PLASMA LUTEINIZING HORMONE (LH) IN *TRYPANOSOMA CONGOLENSE* INFECTED FEMALE GOATS AND THE RESPONSE TO GONADOTROPIN RELEASING HORMONE (GnRH)-AGONIST AND CLONIDINE

BY

NG'WENA, A.G.M.

DEPARTMENT OF MEDICAL PHYSIOLOGY

UNIVERSITY OF NAIROBI

KENYA

THESIS SUBMITTED IN PART FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL PHYSIOLOGY IN THE UNIVERSITY OF NAIROBI

1996

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature _____ Date 21/11/97

NG'WENA, A. G. M.

This thesis has been submitted for examination with our approval as University supervisors

Signature _____ Date 21·11·97

DR. E. O. WANGO
Department of Animal Physiology
University of Nairobi

Signature _____ Date 21/11/97

DR. N. B. PATEL
Department of Medical Physiology
University of Nairobi

DEDICATION

This thesis is a special dedication to my dear wife Grace Magak and children, Shem, Simon, and Jane.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENT

I wish to thank Dr. N. B. Patel and Dr. E. O. Wango, my supervisors, for suggestions and comments pertinent to this work, Messers H. Odongo, J. Owiti, C. Nzivo, and J. Winga for their technical assistance, and my wife and children for their patience during my absence from the family. This work received support from: Reproductive Biology Unit (RBU) of the University of Nairobi, Avenue Nursing Home, Dr. N. B. Patel, and the University of Nairobi.

ABSTRACT

Plasma Levels of Luteinizing Hormone (LH) in *Trypanosoma congolense* Infected Female Goats Challenged with Gonadotropin Releasing Hormone (GnRH)-agonist and Clonidine.

Trypanosomosis is a widespread tropical disease of humans and animals in Africa. Besides its reversal of sleep/wake cycle, trypanosomosis also causes reproductive dysfunctions. In trypanosome infected females, decrease in the plasma levels of oestrogen and progestrone have been reported as well as ovarian damage and lack of corpus luteum formation. However, while studies in males report no change or decrease in plasma LH, there are no reports on the effect of trypanosomosis on plasma LH levels in females during oestrus cycles.

Therefore plasma LH levels were measured by the use of bioassay and radioimmunoassay, over several oestrus cycles in non-infected and *T. congolense* infected female goats. The infected goats became parasitaemic 5-9 days after the infection and as the infection progressed they became anaemic, emaciated, and showed elevated rectal temperature. The white blood cell (WBC) count showed a sharp increase from a pre-infection value of $11.53 \times 10^3 \pm 1.1$ to $16.5 \times 10^3 \pm 0.76$ ($p<0.001$) between 20 and 30 days post infection. While in non-infected goats, the plasma LH showed a singular peak every 20-24 days (normal goat oestrus cycle range), in infected goats, a clear singular LH peak was not evident. Instead the plasma LH level showed rises at interval of 14-18 days in the first post-infection oestrus cycle and no LH rise in the subsequent cycle. There was a significant ($p<0.02$) difference in plasma LH basal level between non-infected and infected goats. To

investigate whether the failure of the LH rise was due to pituitary or hypothalamic dysfunction, the plasma LH levels were measured in response to a GnRH-agonist ([D-Ala6]-Luteinizing hormone releasing hormone) and clonidine (Catapres). With GnRH-agonist injection (20 μg) in the post-infection period, there was a significant ($p<0.01$) increase in plasma LH from the basal level in both infected (day 23: from 2.9 ± 0.95 IU/l to 11.2 ± 3.6 IU/l; day 60: from 4.8 ± 0.4 IU/l to 10.25 ± 2.5 IU/l) and non-infected goats (day 23: from 3.5 ± 0.5 IU/l to 6.2 ± 0.9 IU/l; day 60: from 2.4 ± 0.3 IU/l to 9.1 ± 2.9 IU/l). With clonidine injection (25 μg), in post-infection period, infected goats showed no pulsatile LH activity while the non-infected goats showed pulsatile changes in plasma LH levels. Peak LH amplitude was 3.1 ± 0.3 IU/l (day 28) and 6.9 ± 1.9 IU/l (day 69) and pulse frequency was 2-3/80 mins for both days of injection.

The results show that in trypanosomosis there is failure in the regular periodic increase in plasma LH during the oestrus cycle. Concidering the plasma LH response to GnRH, this failure cannot be attributed to the impairment of LH synthesis and secretion by the pituitary. However, the LH lack of response to clonidine injection, suggests that trypanosomosis causes disruption of the synchronised release of GnRH by the hypothalamic neurons which could possibly account for the absence of the regular periodic LH surge in the oestrus cycle.

