4.10), the PCV of the infected and control group was not significantly ($p>0.05$) different until week 4 and 6pi when they differed significantly ($p<0.05$).

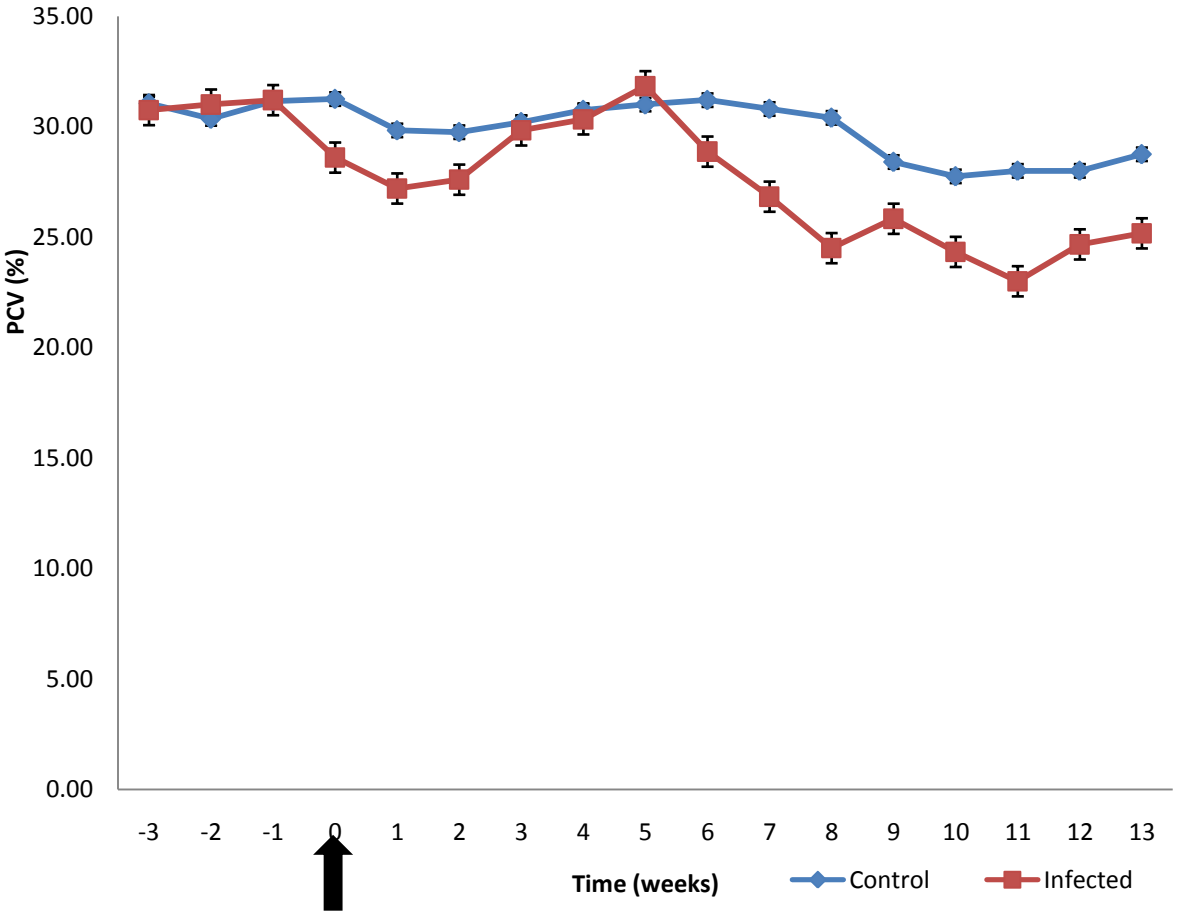

Figure 4.9: Mean ± SEMpacked cell volume (PCV) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

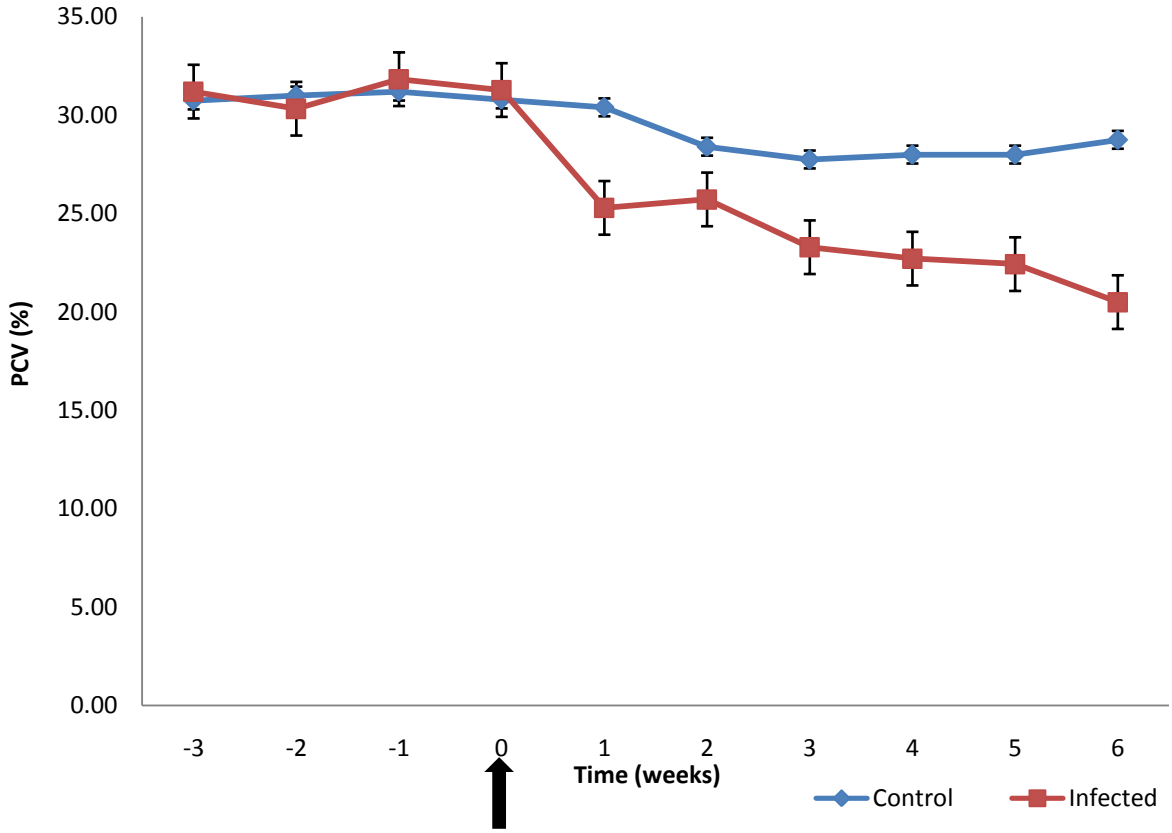

Figure 4.10: Mean ± SEMpacked cell volume (PCV) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.8 Total Leukocyte Count

The mean leukocyte count of ewes infected at the second trimester of pregnancy is presented in figure 4.11. There was leukopaeniaat week 1 pi. This was followed by leukocytosis at 4 weekpiand a leukopaenia at week 7. In the group infected at the third trimester of pregnancy (figure 4.12), there was leukopaenia at week 3 pi.

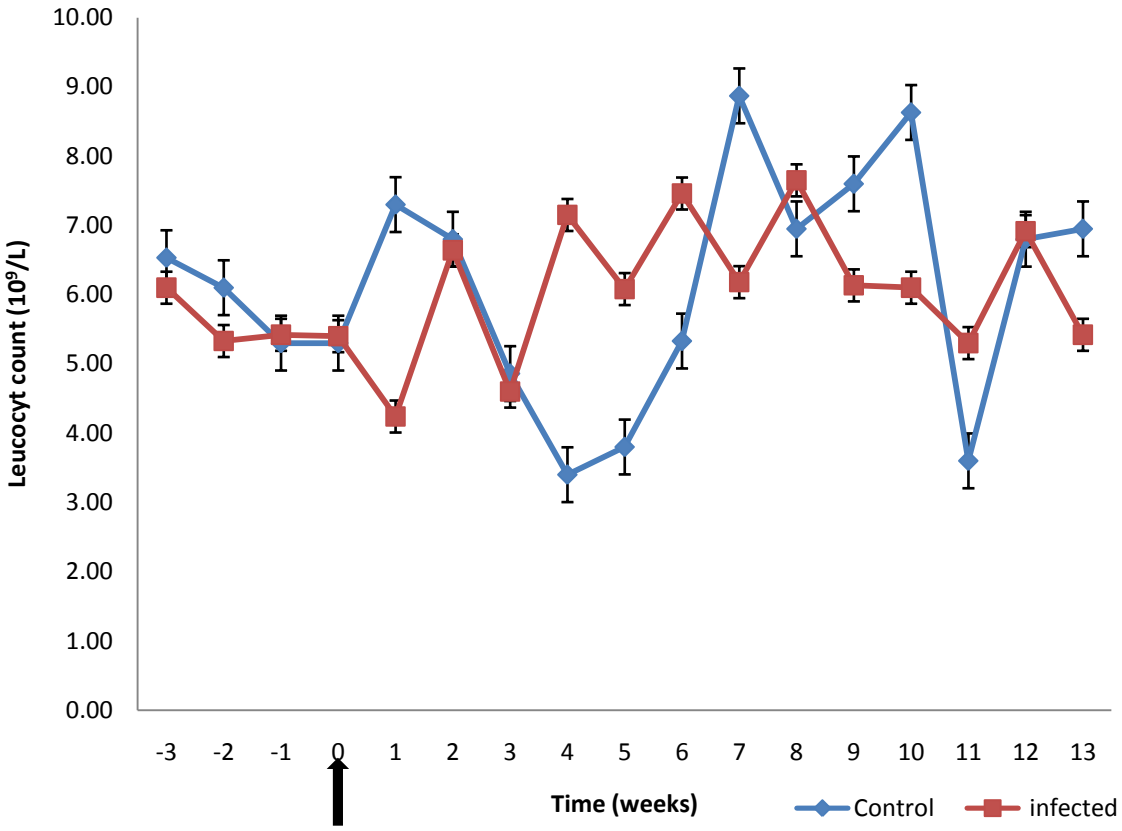

Figure 4.11: Mean ± SEMleukocytecount of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

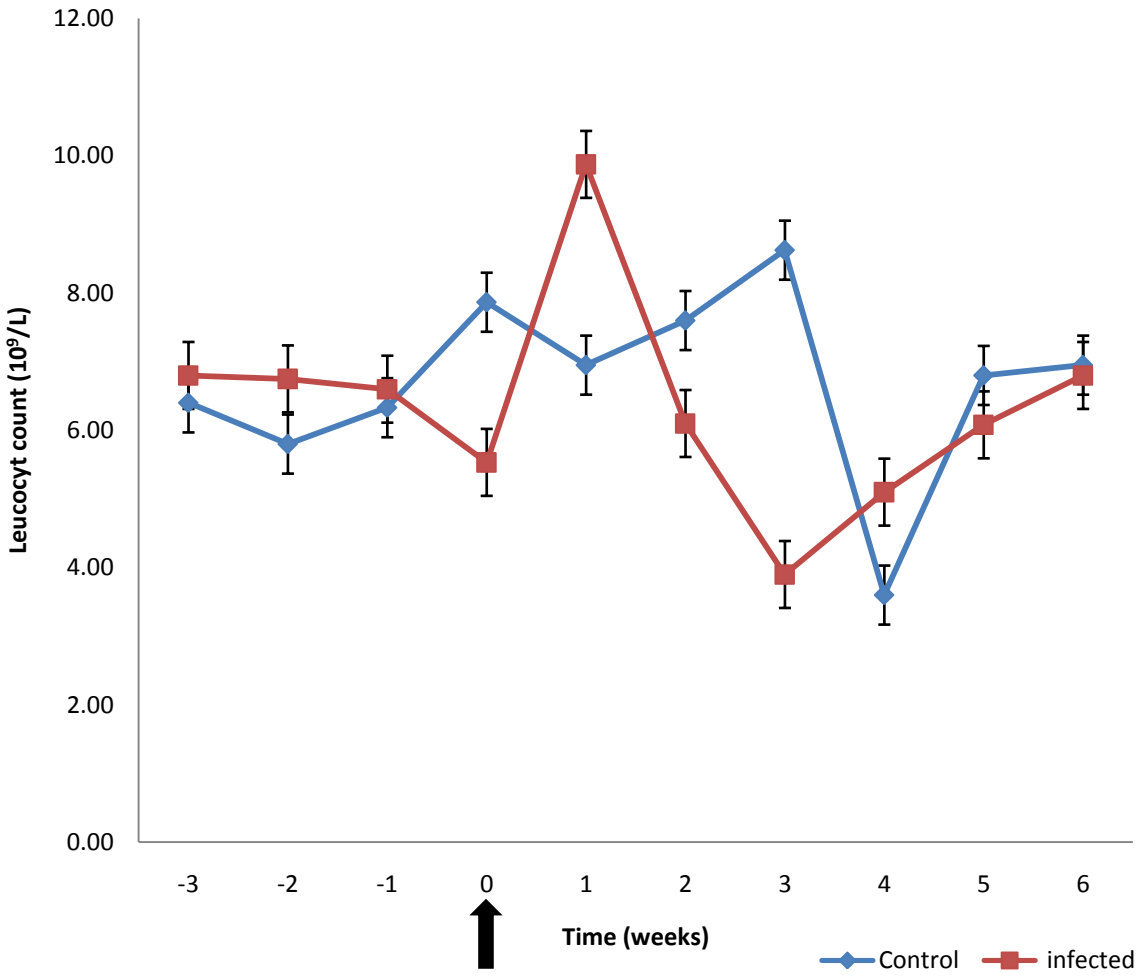

Figure 4.12: Mean ± SEMleukocytecount of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.9 Mean Total Plasma Protein

The mean total plasma protein of Yankasa ewes infected at the second trimester of pregnancy is presented in figure 4.13. Therewas significant ($p<0.05$)increase in the mean total plasma protein values of the infected groupcompared to the control group at weeks 7, 11, 12 and13 pi. In the group infected at the third trimester of pregnancy, the mean total plasma protein significantly($p<0.05$)decreasedand increased atweeks 2 and 6 pi, respectively (figure 4.14) when compared with the control group.

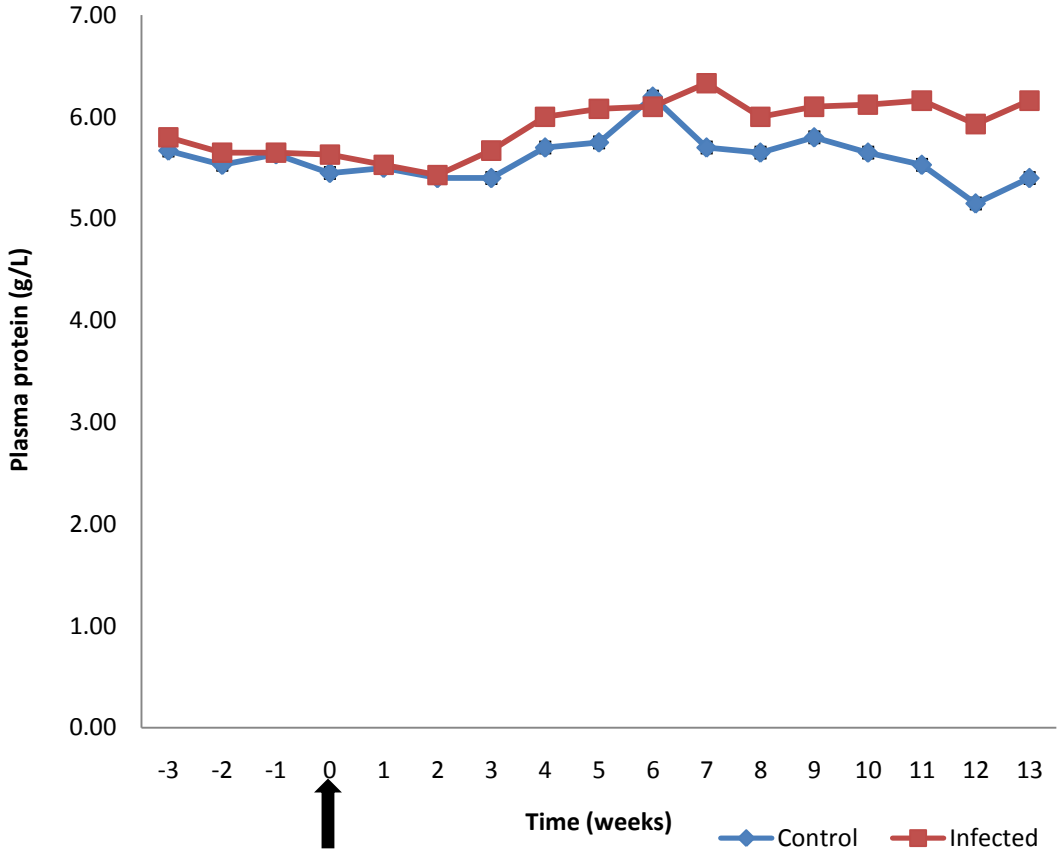

Figure 4.13: Mean ± SEM Plasma Protein level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

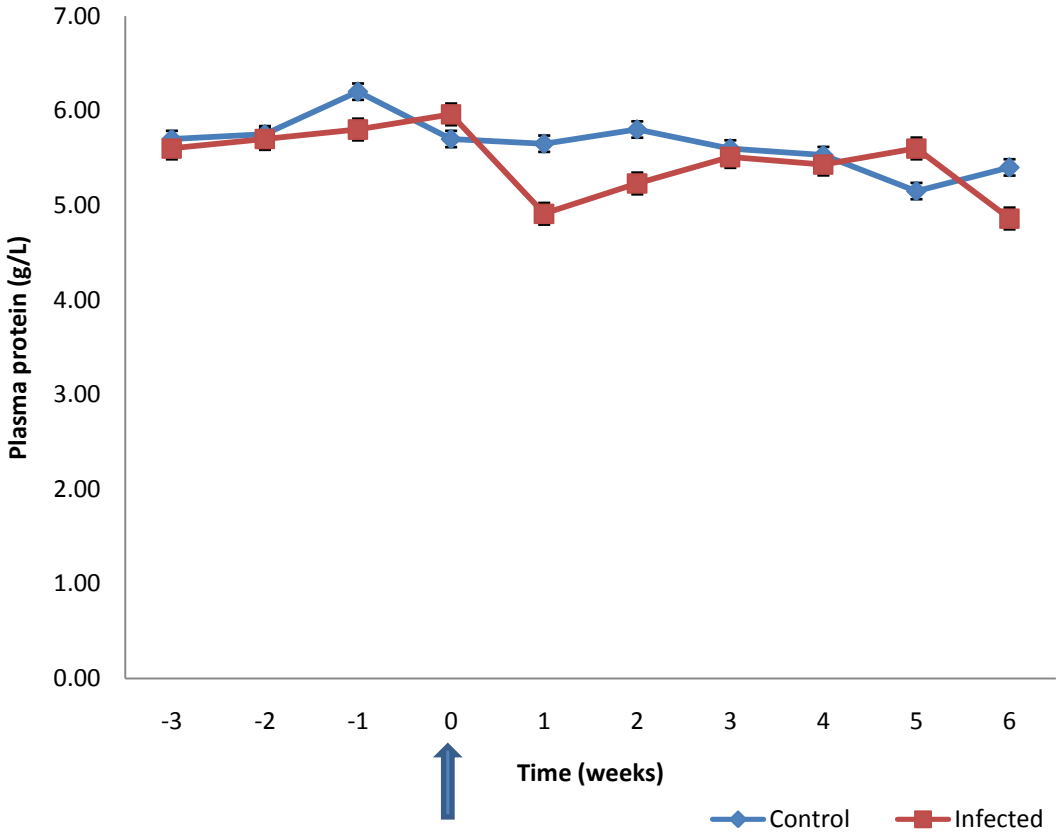

Figure 4.14: Mean ± SEMplasma protein level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.10 Mean Serum Progesterone

The mean serum progesterone level of Yankasa ewes infected at the second trimester of pregnancy is presented in figure 4.15. There was significant ($p<0.05$) increase in the infected group compared to the control group at week 4 pi, subsequently, no significant ($p>0.05$) differences were observed between infected and control groupstill the end of the study.