CHAPTER 1

INTRODUCTION

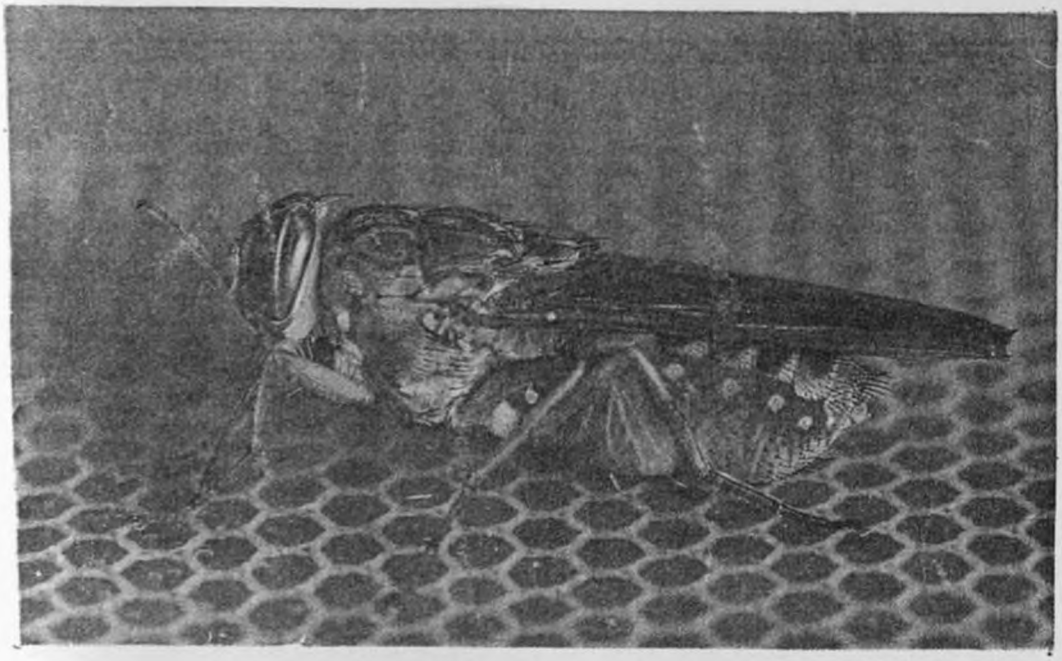
Trypanosomes are vertebrate parasites of wild and domestic animals as well as humans. They are found mainly in Sub-Saharan Africa, S. America and Asia. In Africa, the *brucei* groups of trypanosomes species are transmitted to their warm blooded hosts by the blood sucking tsetse flies of the *Glossina* species (Fig 1). *Trypanosoma cruzi (T. cruzi),* which is the main trypanosome species in S. America and Asia, is transmitted by the *Reduviid* flies. However, in some parts of S. America, *T. cruzi* can be transmitted to rats by fleas.

Trypanosomes, depending on the species, either live extracellularly in the blood or intracellularly, especially in reticuloendothelial tissues of the heart tissue (Vickerman, 1985). *Trypanosoma brucei brucei* (*T.b. brucei*), *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*), *Trypanosoma brucei gambiense* (*T.b. gambiense*), *Trypanosoma congolense* (*T. congolense)* and *Trypanosoma vivax (T. vivax),* are mainly heamatic trypanosomes. *T. congolense* undergoes extravascular development at the site of tsetse fly bite and in associated lymph nodes (Grays and Luckins, 1980) before entering the blood stream (Fig 1). Trypanosomes will localise to particular tissues of the body. *T. vivax* has been found extravascularly in the pituitary gland (Fiennes, 1950), cardiac muscles (Kimeto *et al.*, 1990), cerebrospinal fluid and aqueous humour (Whitelaw *et al.*, 1988).

In Africa, trypanosomes cause trypanosomosis*, a disease commonly known as African sleeping sickness in humans and related primates. The disease also affects

* Change of scientific name: From Trypanosomiasis to Trypanosomosis by the The International Commission on Nomenclature to bring it in line with the names given to similar diseases. Trypanosomosis has been adopted by a number of committees and organizations including the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC).

Figure 1. Tsetse fly of the Glossina species (upper figure) and trypanosomes in a blood specimen (lower figure). The round cells are red blood cells.