In the group infected at thethird trimester of pregnancy, no significant ($p>0.05$) difference was observed between infected and control groups throughout the study (figure 4.16).

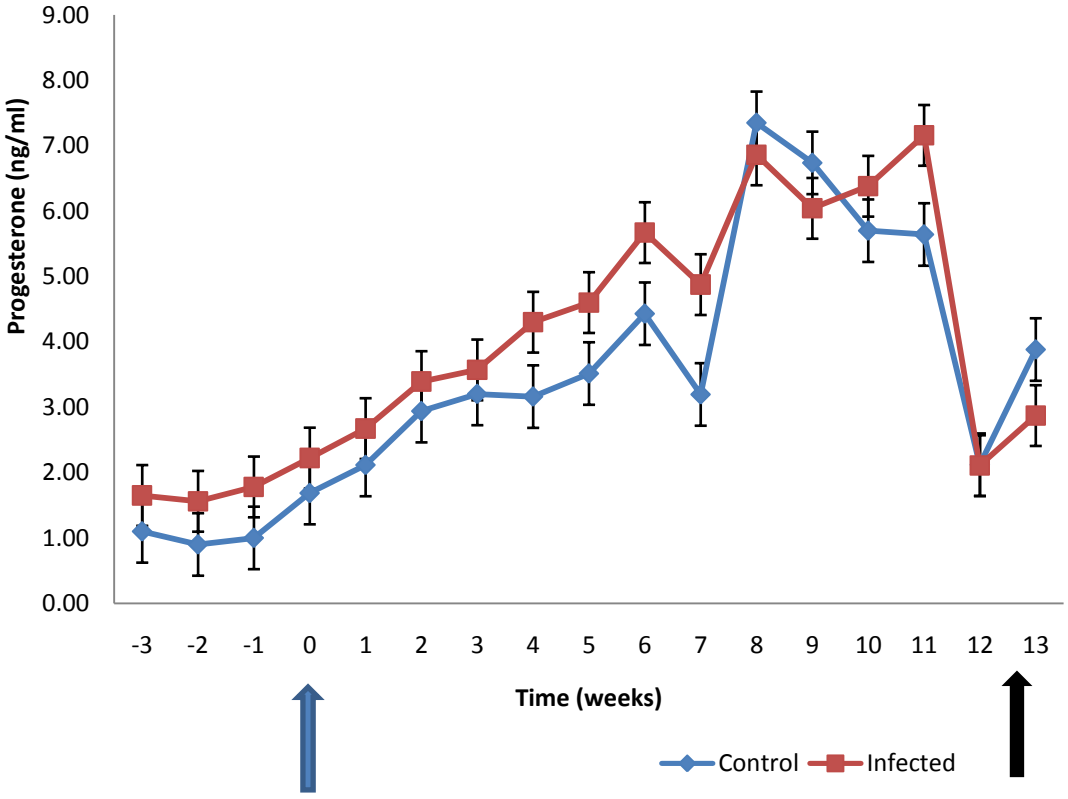

Figure 4.15: Mean ± SEMserum progesterone level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Blue arrow – Day of infection (Day 59 of pregnancy)

Black arrow – Day of lambing

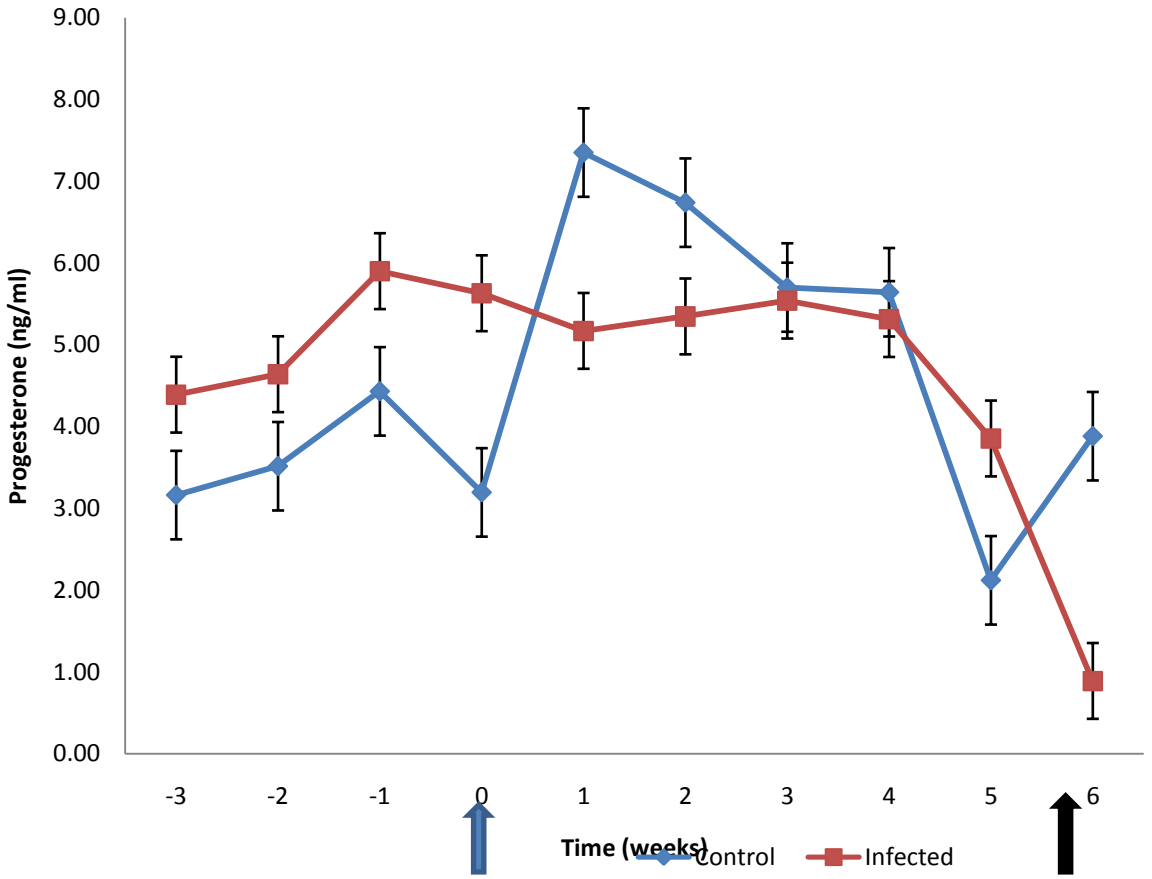

Figure 4.16: Mean ± SEMserum progesterone level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Blue arrow – Day of infection (Day 110 of pregnancy)

Black arrow – Day of lambing

4.11 Mean Serum Pregnancy Associated Glycoprotein

The mean pregnancy associated glycoprotein (PAG) level of Yankasa ewes infected at the second trimester of pregnancy is presented in figure 4.17. There was an initial decrease in PAG following infection and an increase towards parturition. However, these were notstatistically significant ($p>0.05$) throughout the study, except at weeks 12 pi (123.02 ± 3.51), when there was a significant increase ($p<0.05$).

In the group infected at the third trimester of pregnancy, the PAG increased from week 2 pi till week 5 before dropping at week 6 pi. However, these changes were not significantly($p>0.05$) different between the infected and control groups throughout the study (figure 4.18).

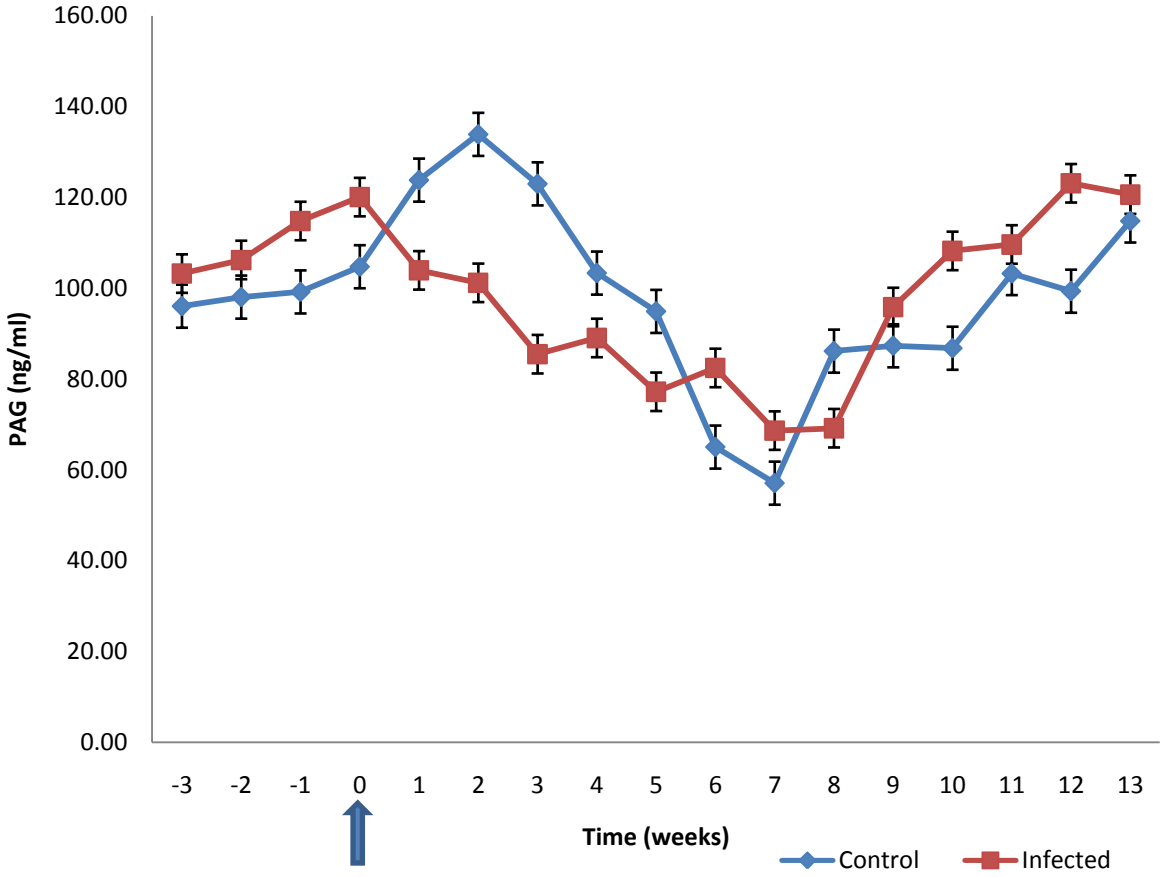

Figure 4.17:Mean ± SEMserum pregnancy associated glycoprotein (PAG) level in pregnant yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

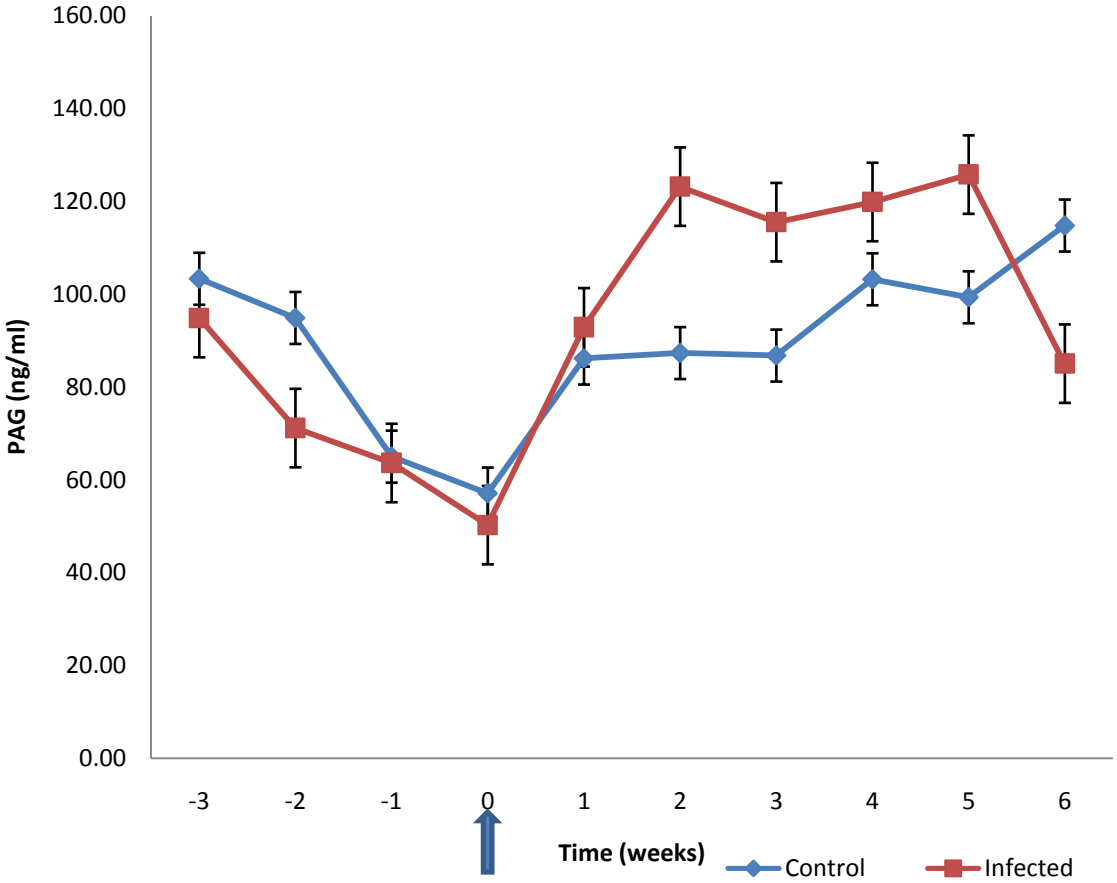

Figure 4.18:Mean ± SEMserum pregnancy associated glycoprotein (PAG) level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.12 Mean Serum Estrone Sulfate

The mean estrone sulfate level of Yankasa ewes infected at the second trimester of pregnancy is presented in figure 4.19. There were significant ($p<0.05$)decrease in the infected group compared with the control groups at week 2 and 11pi. There were also decreases in the infected group at week 3, 8 and 9 pi but they were not significantly($p>0.05$) different from the control group. In the group infected at the third trimester of pregnancy, there was a decrease at week 1 pi and increase at week 3 and 4, although they were not significantly($p>0.05$) differentfrom the control group (figure 4.32).