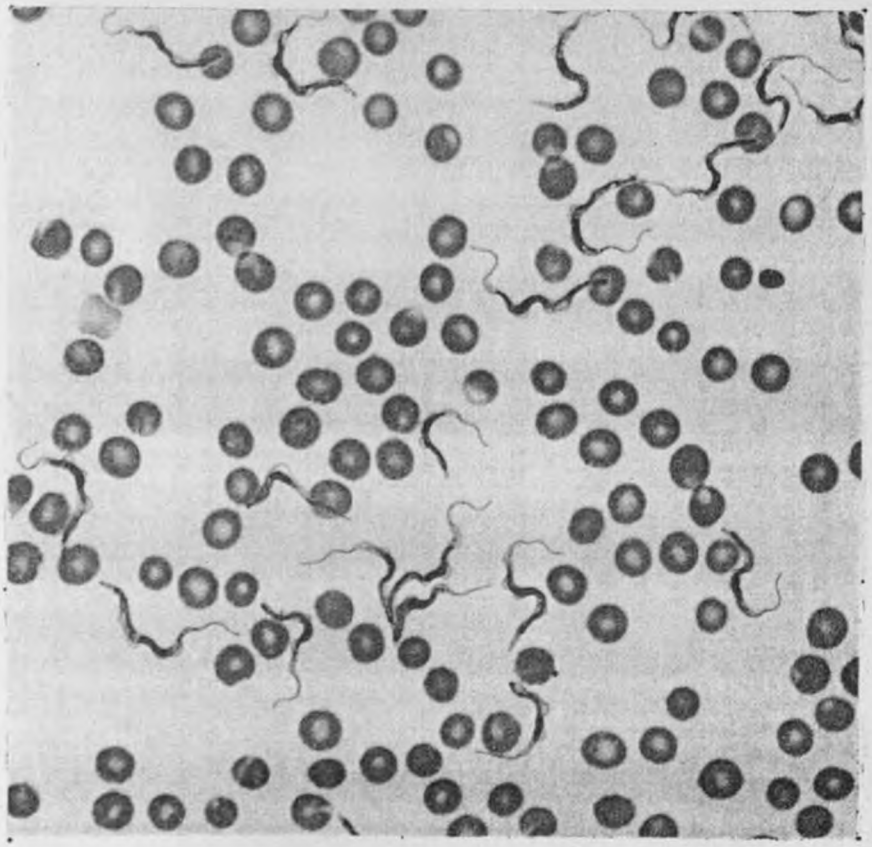


Fig. 1

Figure 2. Distribution of tsetse fly in the African continent. Major cattle ranching is in the tsetse fly free regions. In the tsetse fly infested region, cattle ranching is limited and suitable only for typanotolerent cattle are found.

Figure 2. Distribution of tsetse fly in the African continent. Major cattle ranching is in the tsetse fly free regions. In the tsetse fly infested region, cattle ranching is limited and suitable only for typanotolerent cattle are found.

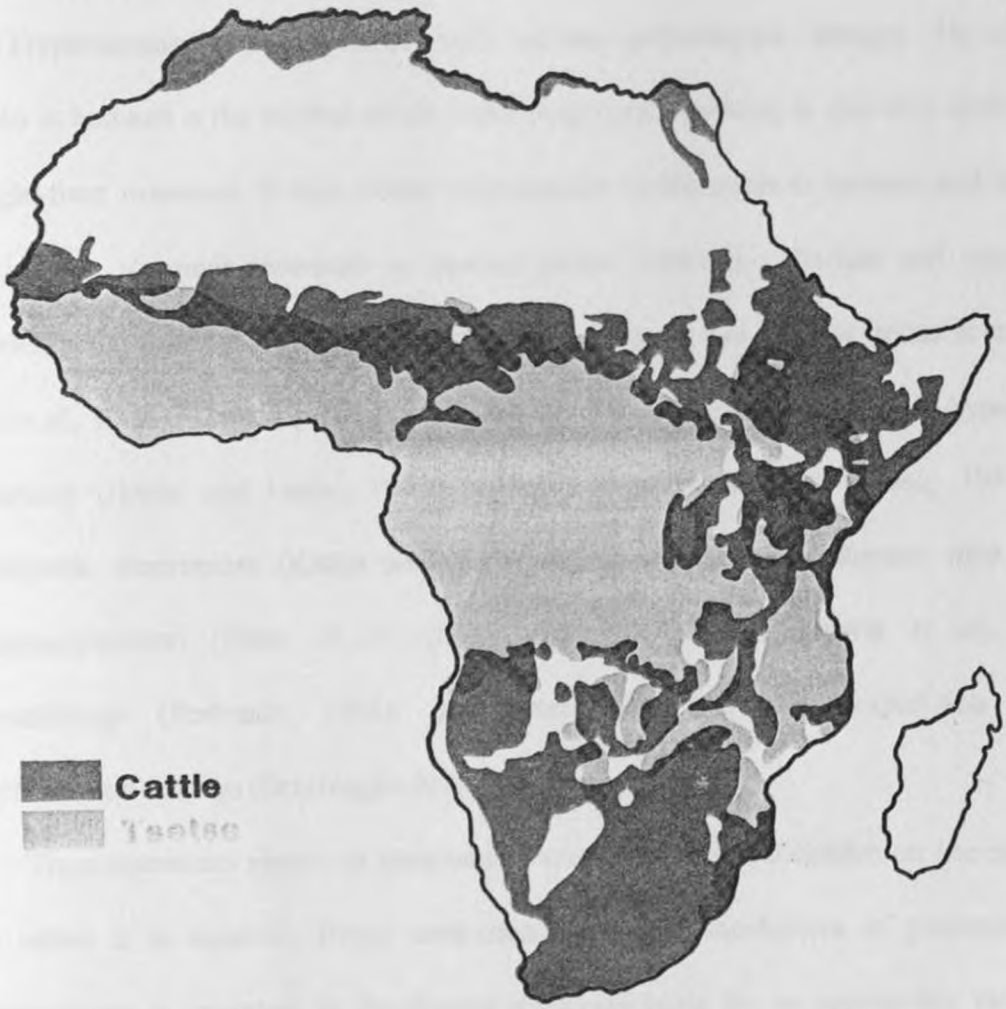


Fig. 2

non-primate animals. It is the most important livestock disease, and one of the major human tropical diseases, on the African continent. The disease is found over more than a third of the continent's area (Fig 2). In S. America, trypanosomes cause Chaga's disease which is quite widespread in that part of the world.