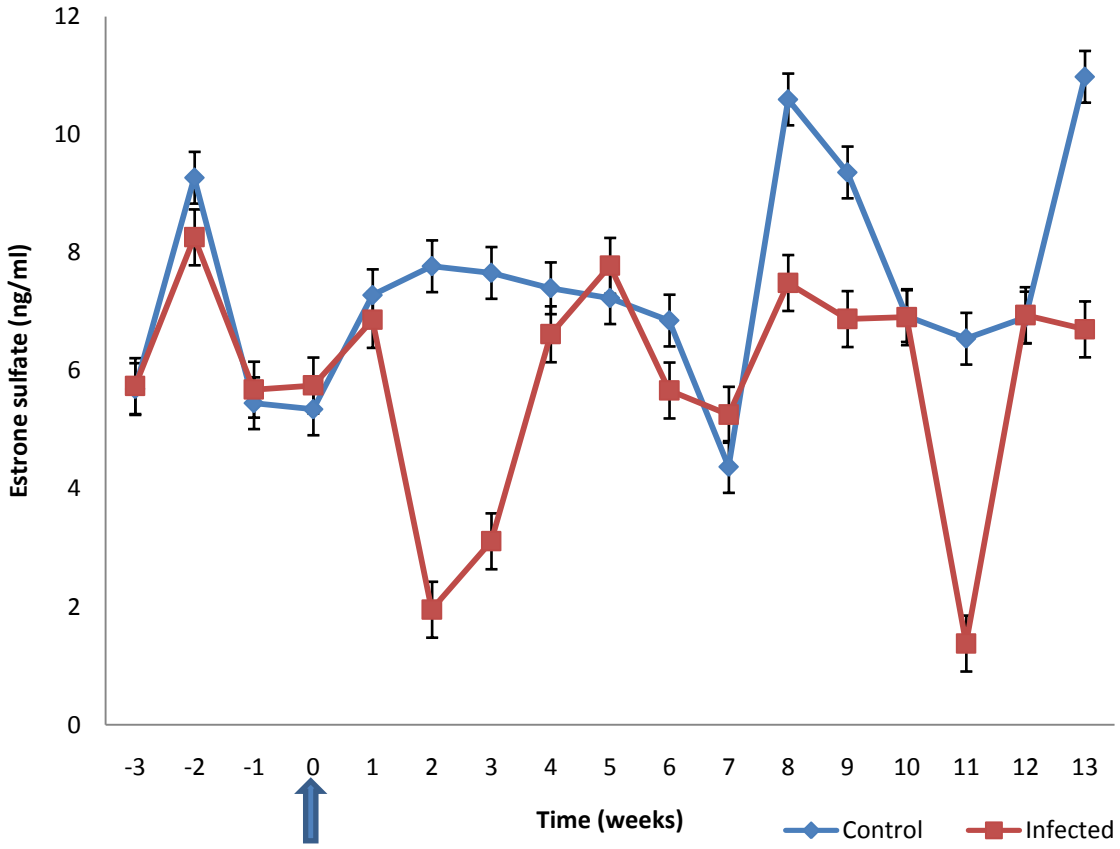

Figure 4.19: Mean ± SEMserum estrone sulfate level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

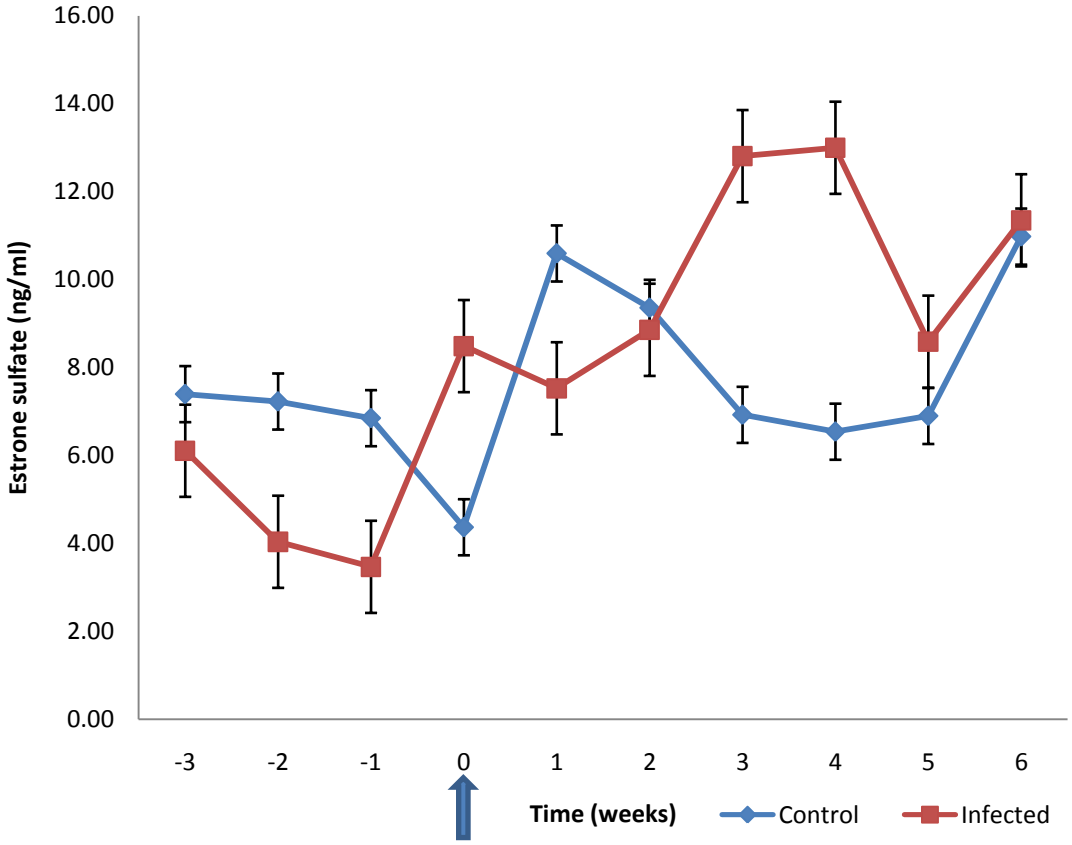

Figure 4.20: Mean ± SEMserum estrone sulfate level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.13 Mean Serum Cortisol

The mean cortisol level of Yankasa ewes infected at the second trimester of pregnancy is presented in figure 4.21. The mean cortisol level was generally lower in the infected group than the control, except at week 10 pi when it was higher although not significantly ($p>0.05$) different from the control. Mean serum cortisol of infected group was significantly($p<0.05$) lower than the control group at week 12 pi but not significant ($p>0.05$) at other periods of the study.

In the group infected at the third trimester of pregnancy, there were increases in mean serum cortisol at week1, 2, 3, 4 and 6 pi but was onlysignificant ($p<0.05$)at week 3 pi. (figure 4.22).

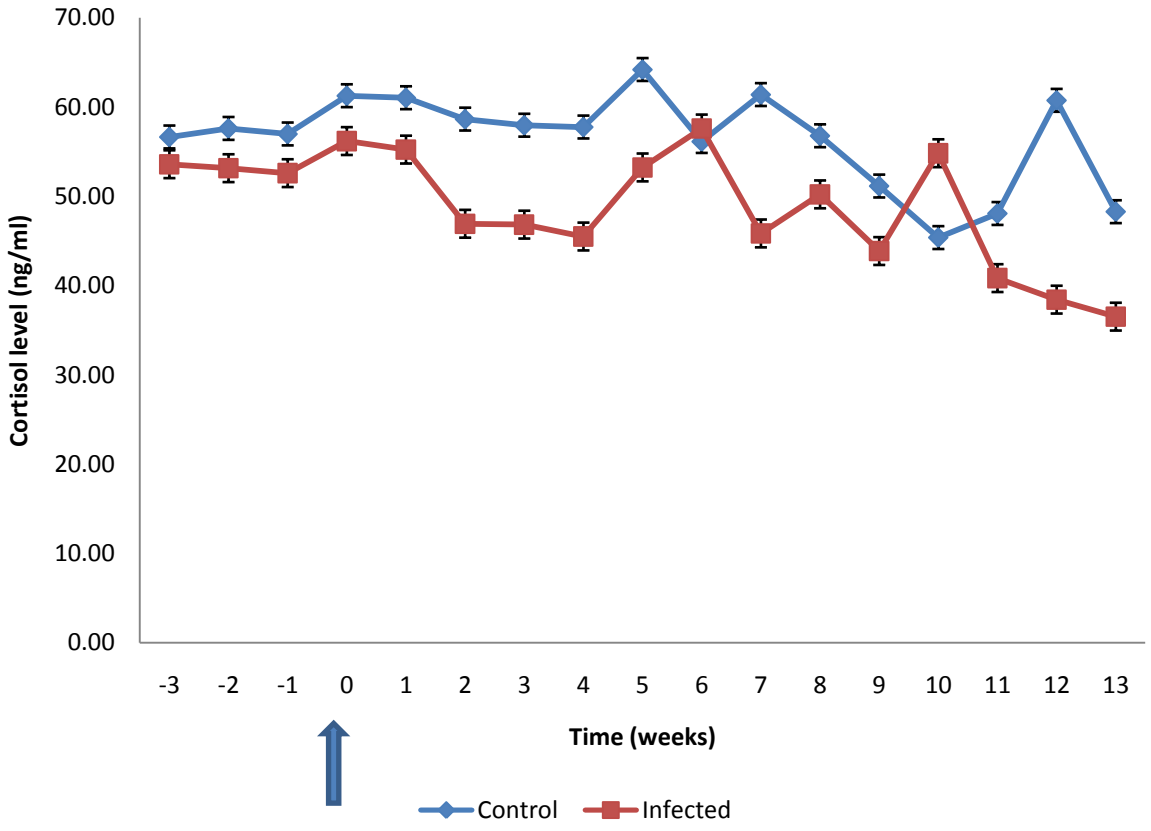

Figure 4.21: Mean ± SEMserum cortisol level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Arrow – Day of infection (Day 59 of pregnancy)

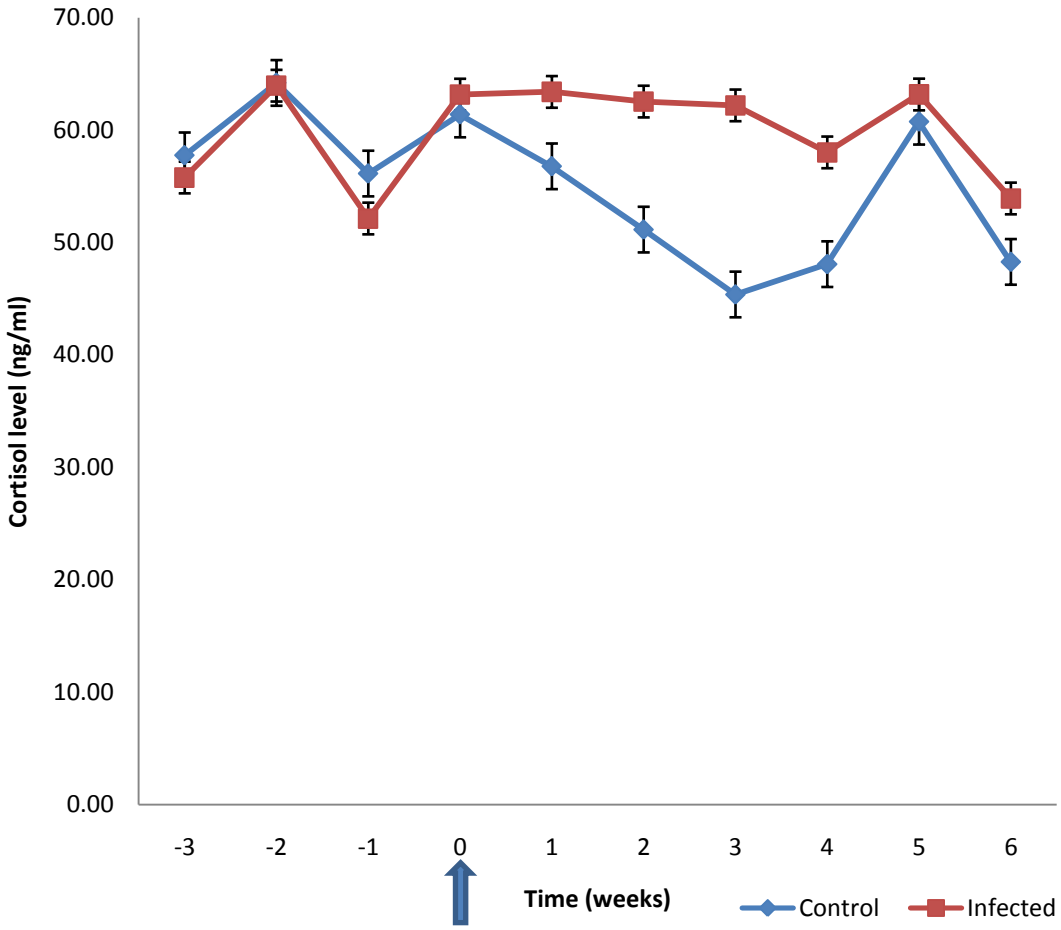

Figure 4.22: Mean ± SEM serum cortisol level in pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Arrow – Day of infection (Day 110 of pregnancy)

4.14 Relationship of the Pregnancy Associated Serum Factors in Pregnant Yankasa Ewes Experimentally infected with *Trypanosoma* evansi

The correlation coefficient among the pregnancy associated serum factors for ewes infected at the second trimester of pregnancy is presented in table 4.2. There was a positive but insignificant ($p>0.05$) correlation coefficient between progesterone and cortisol ($r=0.11$), pregnancy associated glycoprotein and estrone sulfate ($r=-0.10$), and between estrone sulfate and cortisol ($r=0.22$). However, there was negative and insignificant ($p>0.05$) correlation coefficient between progesterone and pregnancy associated glycoprotein ($r=-0.48$), progesterone and estrone sulfate ($r=-0.08$) as well as pregnancy associated glycoproteinand cortisol ($r=0.31$).

In the ewes infected at third trimester of pregnancy (table 4.3), there was a positive and significant ($p<0.05$) correlation coefficient between progesterone and cortisol ($r=0.77$). The correlation coefficient was positive but insignificant ($p>0.05$) betweenprogesterone and pregnancy associated glycoprotein($r=0.10$), estrone sulfate and pregnancy associated glycoprotein ($r=-0.30$) and between cortisol and pregnancy associated glycoprotein ($r=0.06$). There was a negative but insignificant ($p>0.05$) correlation between estrone sulfate and progesterone ($r= -0.13$) and between estrone sulfate and cortisol ($r= -0.60$).

Table 4.2: Correlation coefficient (r) of P_4, PAG, E_1S and C of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

	P_4	PAG	E_1S	C
P_4	-	$- 0.48^{ns}$	$- 0.08^{ns}$	0.11^{ns}
PAG	$- 0.48^{ns}$	-	0.10^{ns}	$- 0.31^{ns}$
E_1S	$- 0.08^{ns}$	0.10^{ns}	-	0.22^{ns}
C	0.11^{ns}	$- 0.31^{ns}$	0.22^{ns}	-

P_4 – Progesterone
PAG – Pregnancy associated glycoprotein
E_1S – Estrone sulfate
C – Cortisol
ns - Non-significant Correlation ($p>0.05$)

Table 4.3: Correlation coefficient (r) of P_4, PAG, E_1S and C of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

	P_4	PAG	E_1S	C
P_4	-	0.10^{ns}	-0.13^{ns}	0.77^{S}
PAG	0.10^{ns}	-	0.30^{ns}	0.06^{ns}
E_1S	-0.13^{ns}	0.30^{ns}	-	-0.60^{ns}
C	0.77^{S}	0.06^{ns}	-0.60^{ns}	-

P_4 – Progesterone
PAG – Pregnancy associated glycoprotein
E_1S – Estrone sulfate
C – Cortisol
s - Significant Correlation ($P<0.05$)
ns - Non-significant Correlation ($P>0.05$)

4.15 Gross Examination

There were no gross lesions seen on the reproductive tract and visceral organs ofall infected ewes sacrificed 10postpartum.

4.16 Histological Examination

There were no observable histological changes in the placenta, spleen, kidney, liver, lungs, heart, ovary, oviduct and uterusof pregnant Yankasa ewes experimentally infected with *Trypanosomaevansi*at the second and third trimesters of pregnancy respectively, and the control group. However, histological changes were observed in the hypothalamus, thyroid gland and pituitary gland of pregnant Yankasa ewes experimentally infected at the second and third trimesters of pregnancy respectively.There wasneuronal degeneration and infiltration of microglia in the hypothalamus of infected ewe (Plate I). These changes were not seen in the hypothalamus of the control group(Plate II). There wasinfiltration of mononuclear cellsin the pituitary gland of infected group (Plate III). These were not observed in the control group (Plate IV).The thyroid gland wasinfiltrated by macrophages and loss its glandular secretions (Plate V). In the control group, the glandular secretions were evident (Plate VI).

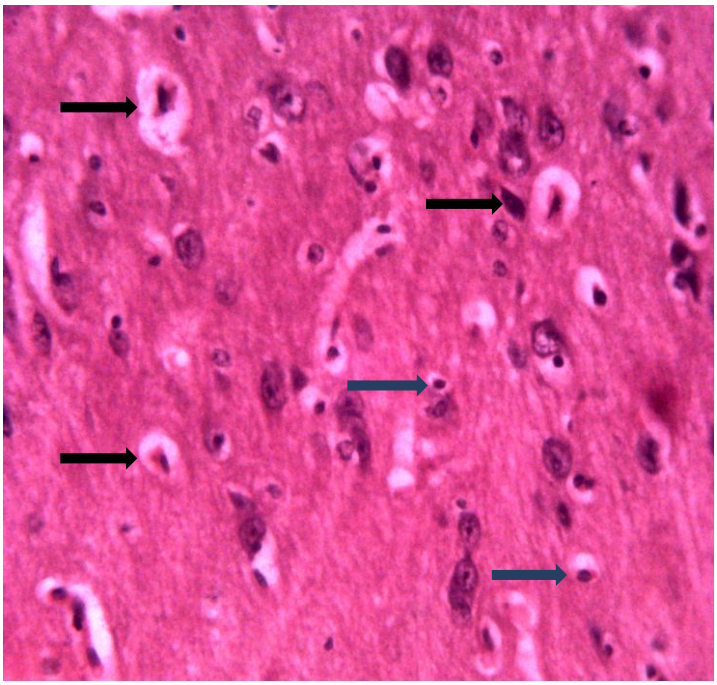

Plate I: Photomicrograph of the hypothalamus of pregnant Yankasa ewe experimentally infected with *Trypanosoma evansi* at the second trimester of pregnancy showing neuronal degeneration (black arrows) and microglia infiltration (blue arrows). H & E stain. X 250

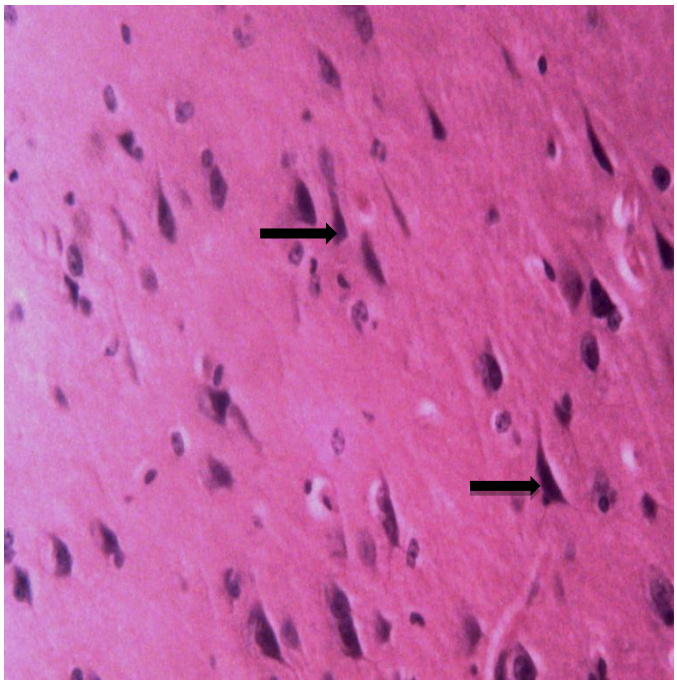

Plate II: Photomicrograph of the hypothalamus of uninfected Yankasa ewe showing normal neurons (black arrows). H & E stain. X 250