Trypanosomosis is associated with various pathological changes. Its classical symptom in humans is the reversal of the wake/sleep cycle resulting in day-time somnolence and night-time insomnia. It also causes reproductive dysfunction in humans and animals. These include irregular menstrual or oestrus cycles, infertility, abortion and impotence. Occasionally intrauterine infections can occur, resulting in still birth or neonatal mortality (Ikede *et al.*, 1988). These reproductive dysfunctions have been associated with hypophyseal malfunction (Ikede and Losos, 1975), gonadal lesion (Isoun and Anosa, 1974), and steroidogenic depression (Kaaya and Oduor-Okelo, 1980). The disease also causes immunosuppression (Blum *et al.*, 1993; Pentreath, 1991; Borowly *et al.*, 1990), neuropathology (Pentreath, 1995), and dysregulation of c-fos expression in the suprachiasmatic nucleus (Bentivoglio *et al.*, 1994).

Trypanosomosis places an enormous economic and social burden on the people in areas where it is endemic. Better understanding of the mechanism of pathogenesis in trypanosomosis is important in developing a rational basis for an appropriate therapeutic strategy to effect a cure and identify areas in which its economic impact can be reduced. Livestock productivity in tsetse fly infested areas is severely affected and finding ways of reducing, controlling, or reversing trypanosomosis related reproductive disorders could lead to a significant improvement in the living conditions in these areas.

Studies on trypanosomosis related reproductive disorders have shown that there is hormonal and gonadal dysfunction. Most of these studies have been carried out on male

animals. There have been no reports on the effect trypanosomosis on the plasma levels of luteinizing hormone (LH) which is essential for normal or regular reproductive cycle in females.

1.1 Aims and objectives of the present study

1.1.1 Broad objective

To determine the effects of trypanosomosis on the female reproductive system through measurement of luteinizing hormone (LH) levels.

1.1.2 Specific objectives

1. To determine the plasma luteinizing hormone (LH) levels and pattern during oestrus cycles in female goats before and after infection with *Trypanosoma congolense.*
2. To determine pituitary response to gonadotropin releasing hormone (GnRH)-agonist challenge in female goats before and after infection with *Trypanosoma congolense,* through measurement of plasma LH concentrations.
3. To determine the hypothalamic response to Clonidine (an alpha-2-adrenergic-agonist) challenge in female goats before and after infection with *Trypanosoma congolense,* through measurement of plasma LH concentration.

CHAPTER 2

LITERATURE REVIEW

2.1 Clinical manifestations of trypanosomosis in the vertebrate hosts.

Trypanosomes, depending on the species, can cause either acute or chronic diseases to their mammalian hosts. At the site of infection oedema may develop due to lymph exudation and cellular infiltration by lymphocytes associated with proliferation of histocytes (Soulsby, 1966). When the parasites enters the blood and the lymph, papulo-erythromatous eruption with cardiovascular involvement, anaemia, increase in serum immunoglobulin M (IgM), and hypoglycaemia can take place. Though, trypanosomes do not enter the red blood cells, they cause anaemia which in some animals results in nearly 50 percent drop in packed cell volume (PCV) within 4 to 6 weeks of infection (Luckins *et al.*, 1986). In the meningoencephalitic phase of the disease, particularly in humans, there is disturbances of the sleep/wake cycle, a confusional state, memory disturbance, behavioural disorders with psychotic manifestations, seizures, hyperthermia and disorders of the muscle tone (Adams *et al.*, 1986). The parasites enter blood vessels of the central nervous system (CNS) and in some cases infiltrate the cerebrospinal fluid (CSF) and multiply there.

Essential features of trypanosomosis include irregular fevers, lymphadenopathy, progressive emaciation and when the nervous system is invaded, drowsiness and stupor (Soulsby, 1966). The irregular fevers are accompanied in humans with headaches and endocrine disorders leading to impotence and ammenorrhea. Pruritus and pain are also common clinical features of the disease. In mammals, endocrine dysfunction and nervous system pathology are prominent clinical features of trypanosomosis and their detailed underlying pathogenic mechanism has not been elucidated completely.

2.2 Effects of trypanosomes on the immune system of the mammalian hosts

The chronic progression of the pathogenesis of trypanosomosis is linked to the inability of the host immune system to eliminate the parasite. In trypanosomes, the surface glycoprotein determinants, which stimulate B cells through T cell-dependent and T cell-independent pathways, change from one generation to the next through variation in their gene sequence (Blum *et al.*, 1993). Hence, by having variant surface glycoproteins (VSG), trypanosomes can evade the immune surveillance of the host. In each cycle, when sufficient antibodies to the surface coat have been produced, the majority of trypanosomes are eliminated and their destruction completed by Kupffer cells in the liver (Pentreath, 1991). In the next reproductive cycle, new antigenic variants repopulate the blood and other tissues and stimulate production of new antibodies. As each variant population is reduced by specific antibodies, variant antigenic type (VAT)- specific immunoglobulin, IgG and IgM levels decline, while the levels of IgG and IgM to the invariant trypanosome antigens remain high.

As parasitaemia develops there is non-specific stimulation of the macrophages and other lymphocyte cells (including B cells and helper and suppressor T cells). Although the B and T cells' responsiveness to a variety of non-parasite related antigens is increased, there is a decline in the ability of the immune system to induce T cell-dependent B cell responses. This is accompanied by progressive impairment of T cell responses, i.e. helper, suppressor and cytotoxic functions, and the infected host only continues with enhanced T cell-independent B cell functions (Pentreath, 1991). Thus trypanosomosis causes progressive immunosuppression which may also involve failure of the macrophages to present antigens normally to the T cells (Borowy *et al*,.1990)

Trypanosomes, especially *T. brucei*, release a Trypanosomes Lymphocyte Triggering Factor (TLTF) which induces interferon (IFN)-γ production from CD8+ T cells. IFN-γ

stimulates the growth and replication of the parasite (Bentivoglio *et al.*, 1994). This bi-directional signalling between the parasite and the CD8+ T cells of the host involving TLTF release and IFN-γ may account for the preferential localization of the trypanosomes in lymphoid tissues (Bakhiet, 1993). Besides the unexpected growth stimulatory effect on trypanosomes, IFN-γ induces macrophages to produce cytokines. These include the interleukin (IL)-1 and tumour necrosis factor (TNF) (Le and Vilcek, 1987), and transforming growth factor (TGF). There is also production of nitric oxide (Bentivoglio *et al.*, 1994). These molecules induced by IFN-γ could be involved in the pathology of trypanosomosis. For example, high level of IL-1 has a sleep and fever promoting effects and may cause hyperalgesia (Wisenfeld-Hallin *et al.* , 1991), and hypogonadism (Kinoshita *et al.*, 1982).

Trypanosomosis also influences prostaglandin secretion. Pentreath *et al.*, (1990) found in patients with sleeping sickness high levels of prostaglandin D_2 (PGD_2) in their CSF compared to normal people. In goats infected with *T. congolense*, there is also a marked increase in plasma prostanglandins especially PG-$F_2\alpha$ (Mutayoba *et al.*, 1989). The increased levels of PGD_2 is probably due to excessive stimulation of astrocytes. Trypanosome derived materials and endotoxins stimulate astrocytes to produce high levels of PGD_2 in *in vivo* preparations. In trypanosomosis there is also marked increase in endotoxin levels in the sera and CSF (Greenwood, 1974) which may cause increase in PG levels. Prostaglandins, especially PGD_2 influence the control of sleep (Hayaishi, 1991), cause immunosuppression (Goodwin and Webb, 1980), hyperalgesia (Malmberg and Yaksh, 1992) and suppression of luteinizing hormone levels (Kinoshta *et al.*, 1982). $PGF_2\alpha$, on the other hand, affects the corpus luteum functions (Mutayoba *et al.*, 1989). Hence, increased prostaglandin secretion in trypanosomosis may enhance immunosuppression and endocrine dysfuctions.

Some studies suggest that autoimmunity could also play a role in chronic trypanosomosis pathology. There is a wide range of autoreactive antibodies and T cells directed towards the host organs. When T cells from mice chronically infected with trypanosomes, are transferred to non-infected mice, inflammatory lesions develop in the nervous tissues (Said *et al.*, 1985; Hontebeyrie Joskowicz *et al.*,1987). However the antigen to which the T cells are directed is not known. The polyclonal activation during the acute phase of the infection may cause production of anti-self clones which could become persistant and aggressive against the host tissues. As the trypanosome has a large variety of antigenic substances, some of which may have cross-reactivity with host antigens, this could lead to an autoimmune response.

Molecular mimicry of the parasite also causes expansion of self-reactive T cells and B cell clones and a very aggressive immune response. Studies suggest the presence of antibodies which cross-react with host vasculature, muscle interstitium, heart, and nervous tissues. Antibodies against neurones seem particularly well represented in *T. cruzi* infection and lead to gastrointestinal tract denervation and contribute to cardiac pathology (Pentreath, 1995). Some epitopes expressed by *T. cruzi* cross-react with the nervous tissues leading to lymphocyte clones which target the nervous system. F1-160 is a *T. cruzi* flagella surface antigen which probably mimics components of the axon filaments of myenteric nervous tissue and its antibodies bind both rodents and human nervous tissues (Petry and Eisen, 1989; Petry and Van voorhis, 1991). It also mimics a 25 KDa protein found in cerebellar and myenteric neurones. This 25 KDa protein isolated from infected mice causes lethal paralysis when transferred to non-infected animals (Petry and Van Voorhis, 1991). Other antigens, found in patients with Chagas' disease, which cross-react with *T. cruzi* include carbohydrate components of sciatic nerve (Gea *et al.*, 1993) and acetylcholinesterase (Ouaissi *et al.*, 1988).

Although the importance of autoimmunity in chronic nervous pathology of Chaga's disease is well documented, it is still not clear what induces loss of self- tolerance which precipitates the damage. Parasitic proteins and lipopolysaccharides (Kierszenbaum and Budzko, 1973; Eakin *et al.*, 1992) together with genetic and other immunological factors have the potential to influence course of the disease. Immune complexes formed between parasite variant antigens and antibody circulating in blood may be deposited in organs invaded by trypanosomes (Poltera, 1980; Lambert, 1981). When these immunoregulatory substances are overproduced, they can have a cytotoxic effect. These effects of trypanosomes on the immune system may account for the enhanced immunopathological changes associated with trypanosomosis.