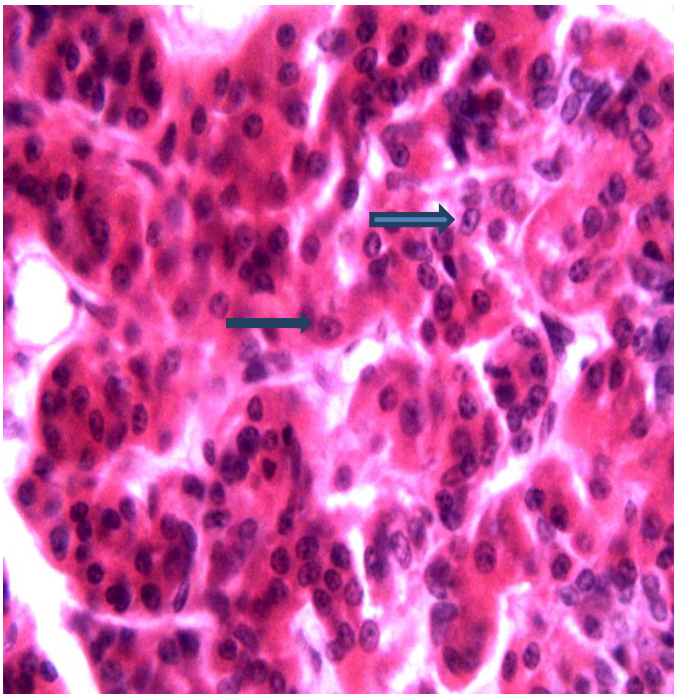

Plate III: Photomicrograph of the pituitary gland of pregnant Yankasa ewes experimentally infected with *Trypanosoma evansi* at the second trimester of pregnancy showing infiltration of mononuclear cells(arrows).H & E stain. X 400

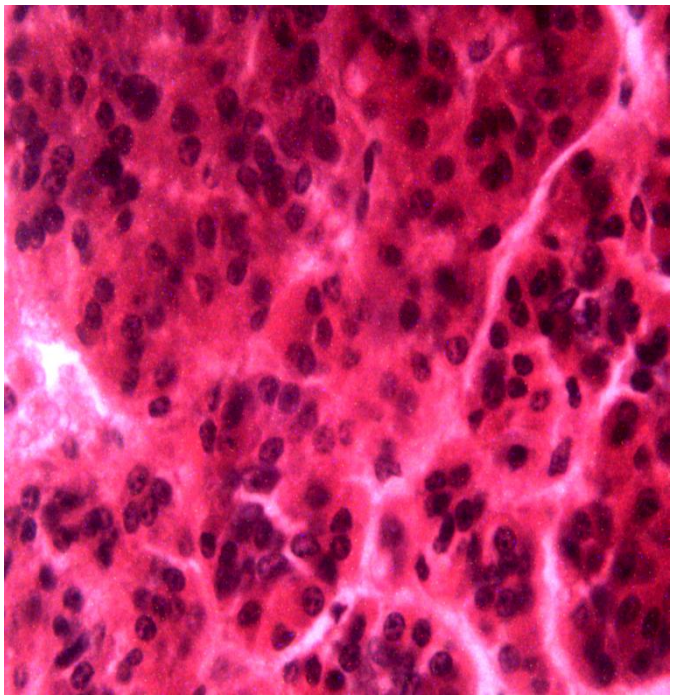

Plate IV: Photomicrograph of the pituitary gland of uninfected ewe showing normal hypothalamus. H & E stain. X 400

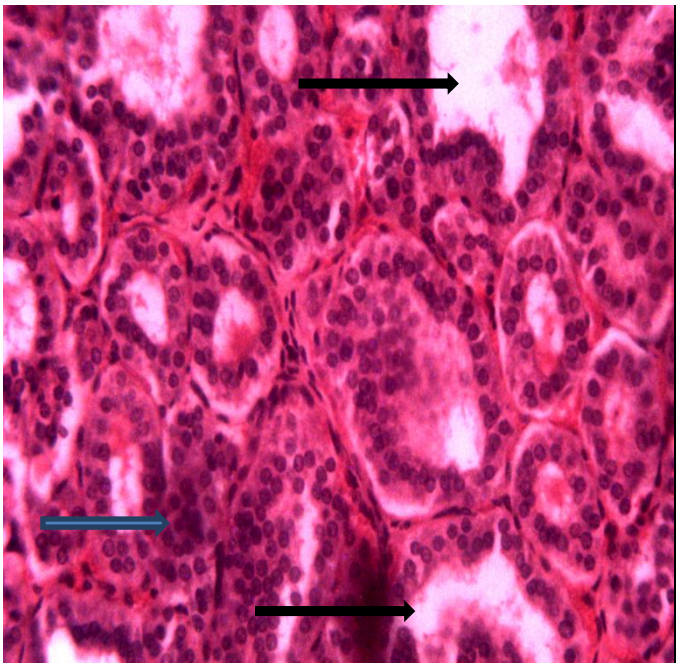

Plate V: Photomicrograph of the thyroid gland of pregnant Yankasa ewes infected with *Trypanosoma evansi*at the second trimester of pregnancy showing presence of inflammatory macrophages (black arrows) and loss of glandular secretions (blue arrow).H & E stain. X 250

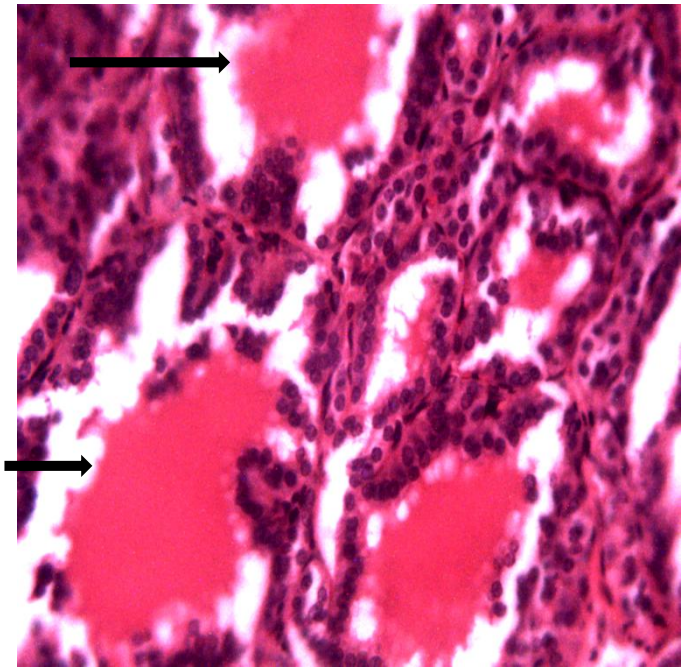

Plate VI: Photomicrograph of the thyroid gland of uninfected ewe showing presence ofglandular secretions.H & E stain. X 250

4.17 Gestation Length and Percentage Lamb Birth-Weights

The gestation length and lamb birth-weight at birth is presented in table 4.4. There were no significant ($p>0.05$) differences in the gestation length and percentage birth-weights between ewes in the infected groups (group A and B) and control group (group C).

Table 4.4: Gestation length and percentage lamb birth-weights of dam in pregnant Yankasa ewes experimentally infected with *T. evansi* at the second and third trimesters

	Gestation length (days)	Percentage lamb birth-weights (%)
Second trimester (n = 7)	151.5 ± 0.85	9.75 ± 0.38
Third trimester (n = 7)	150.8 ± 1.01	9.00 ± 0.68
Control (n = 6)	150.8 ± 0.86	10.15 ± 0.70

P > 0.05

CHAPTER FIVE

DISCUSSION

The prepatent periods observed in this study were 11-25 days and 13-22 days forewes infected in second and third trimesters, respectively. Thesearelonger than previousstudies in pregnant ewes infected with*T. evansi* (Dalal *et al*., 2008). It is also longer than earlier reports in *T. evansi* infected sheep (Onah *et al*., 1996; Audu *et al*.,1999),goats (Dargantes *et al*., 2005; Shehu *et al*., 2006b; Mohammed, 2009;Ogbaje *et al*., 2011) and rabbits (Alsaffar *et al*., 2007; Jatau *et al*., 2013; Yasameen*et al*., 2015). The relative longer prepatent period observed in this study may suggest that the Sokoto isolate used in this study is less virulent compared to the isolate used by thepreviousauthors mentioned above. The more virulent a parasite is, the shorter would be its prepatent period (Basista *et al*., 2007), which determines its severity and ability to multiply and establish itself in the host (Trail *et al*., 1991). Mohammed (2009) had earlier reported thatSokoto isolate of*T. evansi* was virulent in goats. This was not the case in this study probably due to animal speciedifferences.Boid *et al*. (1981) observed that sheep were more resistant to *T. evansi* infection than goats, further confirmingprevious reports that the susceptibility to trypanosome infection may be partly determined by the specie of the animal host (Anosa, 1988b; Igbokwe and Mohammed, 1991).

In this study, the stage of pregnancy did not affect the prepatent period. This is contrary to the reports of Elhassan *et al*. (1995) and Bawa *et al*. (2005) in ewes infected with *T. brucei* and *T.vivax*, respectively. It also contradicts the reports of Ogwu *et al*.(1984) in *T. vivax* infected cows. The difference may be attributed to thevariation in trypanosome specie used. *Trypanosoma brucei* and *T.vivax*are highly pathogenic species of trypanosomes (Desquesnes, 2004). In addition, themildly pathogenic trypanosome used in this studymay bea contributory

factor. Thismay also explain the reasons for the absence ofmost clinical signspreviously reportedin trypanosomosis of pregnancy, such as stillbirth, neonatal death, foetal resorption, abortion or death of the dam(Paikne and Dhake, 1972; Anene and Omamegbe, 1984;Ogwu *et al*., 1984; Luckins *et al*., 1986; Anene *et al*., 1991; Waitumbi and Conor, 1991; Elhassan *et al*., 1994; Elhassan *et al*., 1995; Faye *et al*., 2004; Bawa *et al*., 2005; Dalal *et al*., 2008; Leigh and Fayemi, 2013; Silva *et al*., 2013; Allam *et al*., 2014).

There was low-level intermittent parasitaemia in all the infected groups. This is consistent with thereports in *T. evansi*infected sheep(Onah *et al*., 1996; Dalal *et al*., 2008) and goats (Ogbaje *et al*., 2011). Intermittent parasitaemia in trypanosome-infected animals has been attributed to the ability of the parasitesto change their antigenic coat during each wave of parasitaemia (Vincendeau and Bouteille, 2006). In addition, Onah *et al*. (1996) described low-level parasitaemia in *T. evansi*infection as“cryptic parasitaemia”and concluded that it was responsible for the chronicity of theinfection in sheep.In this study, mice inoculation with blood from infected ewes became parasitaemic for the group infected at the third trimestersignifying chronic trypanosomosis. In the group infected at the second trimester, no mouse was parasitaemic, perhapssuggesting self-cure or resistance to the infection.

In the group infected atthe second trimester of pregnancy, temperature fluctuated throughout the studywith increases recorded at week 4, 6 and 12pi (pi),although itdecreased significantly at week 11 pito valuescomparable with that of the control group. However, thelow value was still significantly ($p<0.05$)higher than the temperature at infection. Similarly, there were fluctuations in temperature of the group infected atthe third trimester, with increases occurring at weeks 1 and 4 pi. Fluctuationssimilar to those observed in this studyhavepreviously been reported in *T. evansi* infected animals (Audu *et al*., 1999; Dalal *et al*.,2008; Jatau*et al*., 2013).

The high temperatures were irregular in pattern,but theypreceded and followed peaks of parasitaemia. This finding agrees with that of Ngeranwa *et al*. (1991) in *T. evansi* infected male goats.High temperature in trypanosomosis is an indication of disturbances in metabolic disorder caused by circulating trypanosomes and their by-products (Audu *et al*., 1999)such as sialidase (Esievo, 1979).

There were negligiblechanges in the weight of ewes infected at the second trimester compared with the controls. These findings are consistent with the observationsmade by Katunguka-Rwakishaya*et al*. (1992) and may be related to the low virulence of the isolate used;since the virulence determines the ability of a parasite to establish itself in the host (Trail *et al*., 1991). However, in the group infected atthe third trimester of pregnancy, there was a gradual decrease in weight which occurred from the third week pi, and coincided with peak parasitaemia. Thisagrees withearlier studies (Edeghere *et al*., 1992; Mutayoba *et al*., 1995b; Audu *et al*., 1999; Bawa *et al*., 2005; Dalal *et al*., 2008; Desquesnes *et al*., 2013, Silva *et al*., 2013).Weight loss is a common finding in trypanosomosis infection(Steverding, 2008). Apart from this, the high demand for energyin the last few months of pregnancy (Henze *et al*., 1998; Van Saun, 2000; Kulcsar *et al*, 2006)may be a contributing factor. There wasdisparity in the pattern of weight loss between the groups infected at the second and third trimesters of pregnancy. This is consistentwith previous reports in *T. vivax* infected ewes (Bawa *et al*., 2005) and cows (Ogwu *et al*., 1986). Thethird trimester is characterized by increasing demand for energy due to rapidly developing foetus (Wallace *et al*., 1996; Firat and Ozpinar, 2002; McCann, 2015).

The glucose level of ewes infected at the second trimester of pregnancy wasnot affected by the trypanosome infection. Similarly in the group infected atthe third trimester, glucose level

was not affected by the infection. However, the glucose level decreasedat 3 weeks pi, although this was not statistically significant. This differs fromthe report of Singh and Gaur(1983); Anosa (1988b); Kadima *et al*. (2000); Gutierrez *et al*. (2006) and Silva *et al*.(2013) who reported hypoglycemia in *Trypanosoma* infections. Trypanosomesare known to cause decline in blood glucose level by utilizingglucose for metabolic process at periods of peak parasitaemia (Anosa, 1988b).Such ability of the trypanosome to cause hypoglycemia has been demonstrated in rats with diabetes mellitus (Ukwueze and Anene,2011) and cattle infused with lactose (Kadima *et al*., 2000). However, the low level parasitaemia observed in this study may be responsible for the insignificant change in blood glucose level.

In thegroup infected at the second trimester of pregnancy, there was decrease in packed cell volume (PCV) from week 6 pi,though statistically insignificant. This is consistent with the reports of Boid *et al*. (1981)in *T. evansi* infected sheep and Ezeokonkwo *et al*. (2010) in dogs co-infected with *T. congolense and T. brucei*, but contradicts the reports of Onah *et al*. (1996); Audu *et al*. (1999) and Dalal *et al*. (2008) in *T. evansi* infected sheep. It also contradicts the reports of*T. evansi* infection in goats (Shehu *et al*., 2006b; Mohammed, 2009; Dargantes *et al*., 2005; Ogbaje *et al*., 2011) and rabbits(Alsaffar *et al*., 2007; Takeet *et al*., 2012; Jatau *et al*., 2013; Yasameen *et al*., 2015).There was significant decrease in the PCV of ewes infected at the third trimester of pregnancy. This is consistent with the observationsmade by Onah *et al*. (1996); Audu *et al*. (1999) and Dalal *et al*.(2008) but differs with the findings of Boid *et al*. (1981). Anaemia is a consistent feature of animal trypanosomosis,usually seen following the first wave of parasitaemia (Anosa, 1988a). This is thought to be caused by haemodilution(Dalal *et al*., 2008) and decline in erythrocyte values following parasitaemia (Anosa, 1988a).The difference in PCV between the two infected groups may be attributed to

varying demands for iron at different stages of pregnancy(Bothwell, 2000; Rodriguez-Bernal *et al*., 2012). Concurrent depletion of iron stores has been postulated as one of the factors responsible for anaemia in trypanosomosis (Connor, 1992).

There was leukopaenia in the first week pi for ewes infected at the second trimester. This was followed by leukocytosis at week 4 pi then leukopaenia at week 7 pi. This variation in the pattern of leukocyte count is consistent with the reports of Esievo and Saror (1983). Early leukopaenia in trypanosomosis is assumed to be due to massive leukophagocytosis in the liver (Esievo and Saror, 1983; Anosa, 1988b; Igbokwe and Anosa, 1989). Leukocytosis coincided with peak parasitaemia, which is a common finding in trypanosomosis infections (Anosa, 1988b). For ewes infected at the third trimester of pregnancy, there was leukopaenia 3 weeks pi whichalso coincided with peak parasitaemia. This is consistent with the reports of Igbokwe and Anosa (1989) in *T. vivax* infected sheep as well as Takeet *et al*. (2012) in co-infection of rabbits with*T. brucei* and *T. congolense*. However, it contradicts the report of El-Baky and Salem (2011) who reported slight leukocytosis in camel and rats infected with *T. evansi*.

There was a significant increase in plasma protein of ewes infected at second trimester of pregnancy compared to the ewes in the control group. This is in agreement withthe observations made in sheep infected with*T. brucei*(Ogunsanmi *et al*., 1994b; Taiwo *et al*., 2003) and*T. evansi*(Dalal *et al*., 2008) as well as camel naturally infected with *T. evansi* (Gutierrez *et al*., 2006). It also agrees with the observations made in rabbits infected with *T. brucei*(Oyewusi and Saba, 2013) and mixed infection of *T. brucei*and*T. congolense*(Takeet *et al*., 2013). However, it disagrees with the observations of Sekoni *et al*. (1990b) in bulls infected with *T. congolense* and *T. vivax*as well as the report of Jatau *et al*. (2013), in rabbits infected with*T. evansi*.Conversely, the total plasma protein for the ewes infected at the third

trimester significantly increased compared to the control group at weeks 2 and 6 pi.The contradicting effect of trypanosomosis on total protein has been attributed to varying degrees of alteration in levels of albumin and globulin, characterized by decrease in albumin and increase in globulin (Anosa, 1988b; Ogunsanmi *et al*., 1994b). Although theindividual levels of these proteins were not determined in this study, it is possible that the rate of globulin increase was higher than the rate of albumin decrease for ewes infected in second trimester, while globulin decrease was higher than the rate of albumin increase for ewes infected in the third trimester of pregnancy. Sulaiman and Adeyemi (2010) associated thevariation in albumin and globulin levels to increase in cell-derived proteins due to haemolysis during anaemia. In addition, Schloesser *et al*.(1993) had observed a higher demand for protein at thethirdtrimester of pregnancy than at the second trimester.

The progesterone (P_4) level of ewes infected at the second trimester of pregnancy was significantly increasedat week 4 pi.However, persistent decrease in P_4 levels have been reported inpregnant ewes infected with *T. vivax* (Bawa*et al*, 2005; Silva *et al*., 2013) and pregnant does infected with *T. congolense* (Faye *et al*., 2004) and *T.brucei* (Leigh and Fayemi, 2013). These reports were characterized by abortion and lesions on the ovary as well as the placenta, but these lesions were absentin this study. Themild virulence of the *T. evansi* isolate used is probably responsible. The mechanism responsible for P_4increase in this study is unknown. However, increase in P_4 may have occurred as a result of stress caused by the parasite leading to release of glucocorticoid. P_4 is an intermediate substance produced during the synthesis of glucocorticoid by the adrenal gland (Mitani, 2014). P_4 is also known to increase in ewes with multiple pregnancies (Noakes*et al*., 2001), but that was not the case in this study as all the ewes had single lambs. Further studies are therefore required to

understand the rational responsible for P_4 increase in ewes infected with *T. evansi* in second trimester. For ewes infected at the third trimester of pregnancy, the P_4 levels were not significantly different from the control. This agrees with previous reports in ewes infected with *T. congolense* (Osaer *et al*., 1998) and *T. evansi* (Dalal *et al*., 2008). It also agrees with the report of Okech*et al*. (1996) in cows infected with *T. vivax* and Leigh and Fayemi (2013) in goats infected with *T. brucei*. The insignificant difference may be attributed to the absence of pathological lesions on the placenta and ovaries where P_4 is produced, probably due to the mild virulence of the parasite.