2.3.0 **Neuropathology of trypanosomosis**

Trypanosomosis affects the nervous system and some of the neurological symptoms are headache, irritability, changes in mood, and an altered EEG which show permanent delta wave forms (Pentreath, 1995). These correlate with meningeal inflammation and the patients experience hyperesthesia and mental deterioration which progresses to a reversal of the sleep/wake cycle. The basic behaviorial pattern is one of lethargy during which bouts of delirium, mania, paranoia, schizoid, and aggressive behaviour, likened to general paralysis of the insane, may occur. The disease progresses from meningitis to encephalitis, and in the absence of treatment, to coma, often followed by death (Apted, 1970).

Chronic trypanosome infections in humans cause diffuse central and peripheral demyelination (Dumas and Boa, 1988). Paraventricular and supraoptic hypothalamic nuclei neurones are the most affected. They are thought to be activated through retrograde signals from their terminals in the neurohypophyseal and median eminence, areas exposed to

trypanosomes or mediator substances. Although in experimental infections, the parasite seems not to penetrate the hosts' blood-brain-barrier, but localises itself in regions such as the choroid plexus, trigerminal and dorsal root ganglia, and the circumventricular organs which include pineal gland, median eminence and area postrema (Schultzberg *et al.*, 1988), the appearance in the brain of the fluorescent tracer, sulphorhodamine B, which normally does not cross the blood-brain-barrier, suggests the possibility of blood-brain barrier damage in chronic trypanosomosis (Philip *et al.*, 1995, in press). Changes in brain tissue density and electrolyte levels characteristic of vasogenic oedema have also been observed (Philip *et al* ., 1995, in press). The affected blood-brain barrier suggests that the nervous system alterations could be provoked by either molecules released by the parasite or by molecules released by host cells in response to the infection. In mice, damage to the CNS, resulting from the progressive infection has been observed and this has been related to autoantibodies to the myelin basic proteins, galactocerebrosides, and gangliosides (Hunter *et al.*, 1992b). Hence, demyelination may contribute to the neuropathology associated with trypanosomosis.

Astrocytes, which are neuroglial cells found in the brain, respond to a wide range of noxious stimuli such as physical trauma, toxic substances and parasitic organisms. The extent of the response and it's reversibility differs between stimuli and the brain area affected (Norton *et al.*, 1992). During reactive gliosis, cytokines and other mediator substances increase in the concentration (Eddlestone and Mucke, 1993). There is a high degree of astrogliosis found in trypanosome infected humans (Adams *et al.*, 1986) and animals (Stevens and Moulton, 1977; Anthoons *et al.*, 1989). It has been suggested that astrocytes being antigen-presenting and cytokine-producing cells, they might initiate and regulate the intracerebral inflammatory response at the blood-CNS interface during trypanosomosis

(Pentreath, 1989). When this immune responses become exaggerated, it could lead to damage of the blood-brain barrier and result in further nervous system complications.

Activation of astrocytes in trypanosome infected mice leads to increased cytokine messenger ribonucleic acid (mRNA) levels in the brain for IL-1α, IL-6, IFN-γ, and tumour necrosis factor (TNF) α (Hunter *et al.*, 1991, 1992a). Transcripts for cytokines associated with activated macrophages and T cells have also been detected. This suggests that altered cytokine production is associated with astrocyte activation at the start of the CNS inflammation. α–Difluoromethylornithine (DFMO) which is the drug of choice in chronic human condition reduces astroglial reaction (O'Callaghan and Seidler, 1992; Zoli *et al.*, 1993). This supports the view that astrogial cells are involved in the neuropathology of the disease. In the presence of *T. b. brucei* products, astrocytes in a cell culture produce prostaglandins PGD_2 and PGE_2 (Alafiatayo *et al.*, 1994), and increased PG production when the parasitic material is combined with a lipopolysaccharide. Thus the neurological symptoms in trypanosomosis show correlation with activated astrocytes products.

2.3.1 **Changes in gene expression: dysregulation of the biological clock.**

Trypanosomosis, in addition to the neurological changes described above, causes disturbances in the state of vigilance and alteration in the sleep/wake cycle. To determine which brain structures are affected in tryponosomosis, the expression of c-fos gene, a transcription regulatory factor involved in the transduction of extracellular events into short-term and long-term intracellular changes (Morgan and Curran, 1989; Schultzberg *et al.*, 1989), was investigated by immunohistochemical detection of the Fos nuclear proteins encoded for by the c-fos gene in trypanosome infected rats during sleep and wakefulness (Grassi-Zucconi *et al.*, 1993). In brains of uninfected rats, c-fos expression followed a

circadian rhytmn, whereas in *T. b. brucei* infected rats, kept under a 12 hr/12hr light/dark cycle, there was reversal of the c-fos gene expression in the suprachiasmatic nuclei (SCN). During the a wake (light off) period, the infected rats showed c-fos positive neurones in the SCN, while non-infected rats showed no c-fos immunoreactive neurones. These observations indicate that there is a selective dysregulation of SCN neuronal function during trypanosomosis.

In mammals the SCN synchronises or entrains the endogenous body rhythms to exogenous environmental cues such as the light/dark cycle (Meijer and Rietveld, 1989). The SCN receives light input from the retina through the retinohypothalamic tract (Morgan and Curran, 1991) and this light stimuli influences SCN activity (Aronin *et al.*, 1990; Korhauser *et al.*, 1990; Rusak *et al.*, 1990). The activity of SCN is also regulated by melatonin secreted from the pineal gland (McArthur *et al.*, 1991). As the trypanosomes have been detected around the pineal gland and ocular area, they could cause alterations in the activity of the ocular-SCN-pineal axis by interference of melatonin secretion by the pineal gland or light transduction by the retina.