There was an insignificant decline in the pregnancy associated glycoprotein (PAG) of ewes infected at the second trimester of pregnancy,3-4weeks pi. Eight days to parturition, the PAG concentrations were significantly elevated compared to the control group. This is contrary to earlier reports in *T. congolense* infected goats (Osaer *et al*., 1998; Faye *et al*., 2004) and in*Actinomyces pyogenes* infected cows (Semambo *et al*., 1992). It is also contrary to the report of Zarrouk*et al*. (1999) in goats infected with *Toxoplasma gondii*or*Listeria monocytogenes*. All these diseases are known to beabortifacient agents. The increase in PAG levels of the infected group before parturition may be attributed to increased protein synthesis at the placenta following recovery from low virulent *T. evansi*. It may also be associated with disproportion between luteotropic and luteolytic factors. Levels of luteotropic factors (placental lactogen and prolactin) decrease in the last weeks of parturition with subsequent increase in luteolytic factors (prostaglandin $F_2\alpha$) (Ford *et al*., 1998).The decrease in the control and infected groups between weeks 6 and 7 pi are similar to the reports Ranilla *et al*. (1994).The PAG in the ewes infected at the third trimesterwas not significantly differentcompared with the control group. The low virulenceof the isolate may be responsible

for this. Previous studies have shown decrease in PAG levels following infection with virulent*T. congolense*in goats (Osaer *et al*., 1998; Faye *et al*., 2004).

The estrone sulfate (E_1S) level of ewes infected at second trimester fluctuated with significant decrease at weeks 2 and 11pi. The depressed E_1S level was probably a threatened abortion.The presence of a viable foetus is usually accompanied by an increase in E_1S concentrations (Karen *et al*., 2001). Conversely, a decrease would be as a result of poor viability of the foetus. However, the resistance of the ewes to the mild *T. evansi* isolate used in this study may have made abortion impossible. There were no significant differences between ewes infected at the third trimester and the control, despite wide variations with the control group. This was probably due to wide individual variations within infectedgroup.

The cortisol level of ewes infected at the second trimester of pregnancyfluctuated from infection till the end of the study, but decreased significantly 12 weeks pi. This is consistent with the report of Mutayoba *et al*. (1995b) in *T. congolense* infected rams. The susceptibility of the host animals to trypanosome isolate has been identified as one of the factors associated with cortisol secretion in trypanosomal infection (Mutayoba and Gombe, 1989). This may also have contributed to the decrease in cortisol level, since a less virulent isolate of trypanosome was used in this study.For ewes infected at the third trimester, cortisol levels were elevated, but were only significant 3 weeks pi.This is similar to the reports of Ogwu *et al*. (1986). The increase coincided with the periods of increased cortisol production in pregnancy (Mescher *et al*., 1975; Jackson, 2004). Though it has been suggested that *Trypanosoma* infection modulates adrenocortical activity (Ikede *et al*., 1988), the physiological endocrine changes in late pregnancy may have resisted this modulatory role by

the parasite, thereby stimulatingincreased release of adrenocorticotropic hormonefrom the anterior pituitary gland of the dam.

There was an insignificantcorrelation among all the pregnancy associated serum factors (P_4, PAG, E_1S and cortisol) in the ewes infected at the second trimester of pregnancy. However, a significant correlation was observed between P_4 and cortisol in ewes infected at the third trimester of pregnancy. This is contrary to the report of Ranilla *et al*. (1997), who observed correlation only between P_4 and PAG in Assaf ewes. The breed variation could be responsible for this difference. In addition, the Assaf breed of sheep used by Ranilla *et al*. (1997) carried single and twin lambs unlike the Yankasa ewes used in this study which all carried single lambs. The significant correlation between P_4 and cortisol in the ewes infected in third trimester mayalso be attributedto the stress caused by the infection coupled with increased secretion of corticotropin releasing hormone by the foetal brain during the last days of parturition (Jackson, 2004), which leads to increased cortisol production by the foetal adrenal glands and is believed to be one of the factors responsible for initiating parturition in animals.

There were no observable pathological lesions in the reproductive tracts of infected ewes postpartum. This agrees with the report of Dalal *et al*. (2008) in pregnant ewes infected with *T. evansi*. However, itdisagrees with the reports ofFaye *et al*. (2004)in pregnant does infected with *T. congolense*andSilva *et al*. (2013) in pregnant ewes infected with*T. vivax*. Genital lesions have also been observed in goats infected with *T. evansi*(Shehu *et al*., 2006) and*T. brucei* (Leigh *et al* 2015); as well as heifers infected with *T. congolense*(Ogwu and Njoku, 1991). The mildpathogenicity of the *T. evansi*isolate used in this study may be responsible for this. Histologically, there was neuronal degeneration and microglia infiltration in the hypothalamus while the pituitary gland wasinfiltrated by mononuclear cells. This is similar to

the reports of Adenowo *et al*. (2005) in *T. vivax* infected ewes as well as Leigh *et al*. (2015) in *T. brucei* infected West African dwarf does.Although the parasite was not seen in the hypothalamus and pituitary gland, the changes observed may be caused by the tissue invasive ability of the brucei group which *T. evansi*belongs too (OIE, 2012). Thyroid gland was infiltrated by macrophages. This is similar to the reports in cattle infected with *T. congolense*(Ogwu *et al*., 1992) and *T. vivax*(Fatihu *et al*., 2007).Although thyroid hormones were not determined in this study, their metabolism may have been altered coupled with the pathological lesions observed in the hypothalamus and pituitary which are involved in the pathway of thyroid hormone secretions (Flamant*et al*., 2015). The thyroid gland is known to play a role in the regulation of theneuroendocrine system at the hypothalamo-pituitary axis (Fonseca*et al*., 2013).

There was no difference between thegestation lengthof the infected groups and the control. Earlier reports haddemonstrated that trypanosomes are capable of disrupting pregnancy by causing abortion and premature parturition (Ogwu *et al*., 1986;Edeghere *et al*., 1992; Elhassan *et al*., 1995; Okech *et al*., 1996 Faye *et al*., 2004; Bawa *et al*., 2005; Silva *et al*., 2013).The low level parasitaemia observed in this study probably due to low virulence may be responsible for non-disruption of pregnancy in the infected groups. There was also no significant difference in the percentage lamb birth-weightsof the infected groups and the control. This disagrees with the reports of Ogwu *et al*.(1986) and Okech *et al*. (1996).Themild pathogenicity of the parasite used may be responsible for this, since healthy lambs were born in both the infected and control groups.

CHAPTER SIX
CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study shows that the *Trypanosoma evansi*isolate used in this study was mildly pathogenic to Yankasa ewes experimentally infected at the second and third trimesters of pregnancy, suggesting that the ewes aretolerant to the infection. The parasite had no effect on all the pregnancy associated serum factors determined throughout the study. However, there were mild histological changes in the hypothalamus, pituitary and thyroid glands.

6.2 Recommendation

Based on the findings in this study, the following are recommended:

1. Further studies are needed to determine the effect of the parasite on the oestrus cycle of Yankasa ewes.

2. Studies should be carried out on the consequence of the parasite at the first pregnancy of sheep.

3. Other pathogenic species of trypanosome should be used to study the pathogenesis of reproductive losses in pregnant Yankasa ewes with the view of establishing their effect on pregnancy associated serum factors.

4. Considering the mild effect of the parasite on the hypothalamus and pituitary gland of infected ewes, rams should be infected to determine the effect of the parasite on the exocrine (spermatogenesis) and endocrine(production of testosterone) of the testes and other aspects of male reproductive functions.

5. The mechanism responsible for progesterone changes in pregnant Yankasa ewes infected with this*T. evansi*in second trimester is not well understood, and requires further studies.

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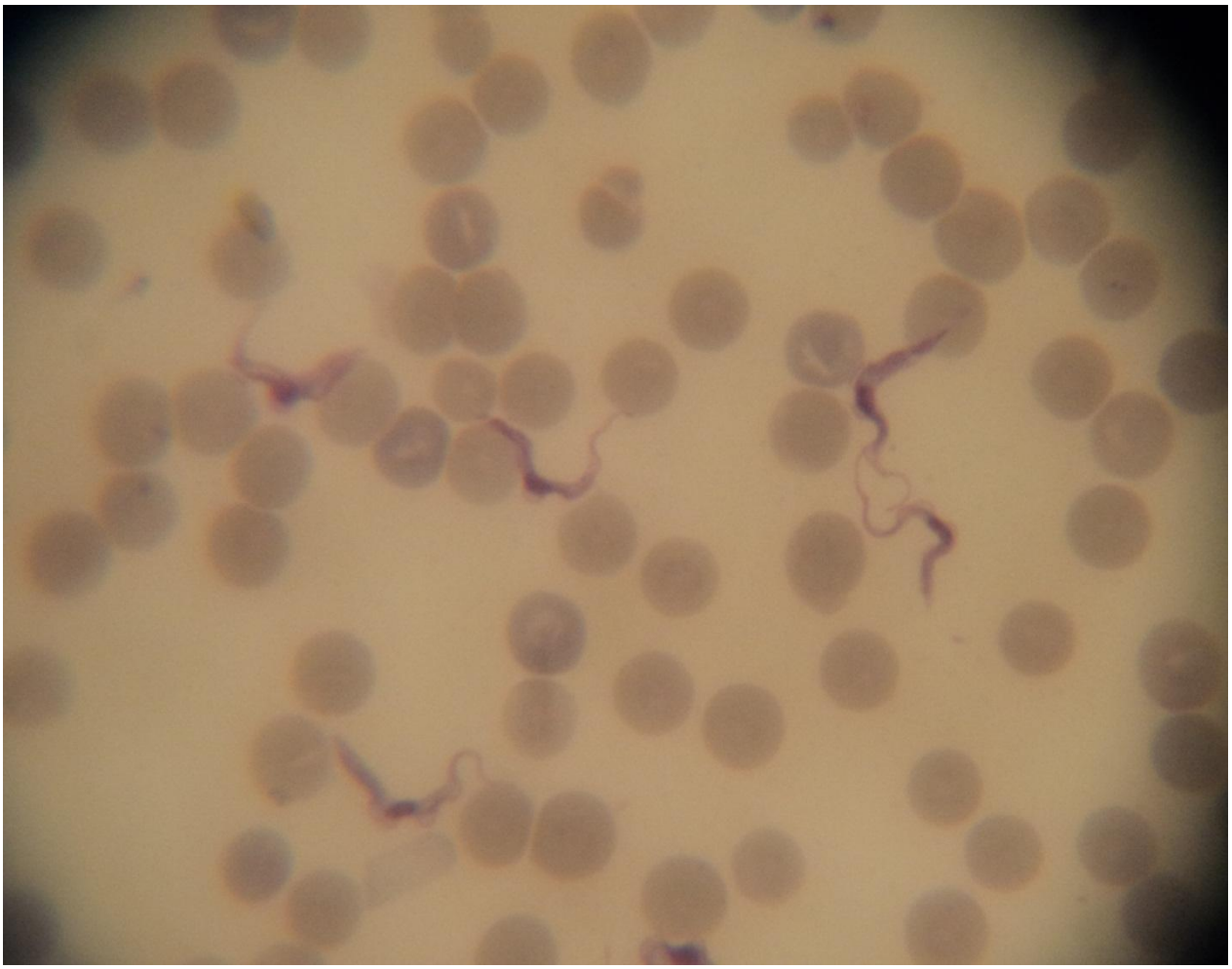
APPENDIX 1:



Thin blood smear of *Trypanosomaevansi*(Sokoto isolate) from albino rats

APPENDIX 2

Lamb born by pregnant Yankasa ewes experimentally infected with *T. evansi*at the second

trimester of pregnancy

APPENDIX 3

Mean ± SEM weekly temperature of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

Week	Control (^{0}C)	Infected (^{0}C)
0	38.00 ± 0.36	38.33 ± 0.11
1	38.60 ± 0.14	38.90 ± 0.04
2	38.25 ± 0.10	38.10 ± 0.07
3	38.45 ± 0.11	38.01 ± 0.18
4	38.37 ± 0.13*	38.90 ± 0.04*
5	38.62 ± 0.21	38.48 ± 0.21
6	38.30 ± 0.16*	38.77 ± 0.09*
7	38.60 ± 0.28	38.00 ± 0.07
8	38.50 ± 0.09*	38.50 ± 0.07*
9	38.60 ± 0.10*	38.78 ± 0.08*
10	38.40 ± 0.09	38.38 ± 0.10
11	37.87 ± 0.15*	37.70 ± 0.04*
12	38.40 ± 0.04*	38.68 ± 0.10*
13	38.95 ± 0.03	38.75 ± 0.13

* $P < 0.05$

APPENDIX 4

Mean ± SEM temperature changes in pregnant ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (^{0}C)	Infected (^{0}C)
0	38.60 ± 0.60	38.84 ± 0.10
1	38.48 ± 0.09	39.07 ± 0.13
2	38.60 ± 0.10	38.78 ± 0.12
3	38.37 ± 0.09	38.41 ± 0.11
4	38.87 ± 0.15*	39.07 ± 0.19*
5	38.20 ± 0.04	38.03 ± 0.11
6	38.95 ± 0.02	38.89 ± 0.07

* $P < 0.05$

APPENDIX 5

Mean ± SEM weekly weight of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

Week	Control (Kg)	Infected (Kg)
0	19.00 ± 1.47	19.17 ± 1.17
1	19.25 ± 1.60	20.00 ± 1.16
2	20.00 ± 1.08	21.17 ± 1.13
3	20.50 ± 1.26	21.33 ± 1.09
4	20.75 ± 1.03	21.17 ± 1.01
5	21.25 ± 1.11	21.33 ± 1.17
6	21.75 ± 0.85	21.83 ± 1.14
7	21.25 ± 0.85	22.67 ± 1.05
8	21.25 ± 0.85	23.50 ± 1.03
9	21.75 ± 1.49	23.33 ± 0.99
10	23.00 ± 0.71	23.67 ± 1.05
11	22.50 ± 0.87	23.00 ± 0.93
12	22.75 ± 1.11	22.83 ± 0.91
13	24.00 ± 1.22	23.50 ± 1.06

APPENDIX 6

Mean ± SEM weekly weight of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (Kg)	Infected (Kg)
0	21.25 ± 0.85	20.71 ± 1.28
1	21.25 ± 0.85	20.57 ± 1.02
2	21.75 ± 1.49*	18.38 ± 0.71*
3	23.00 ± 0.71	19.00 ± 1.51
4	22.50 ± 0.87*	17.86 ± 1.08*
5	22.75 ± 1.11*	16.57 ± 1.00*
6	24.00 ± 1.22*	17.25 ± 1.08*

* - $P < 0.05$

APPENDIX 7

Mean ± SEM blood glucose of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

Week	Control (mg/dL)	Infected (mg/dL)
0	72.00 ± 4.10	68.00 ± 2.42
1	67.20 ± 6.67	64.17 ± 2.95
2	67.75 ± 5.92	71.20 ± 4.00
3	69.00 ± 7.93	63.60 ± 3.12
4	56.50 ± 5.52	60.20 ± 3.09
5	64.40 ± 5.72	61.40 ± 4.82
6	63.00 ± 5.34	61.33 ± 6.14
7	62.25 ± 2.87	55.67 ± 3.50
8	65.50 ± 4.52	62.17 ± 2.60
9	67.25 ± 4.39	59.67 ± 4.27
10	64.67 ± 6.36	61.00 ± 5.36
11	64.80 ± 3.22	59.67 ± 5.36
12	73.20 ± 6.72	63.67 ± 2.86
13	69.60 ± 6.88	63.33 ± 2.97

APPENDIX 8

Mean ± SEM blood glucose of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (Kg)	Infected (Kg)
0	62.25 ± 2.87	54.33 ± 2.87
1	65.50 ± 4.52	55.67 ± 1.33
2	67.25 ± 4.39	56.25 ± 2.14
3	64.67 ± 6.36	56.63 ± 3.83
4	64.80 ± 3.22	57.57 ± 3.78
5	73.20 ± 6.72	65.25 ± 8.29
6	69.60 ± 6.88	62.40 ± 3.70

APPENDIX 9

Mean ± SEM Packed Cell Volume of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

Week	Control (%)	Infected (%)
0	31.25 ± 1.50	28.60 ± 0.98
1	29.67 ± 1.20	27.20 ± 0.49
2	29.75 ± 2.75	27.60 ± 1.20
3	30.20 ± 1.93	29.83 ± 1.44
4	30.75 ± 1.49	30.33± 0.84
5	31.00 ± 1.00	31.85 ± 1.11
6	31.20 ± 1.24	28.87 ± 2.65
7	30.80 ± 2.20	26.83 ± 2.98
8	30.40 ± 2.14	24.50 ± 1.73
9	28.40 ± 1.40	25.83 ± 1.78
10	27.75 ± 1.11	24.33 ± 1.69
11	28.00 ± 1.67	23.00 ± 1.79
12	28.00 ± 1.41	24.67 ± 1.87
13	28.75 ± 2.32	25.17 ± 1.52

APPENDIX 10

Mean ± SEM Packed Cell volume of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (Kg)	Infected (Kg)
0	30.80 ± 2.20	31.28 ± 2.32
1	30.40 ± 2.14	25.29 ± 1.51
2	28.40 ± 1.40	25.72 ± 1.52
3	27.75 ± 1.11	23.29 ± 2.49
4	28.00 ± 1.67*	22.71 ± 1.40*
5	28.00 ± 1.41	22.43 ± 1.89
6	28.75 ± 2.32*	20.50 ± 1.26*

* $p < 0.05$

APPENDIX 11

Mean ± SEM Leukocyte count of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

Week	Control (%)	Infected (%)
0	5.00 ± 0.11	5.72 ± 0.69
1	7.30 ± 1.20	4.24 ± 0.38
2	6.80 ± 2.16	6.64 ± 0.79
3	4.86 ± 1.04	4.60 ± 0.95
4	3.40 ± 0.50*	7.15 ± 0.76*
5	3.80 ± 1.70	6.08 ± 0.38
6	5.33 ± 0.87	7.46 ± 2.62
7	8.87 ± 0.47*	6.18 ± 0.71*
8	6.95 ± 1.06	7.65 ± 0.77
9	7.60 ± 2.16	6.13 ± 1.11
10	8.63 ± 0.90	6.10 ± 0.70
11	3.60 ± 0.95	5.30 ± 2.05
12	6.80 ± 1.00	6.92 ± 0.96
13	6.95 ± 0.15	5.42 ± 1.01

* $p < 0.05$

APPENDIX 12

Mean ± SEM Leukocyte count of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (Kg)	Infected (Kg)
0	8.87 ± 0.47	5.53 ± 1.08
1	6.95 ± 1.06	9.88 ± 1.12
2	7.60 ± 1.16	6.10 ± 0.01
3	8.63 ± 0.90*	3.90 ± 0.01*
4	3.60 ± 0.95	5.10 ± 0.01
5	6.80 ± 1.01	6.08 ± 0.01
6	6.95 ± 0.15	6.80 ± 0.03

* $p < 0.05$

APPENDIX 13

Mean ± SEM total plasma protein level of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

Week	Control (g/L)	Infected (g/L)
0	5.45 ± 0.22	5.63 ± 0.19
1	5.50 ± 0.19	5.53 ± 0.18
2	5.40 ± 0.22	5.43 ± 0.12
3	5.40 ± 0.52	5.67 ± 0.15
4	5.70 ± 0.37	6.00 ± 0.16
5	5.75 ± 0.29	6.08 ± 0.05
6	6.20 ± 0.59	6.10 ± 0.04
7	5.70 ± 0.13*	6.33 ± 0.14*
8	5.65 ± 0.22	6.00 ± 0.05
9	5.80 ± 0.20	6.10 ± 0.09
10	5.60 ± 0.24	6.12 ± 0.20
11	5.53 ± 0.24*	6.16 ± 0.10*
12	5.15 ± 0.10*	5.93 ± 0.13*
13	5.40 ± 0.14*	6.16 ± 0.07*

* $P < 0.05$

APPENDIX 14

Mean ± SEM total plasma protein level in pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (g/L)	Infected (g/L)
0	5.70 ± 0.13	5.96 ± 0.09
1	5.65 ± 0.22	4.91 ± 0.21
2	5.80 ± 0.20*	5.23 ± 0.14*
3	5.60 ± 0.24	5.51 ± 0.15
4	5.33 ± 0.24	5.43 ± 0.17
5	5.15 ± 0.10	5.60 ± 0.20
6	5.40 ± 0.14*	4.86 ± 0.13*

* $P < 0.05$

APPENDIX 15

Mean ± SEM serum progesterone level of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

Week	Control (ng/mL)	Infected (ng/mL)
0	1.69 ± 0.63	2.22 ± 0.30
1	2.12 ± 0.26	2.67 ± 0.28
2	2.94 ± 0.64	3.39 ± 0.31
3	3.20 ± 0.63	3.57 ± 0.31
4	3.16 ± 0.40*	4.30 ± 0.25*
5	3.52 ± 0.83	4.60 ± 0.55
6	4.43 ± 0.60	5.67 ± 0.68
7	3.20 ± 1.15	4.88 ± 1.09
8	7.35 ± 1.00	6.86 ± 1.79
9	6.74 ± 1.22	6.04 ± 1.57
10	5.70 ± 0.54	6.38 ± 0.09
11	5.64 ± 1.01	7.16 ± 1.22
12	2.12 ± 1.03	2.11 ± 1.24
13	3.88 ± 1.51	2.87 ± 0.48

* $P < 0.05$

APPENDIX 16

Mean ± SEM serum progesterone level of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (ng/mL)	Infected (ng/mL)
0	3.20 ± 1.15	5.63 ± 2.33
1	7.35 ± 1.00	5.17 ± 1.66
2	6.74 ± 1.22	5.35 ± 2.10
3	5.70 ± 0.54	5.54 ± 1.01
4	5.64 ± 1.01	5.31 ± 1.66
5	2.12 ± 1.03	3.85 ± 2.14
6	3.88 ± 1.51	0.89 ± 0.54

APPENDIX 17

Mean ± SEM serum Pregnancy associated glycoprotein level of pregnant Yankasa ewes experimentally infected with *T. evansi* at the second trimester of pregnancy compared with the control

Week	Control (ng/mL)	Infected (ng/mL)
0	104.75 ± 14.46	120.09 ± 7.12
1	123.82 ± 10.46	103.95 ± 10.02
2	133.90 ± 22.15	101.20 ± 13.65
3	123.00 ± 24.11	85.49 ± 10.56
4	103.35 ± 12.35*	89.06 ± 7.93*
5	94.91 ± 1.12	77.21 ± 6.75
6	65.03 ± 9.37	82.46 ± 8.28
7	57.08 ± 0.53	68.66 ± 5.57
8	86.16 ± 5.50	69.19 ± 3.95
9	87.34 ± 8.07	95.88 ± 8.87
10	86.80 ± 7.09	108.23 ± 10.61
11	103.24 ± 11.64	109.65 ± 14.72
12	99.36 ± 6.32*	123.02 ± 3.51*
13	114.82 ± 10.70	120.64 ± 24.96

* $P < 0.05$

APPENDIX 18

Mean ± SEM serum Pregnancy associated glycoprotein level of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (ng/mL)	Infected (ng/mL)
0	57.08 ± 0.53	50.27 ± 5.26
1	86.16 ± 5.50	92.89 ± 18.06
2	87.34 ± 8.07	123.20 ± 17.00
3	86.80 ± 7.09	115.55 ± 26.16
4	103.24 ± 11.64	119.89 ± 4.39
5	99.36 ± 6.32	125.80 ± 19.58
6	114.82 ± 10.70	85.06 ± 10.87

APPENDIX 19

Mean ± SEM seum estrone sulfatelevel of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Week	Control (ng/mL)	Infected (ng/mL)
0	5.35 ± 1.03	5.75 ± 1.23
1	7.29 ± 1.81	6.86 ± 2.03
2	7.77 ± 1.95*	1.95 ± 0.83*
3	7.66 ± 2.52	3.11 ± 1.31
4	7.40 ± 1.06	6.62 ± 1.20
5	7.23 ± 0.40	7.78 ± 1.96
6	6.85 ± 2.05	5.67 ± 1.18
7	4.37 ± 1.76	5.25 ± 1.26
8	10.60 ± 2.21	7.49 ± 1.88
9	9.36 ± 3.32	6.88 ± 2.00
10	6.93 ± 1.08	6.91± 2.18
11	6.54 ± 1.62*	1.38 ± 0.75*
12	6.90 ± 1.61	6.94 ± 1.84
13	10.98 ± 1.97	6.70 ± 0.96

*P < 0.05

APPENDIX 20

Mean ± SEM serum estrone sulfatelevel of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

Week	Control (ng/mL)	Infected (ng/mL)
0	4.37 ± 1.76	8.49 ± 1.81
1	10.60 ± 2.21	7.53 ± 1.96
2	9.36 ± 3.32	8.86 ± 2.65
3	6.93 ± 1.08	12.81 ± 3.73
4	6.54 ± 1.62	13.00 ± 2.50
5	6.90 ± 1.61	8.59 ± 1.16
6	10.98 ± 1.97	11.35 ± 2.73

APPENDIX 21

Mean ± SEM serum cortisollevel of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

Week	Control (ng/mL)	Infected (ng/mL)
0	61.25 ± 2.95	56.18 ± 4.97
1	61.03 ± 3.06	55.22 ± 5.84
2	58.64 ± 6.60	46.92 ± 7.39
3	57.95 ± 3.87	46.82 ± 7.00
4	57.75 ± 3.62	45.49 ± 5.95
5	64.19 ± 3.92	53.22 ± 6.44
6	56.13 ± 5.01	57.58 ± 3.15
7	61.38 ± 3.28	45.83 ± 5.82
8	56.77 ± 5.93	50.21 ± 7.28
9	51.14 ± 5.72	43.86 ± 5.99
10	43.37 ± 5.35	54.82 ± 4.72
11	48.07 ± 2.62	40.86 ± 7.46
12	60.74 ± 6.01*	38.42 ± 5.17*
13	48.27 ± 6.47	36.52 ± 5.94

*$P < 0.05$

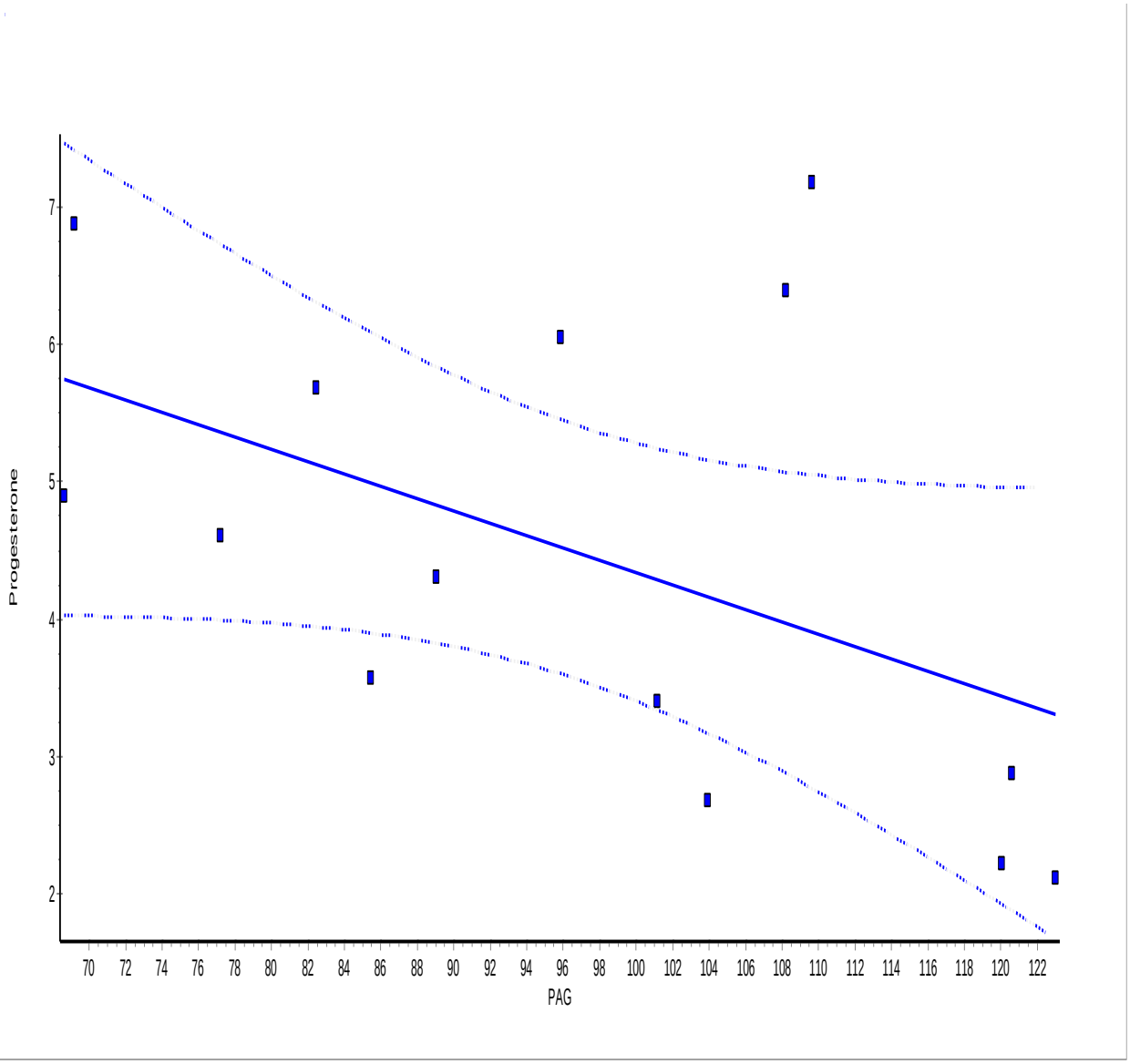
APPENDIX 22

Mean ± SEM serum cortisollevel of pregnant Yankasa ewes experimentally infected with *T. evansi* at the third trimester of pregnancy compared with the control

Week	Control (ng/mL)	Infected (ng/mL)
0	61.38 ± 3.28	63.15 ± 2.64
1	56.77 ± 5.93	63.39 ± 3.69
2	51.14 ± 5.72	62.53 ± 9.77
3	43.37 ± 5.35*	62.19 ± 2.59*
4	48.07 ± 2.62	58.01 ± 3.20
5	60.74 ± 6.01	63.16 ± 2.46
6	48.27 ± 6.47	53.91 ± 7.89

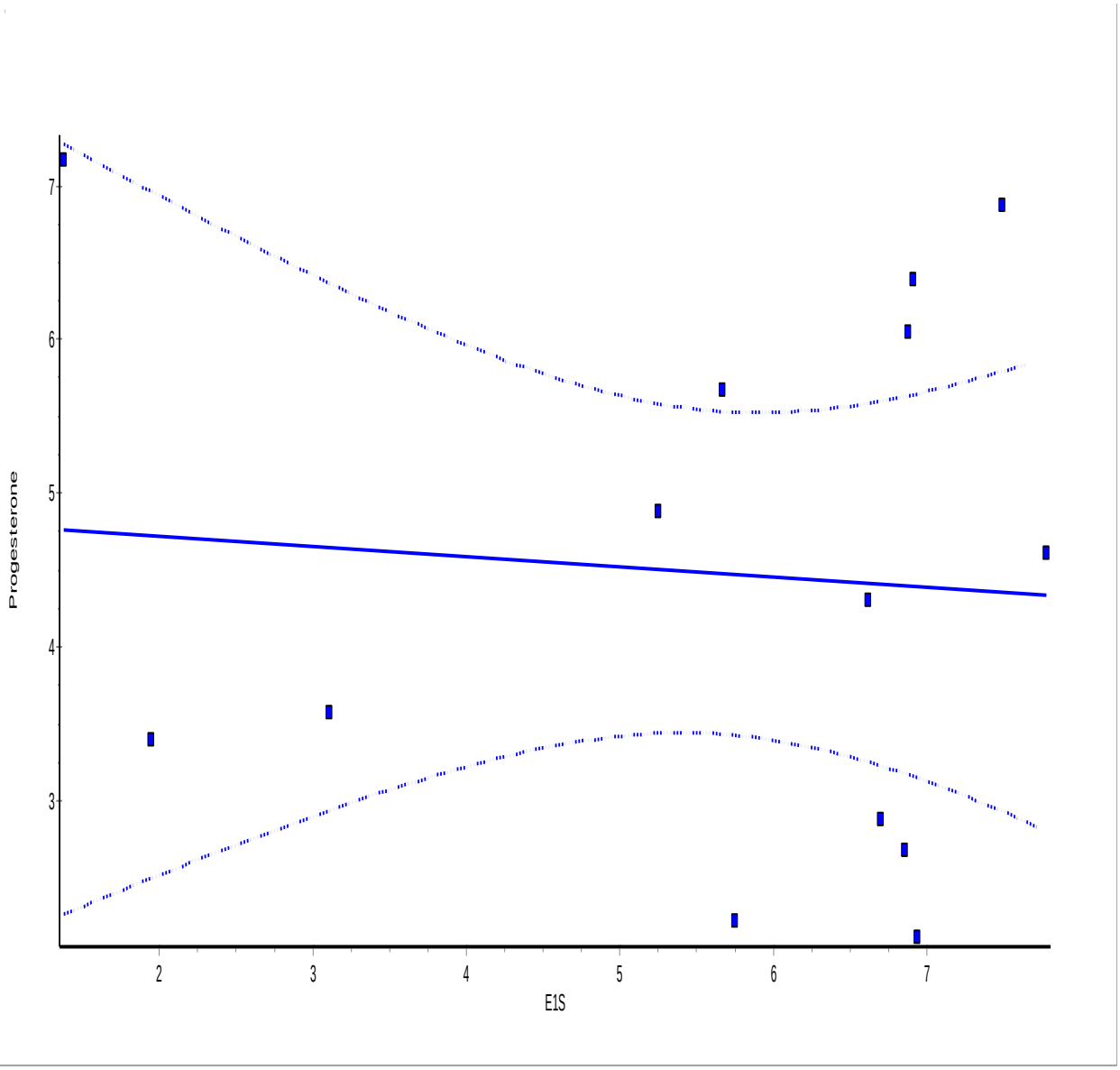
* $P < 0.05$

APPENDIX 23



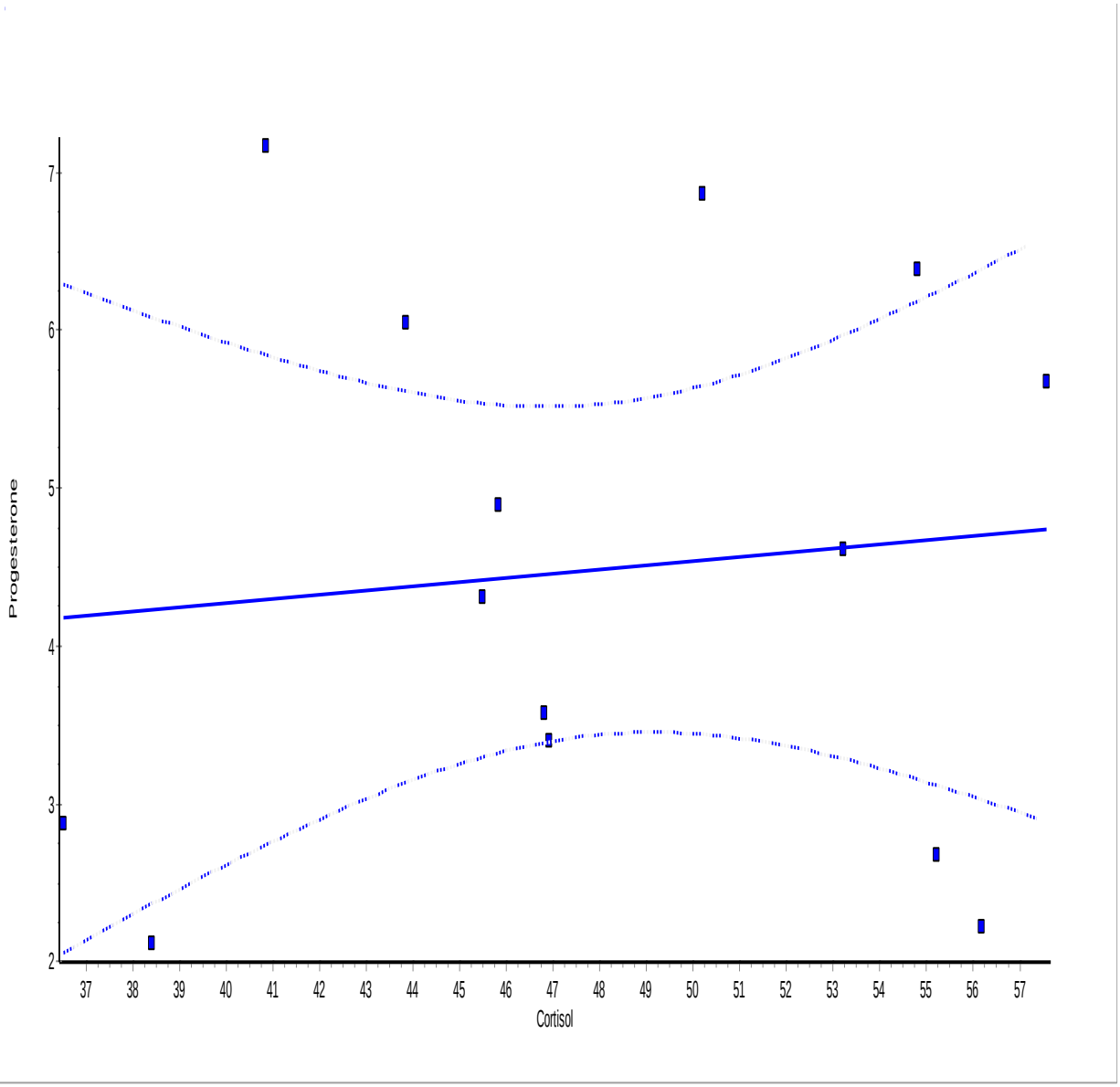
Diagrammatic expression of the correlation coefficient of progesterone and pregnancy associated glycoprotein(PAG) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