When the SCN is destroyed by means of electrolytic lesions, the circadian rest-activity and sleep-wake cycle are abolished without altering the total sleep time (Meijer and Rietveld, 1989; Mistlberger *et al.*, 1983; Ibuka and Kawamura, 1975). In a diurnal primate, SCN lesions alter the daily total wake and sleep times and facilitate the initiation and maintenance of wakefulness (Edgar *et al.*, 1993). Therefore the dysregulation, in an otherwise structurally intact SCN, could affect the SCN's control of endogenous rhythms and thus lead to impaired functions of the organs that are influenced by SCN activity.

As the SCN and periventricular nuclei (PVN) are interconnected, the whole periventricular-hypothalamic axis could be affected in trypanosomosis. This pathological

effect may account in part for the neurological and psychiatric symptoms associated with African sleeping sickness. Although SCN axons do not have a direct access to the limbic and extrapyramidal systems (Watts *et al.*, 1987), their major target outside the hypothalamus is the PVN of the thalamic midline (Kalsbeek *et al.*, 1993). The PVN has projections to the amygdala, ventral striatum and the limbic cortex (Bentivoglio *et al.*, 1991). The septum, to which the SCN sends neuronal projections, is connected with the hippocampus. Therefore, alterations in the neuronal activity of these circuits could cause changes in affective and instinctual behaviour, locomotor activity as well as cognitive functions. As the reproductive system depends on the endogenous rhythms in hormone levels and neuronal activity, the effect of trypanosomosis on the biological clock could lead to disruption of reproductive function.

2.4 Effects of trypanosomosis on the responsiveness of the hypothalamic-pituitary -adrenal axis.

The hypothalamic-pituitary-adrenal axis, one of the hormonal axis affected in trypanosomosis (Abebe *et al.*, 1993), co-ordinates the production of several hormones in the body. In infected Boran (*Bos indicus)* cattle, the pituitary response to corticotropin releasing hormone (CRH) challenge is reduced. The change in plasma adrenocorticotropic hormone (ACTH) and cortisol concentration to CRH challenge is lower after infection than before (Abebe *et al.*, 1993). The adrenal gland response to ACTH however is the same in pre-infection, infection and post-infection periods. The reduced pituitary response to CRH challenge and the normal adrenal gland response to ACTH challenge suggests that pituitary dysfunction could be the primary cause of endocrine dysfunction in trypanosome infected animals. There are reports showing degenerative changes in the anterior pituitary glands of

infected animals (Isoun and Anosa, 1974). Lesions have also been observed in the pituitary glands of infected goats and sheep (Hawking and Greenfield, 1941; Losos and Ikede, 1970, 1972; Moulton and Sollod, 1976; Ikede *et al.*, 1977; Morrison *et al.*, 1981).

Trypanosomosis also causes marked pathological changes in the adrenal cortex (Mutayoba *et al.*, 1995). It enhances the adrenocortical secretion of cortisol and probably other steroid hormones, but lowers thyroxine (T_4) production (Mutayoba and Gombe, 1989). Cortisol secretion is induced by ACTH secreted from the anterior pituitary which in turn is stimulated by hypothalamic CRH as well as other mediators including vasopressin, noradrenaline and adrenaline all of which increase during stress (Moberg, 1985; Imura, 1985). The increased adrenocortical activity in the host is a response to the stressful condition caused by trypanosome infection. The increase in plasma adrenocortical hormone levels facilitates the various metabolic and defence mechanisms that aid the host to combat the infection.

In normal body function one of the roles of the hypothalamus is in stress mediation. Since trypanosomosis causes stress, the involvement of the hypothalamus in the observed endocrine dysfunction is possible. The dysregulation of the biological clock in trypanosomosis (Grassi-Zucconi *et al.*, 1995, in press; Bentivoglio *et al.*, 1995, in press) could disrupt hypothalamus function and along with the degenerative changes seen in the anterior pituitary glands could account for the low levels of plasma pituitary hormone in infected animals. However, it is not clear what pathogenic mechanisms lead to pituitary impairment, but since the pituitary is under the direct control of the hypothalamus, any changes in the hypothalamic function would be reflected in changes in the pituitary function first before being observed at other levels.

2.5. Effects of trypanosomosis on mammalian reproductive system.

2.5.0 Effects of trypanosomosis on the male reproductive system.

Male animals infected with trypanosomes show lesions in the gonads. There is more connective tissues seen in the caput and the cauda epididymis and the tubules are smaller in diameter with some of the cauda epididymis filled with cell debris (O'Hara and Gombe, 1989). The testes show marked reduction in spermatogenesis (Waindi *et al.*, 1986; Kaaya and Oduor-Okelo, 1980; Isoun and Anosa, 1974, ; Sekoni *et al.*,1988, 1990b) and spermatozoa are not seen in the lumina. In rams, there is a drastic and progressive deterioration in semen quality in infected animals. There is decrease in semen volume, cessation of semen production, oligozoospermia, a sharp decrease in progressively motile sperm, elevated number of dead (eosinophilic) sperm cells, and morphological abnormalities in all the sperms examined (Sekoni, 1992).