APPENDIX 24



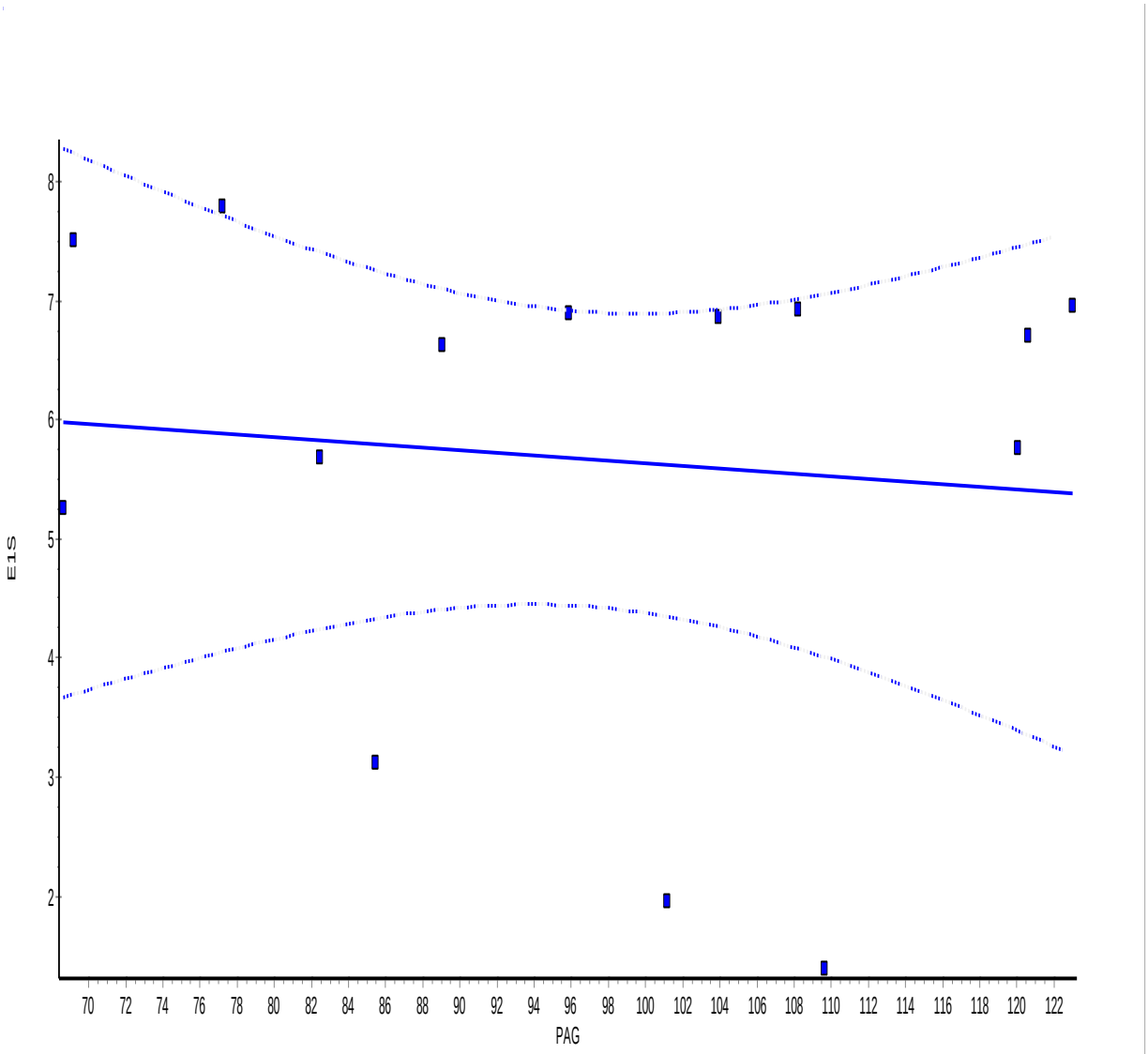
Diagrammatic expression of the correlation coefficient of progesterone and estrone sulfate (E_1S) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

APPENDIX 25



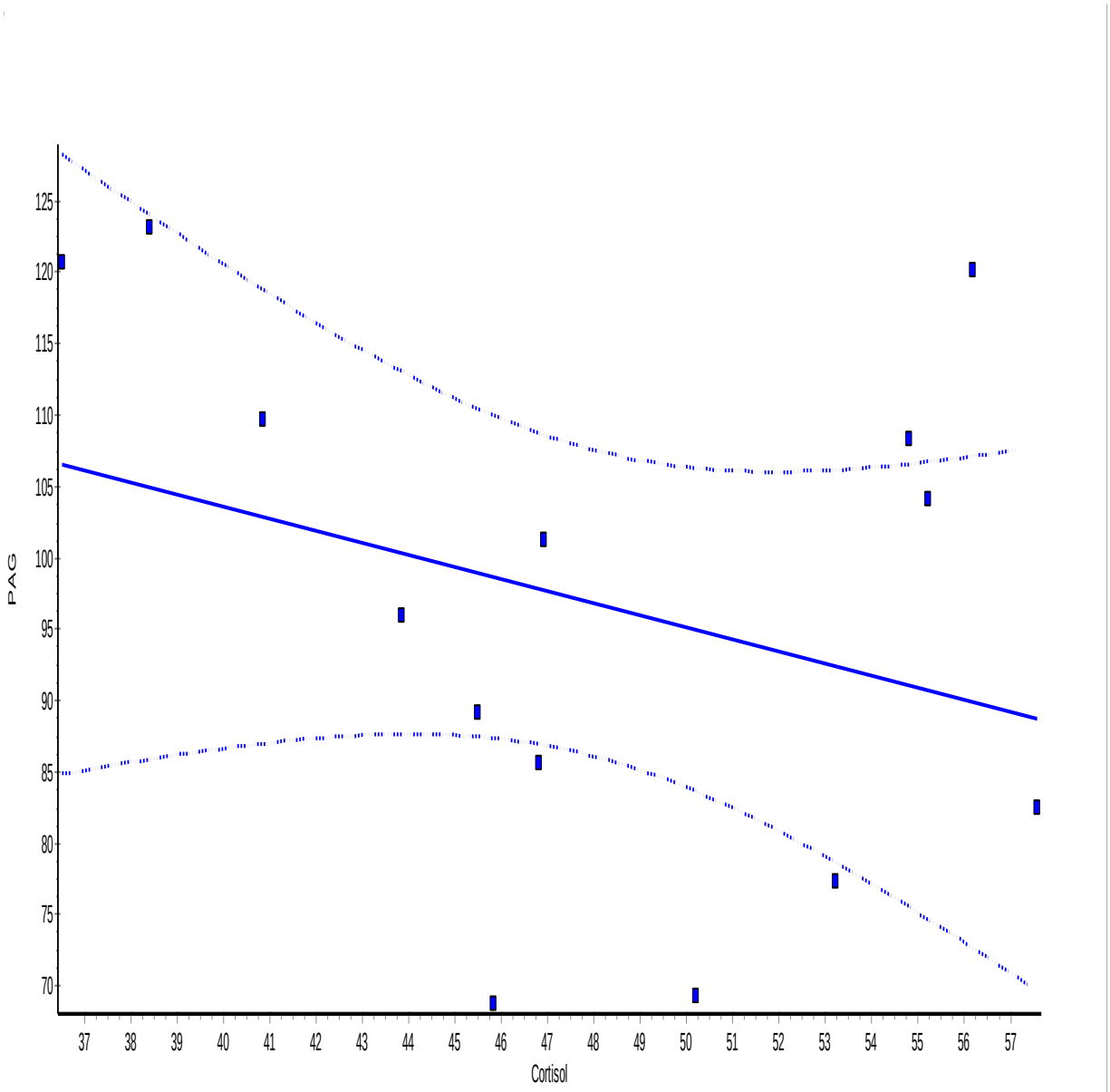
Diagrammatic expression of the correlation coefficient of progesterone and cortisol of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

APPENDIX 26



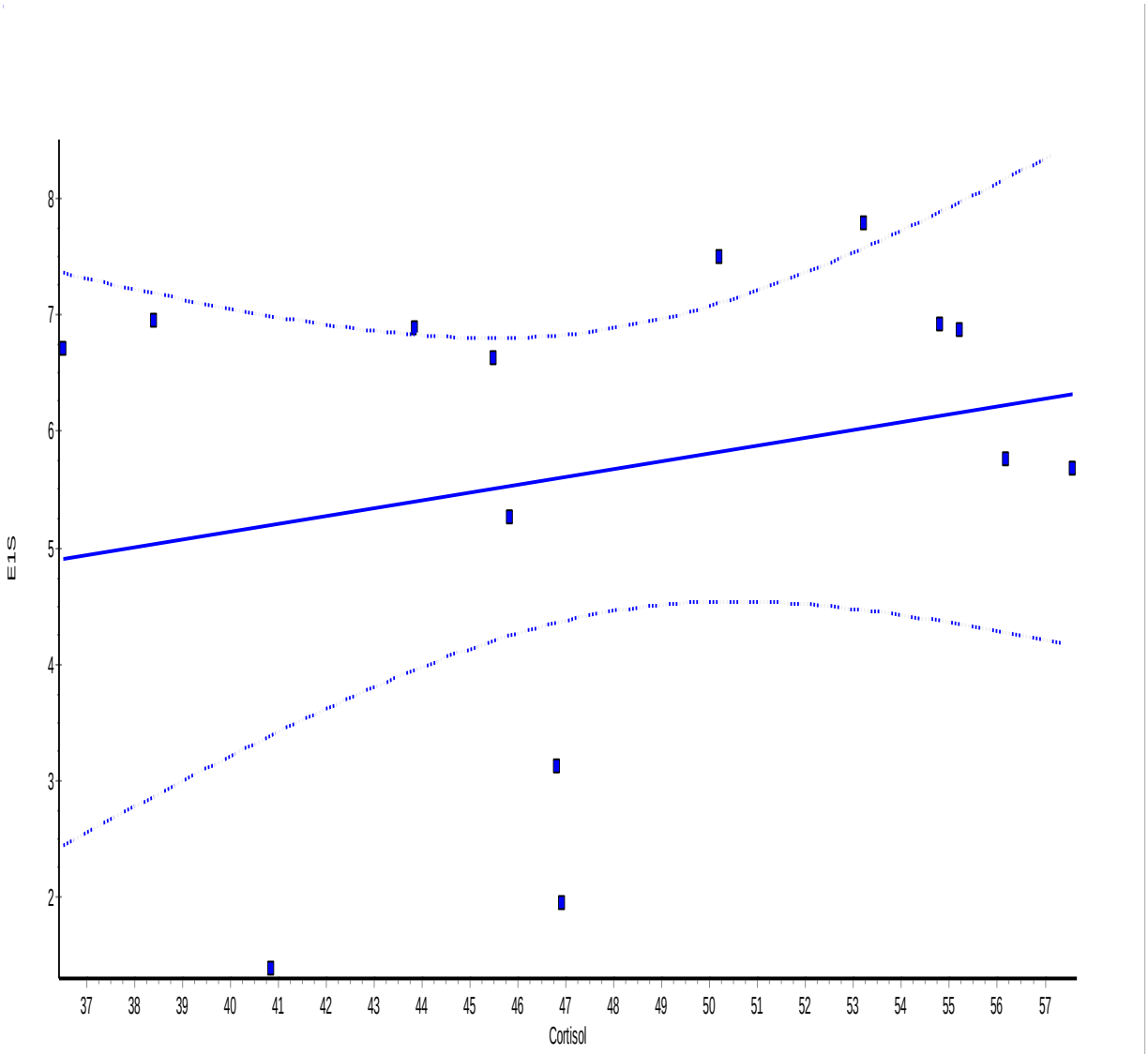
Diagrammatic expression of the correlation coefficient of estrone sulfate and pregnancy associated glycoprotein(PAG) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

APPENDIX 27



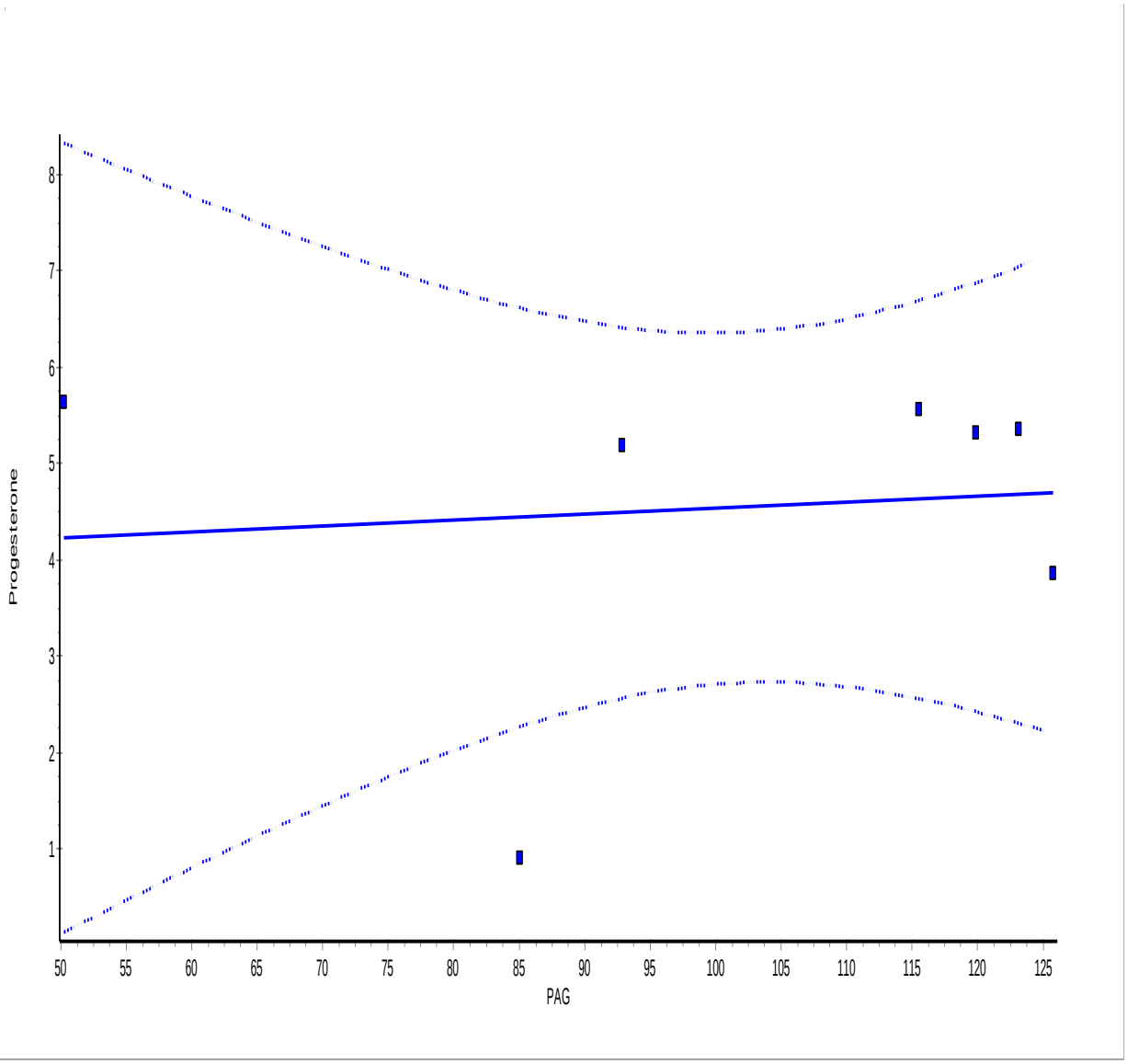
Diagrammatic expression of the correlation coefficient of cortisol and pregnancy associated glycoprotein(PAG) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

APPENDIX 28



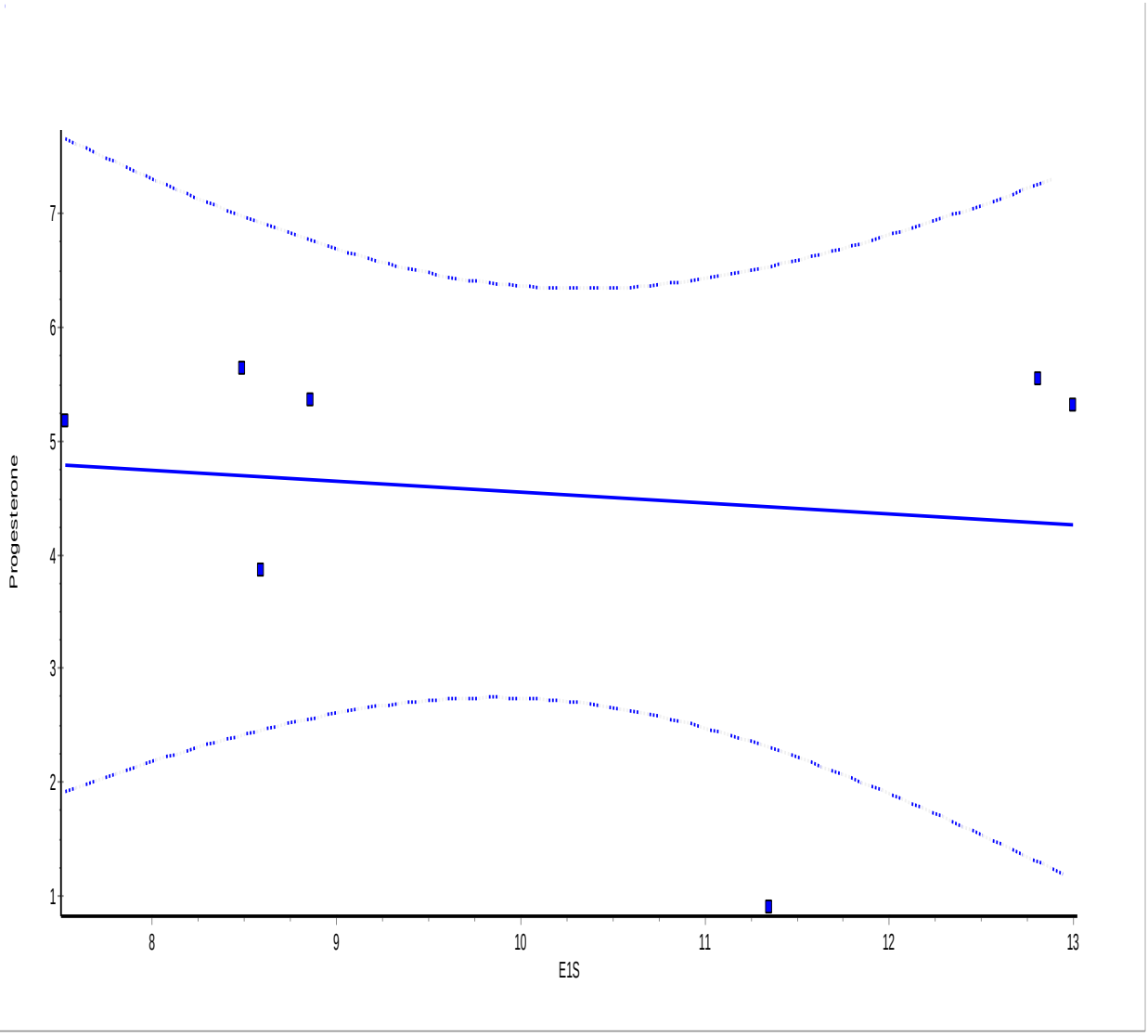
Diagrammatic expression of the correlation coefficient of cortisol and estrone sulfate (E_1S) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the second trimester of pregnancy compared with the control

APPENDIX 29



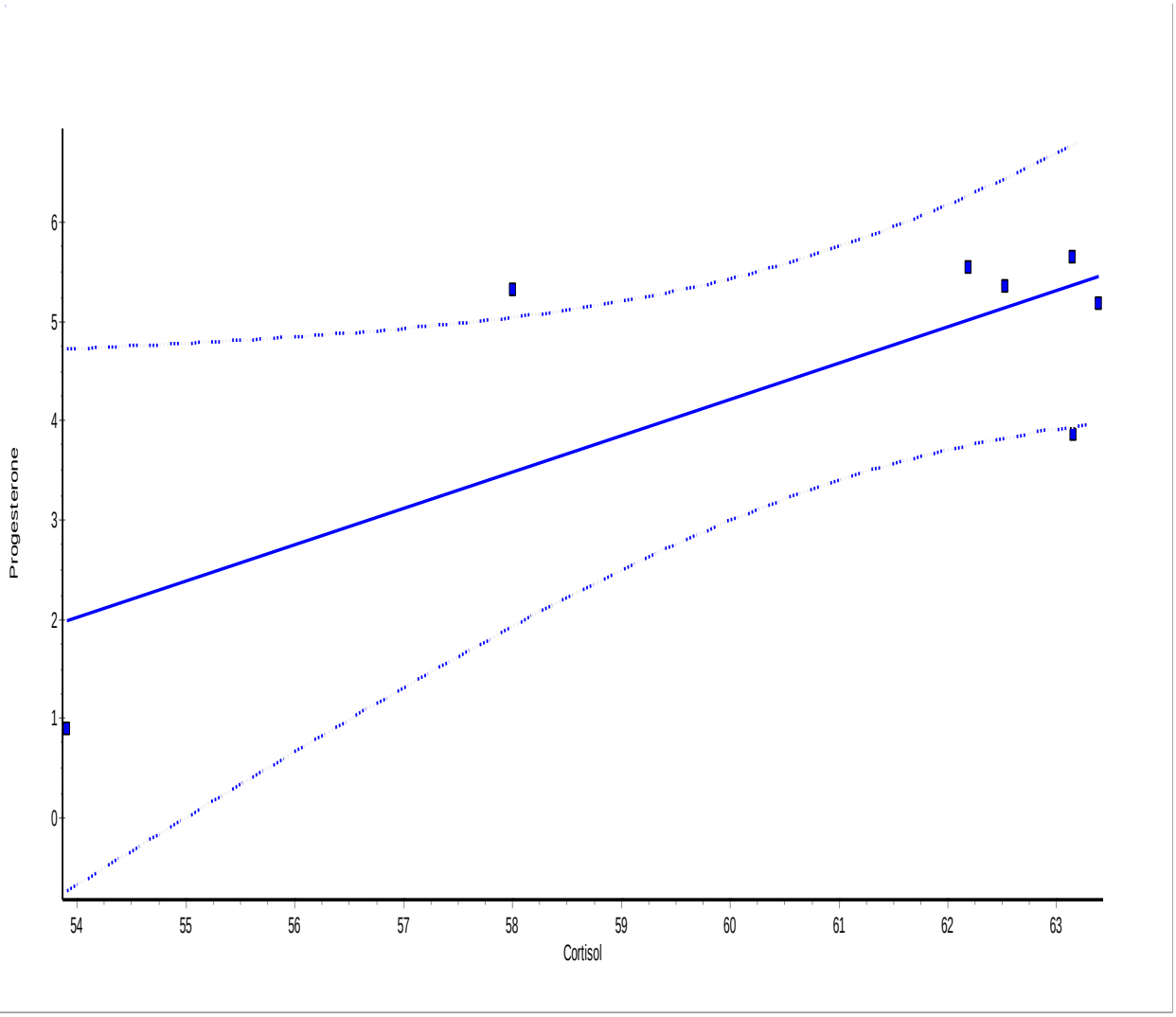
Diagrammatic expression of the correlation coefficient of progesterone and pregnancy associated glycoprotein(PAG) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

APPENDIX 30



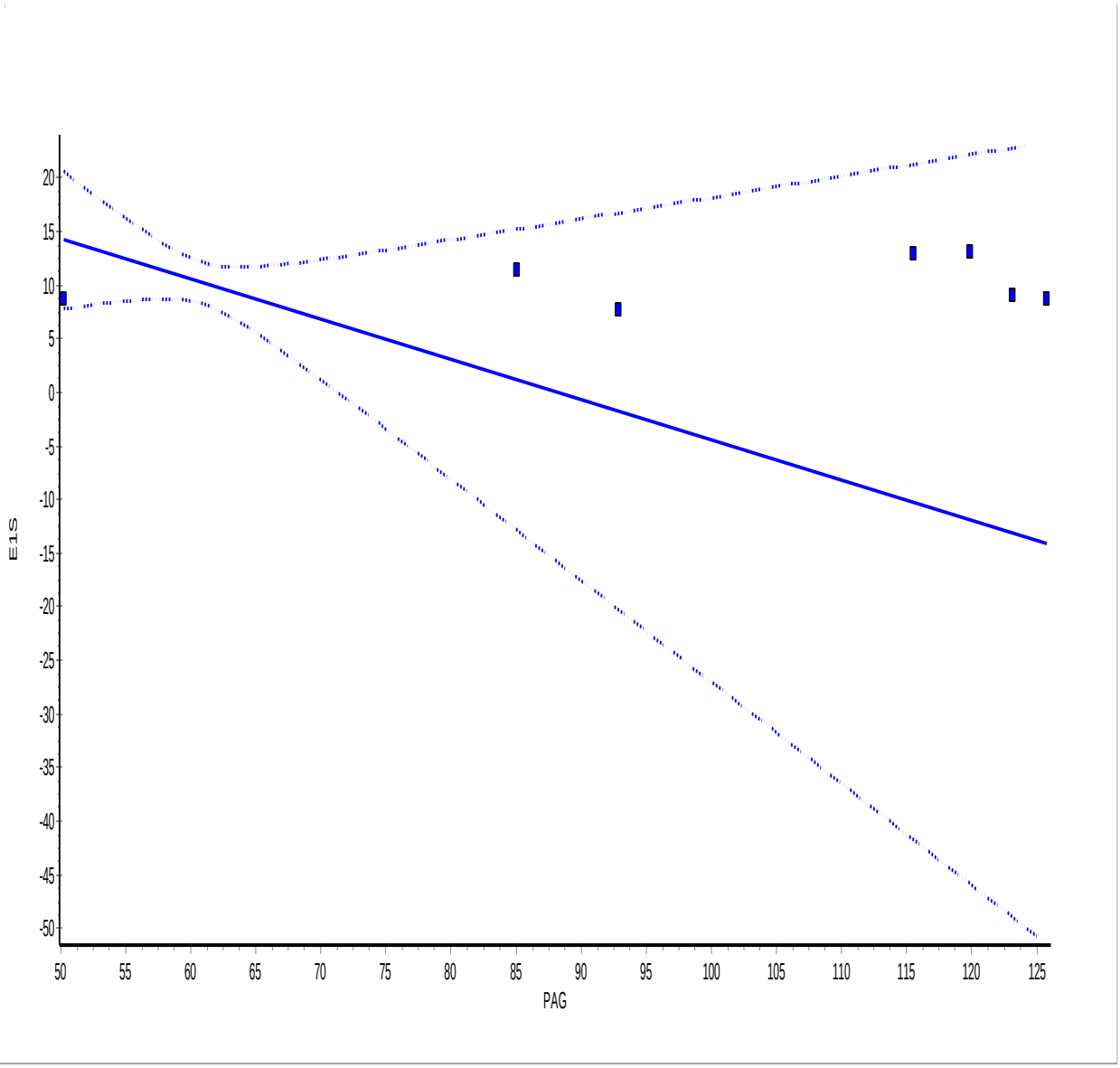
Diagrammatic expression of the correlation coefficient of progesterone and estrone sulfate (E_1S) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

APPENDIX 31



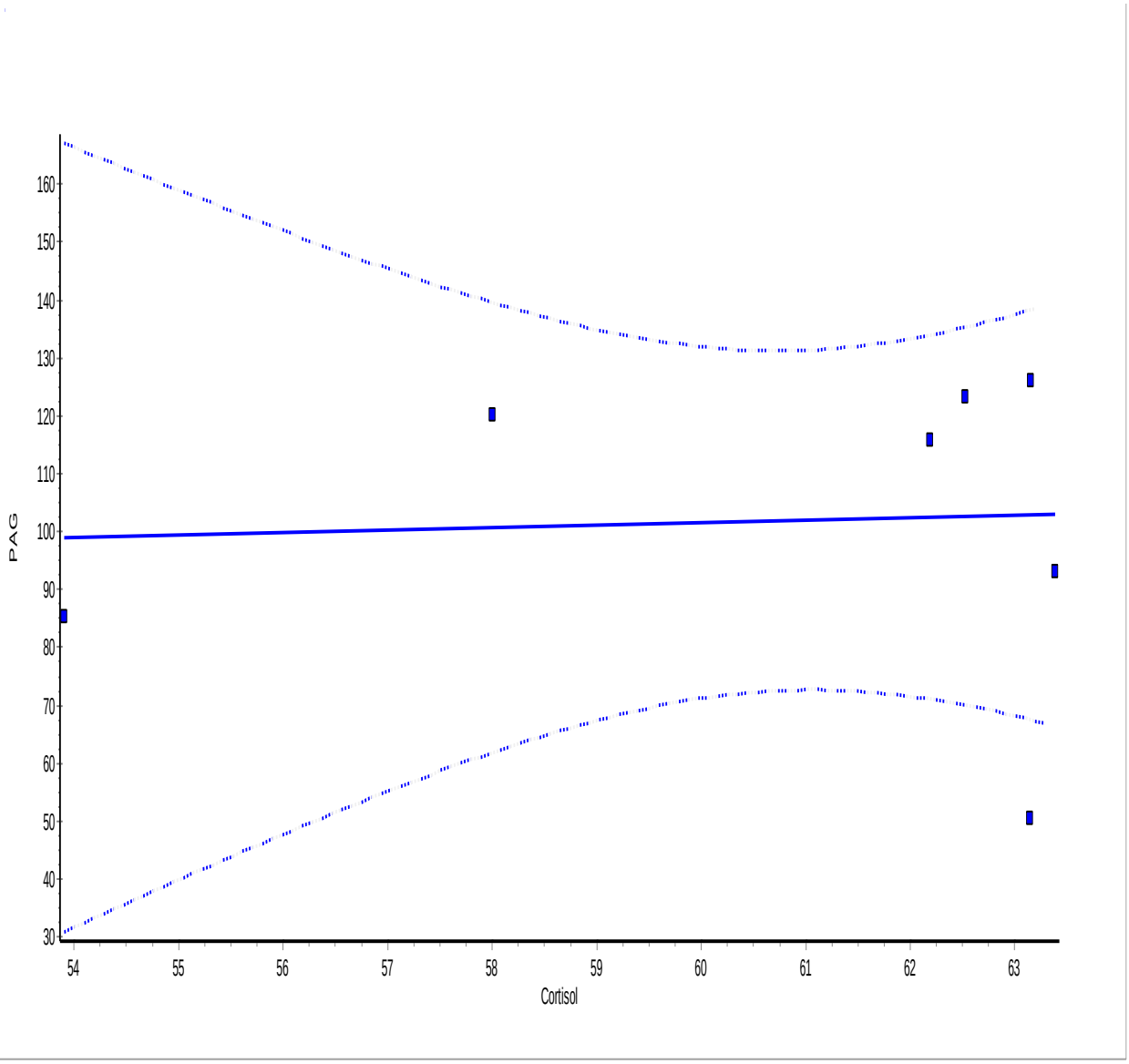
Diagrammatic expression of the correlation coefficient of progesterone and cortisol of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

APPENDIX 32



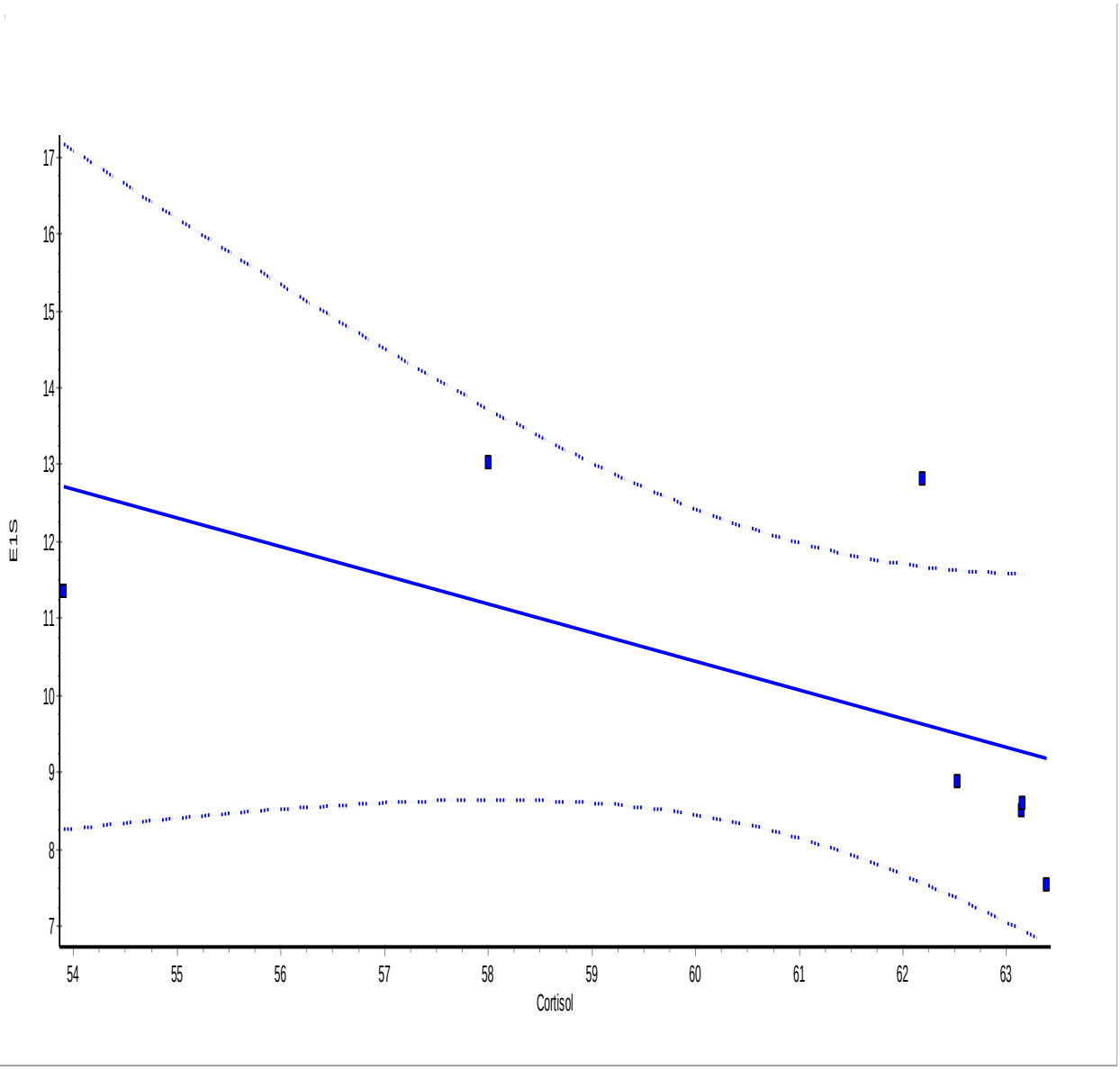
Diagrammatic expression of the correlation coefficient of estrone sulfate (E_1S) and pregnancy associated glycoprotein(PAG) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

APPENDIX 33



Diagrammatic expression of the correlation coefficient of cortisol and pregnancy associated glycoprotein(PAG) of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy compared with the control

APPENDIX 34



Diagrammatic expression of the correlation coefficient of estrone sulfate (E_1S) and cortisol of pregnant Yankasa ewes experimentally infected with *T. evansi*at the third trimester of pregnancy