Initially, infection with *T. congolense* increases the mean plasma LH concentrations in rams which then decreases with the progression of the infection (Mutayoba *et al.*, 1994). However, there is a rapid decline in plasma testosterone concentration from the onset of parasitaemia to the final stages of the disease. The initially high levels of LH accompanying the decline in the plasma testosterone is due to the activity of the negative feedback mechanism. The low LH levels in the late stages of the infection appear not to be corrected for by the negative feedback mechanism. The LH levels remain low despite low testosterone levels. These observations suggest either a pituitary dysfunction or failure of the hypothalamus to release GnRH during trypanosomosis.

Mutayoba *et al.*, (1994) found that in rams the decline in plasma LH concentration was not associated with reduced sensitivity of the pituitary to GnRH or it's ability to release

LH since the LH response to exogenous GnRH was not impaired in the infected animals. However, testosterone levels did not increase with GnRH-induced LH release during the infection. These findings indicate that the decline in plasma LH concentration in infected rams could be due to reduced GnRH stimulation of the pituitary while the decline in plasma testosterone could be due, in part, to reduced sensitivity of the Leydig cells to circulating LH (Mutayoba *et al.*, 1994) or reduced LH receptors in gonadal tissues (Boly et al., 1994). Thus trypanonomosis may affect the release of hypothalamic releasing hormones resulting in reduced stimulation of the pituitary gonadotrophs to release LH.

2.5.1 **Effects of trypanosomosis on the fertility and oestrous cycles in female.**

Trypanosomosis affects the reproductive function of both trypanotolerant and trypanosensitive cows (Chicoteau *et al.*, 1990). Sterility, menstrual disorder, premature births, still births and high abortion rates have been reported (Apted, 1970; Parkne and Dhake, 1972; Murray *et al.*, 1981; Kanyari *et al.*, 1983; O'Hara *et al.*, 1985) in both laboratory and field conditions. The disease exerts a long lasting effect on ovarian function and, in some cases, the hosts become acyclic. Luckins *et al.*, (1986) found that while in non-infected female goats the normal oestrus cycle is 19-23 days, in infected goats the cycles are irregular varying in duration (53-97 days) as determined by oestrus behaviour and plasma progesterone levels. The low progesterone levels in the infected animals suggests that the luteal phase of oestrus cycle was prolonged. Low estradiol-17β levels have also been measured in trypanosomosis (Mutayoba *et al.*, 1988b). Histological examination of the ovaries (O'Hara and Gombe, 1989) showed partially denuded ovarian germinal epithelium, few primary follicles and many degenerative tertiary follicles without corpora luteum formation. Extensive lesions of the pituitary glands have also been observed (Hawking and Greenfield, 1941; Losos and Ikede, 1970, 1972; Moulton and Sollod, 1976; Ikede *et al.*,

1977; Morrison *et al.*, 1981), which could account for the disruption in gonadotropin pattern and gonadal dysfunction. Thus in trypanosomosis, the ovaries may not be stimulated to synthesize or to secrete ovarian steroids, or both. However as the level of plasma LH in trypanosome infected female animals have not been measured, it is not possible to determine the cause of reduced ovarian hormones.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental animals

Ten female goats of reproductive age were used in this study. These were bought from Karaba, Embu District, about 120 Km from Nairobi and transported to Chiromo campus, University of Nairobi where they were housed. Immediately on arrival, the goats were numbered with ear tags for identification. The animals were allowed 4 weeks acclimatization period during which time they were made accustomed to routine handling and also screened for parasites and other diseases. During the first week of acclimatization all the goats were given an antibiotic (Combiotic, 5 ml i.m., Bimeda) and dewormed with Combatrin (125 mg per goat). Two goats developed eye problem and were treated with 5 ml i.m. Tetroxy A (Bimeda) and a few drops were applied to their eyes. This treatment corrected the infection within three days. The animals were allowed a period of up to 7 hours grazing time every day and were housed in a fly free house in the night. Ewe pellets were given as a supplement feed and clean drinking water was available *ad libitum*.

3.1.1 Trypanosomes and goat infection

Trypanosoma congolense stabilate (EATRO 1753) was obtained from the Kenya Trypanosomiasis Research Institute (KETRI) in rat reservoirs. The parasites were passaged twice in the rats before infection of the goats. The rats were bled at peak parasitaemia by heart puncture under ether anaesthesia and the blood diluted in phosphate buffered saline

containing ethylenediamine tetra-acetic acid (EDTA) (Walker, 1970) to give a final haemocytometer count of 2.5×10^4 trypanosomes/ml.

Four goats selected randomly from the flock were each infected via the jugular vein with approximately 2.5×10^4 trypanosomes. Three goats served as controls. The infected and non-infected goats were housed and grazed together over the next 69 days, the duration of the study.

Of the ten starting goats, one became pregnant from a male in another flock during the acclimatization period. This goat was replaced. Three other goats died: one from unidentifiable causes during the pre-infection phase of the study, another from gastric obstruction due to swallowing a piece of cloth in the post-infection phase of the study, and the third due to the trypanosome infection three weeks after the infection. These three goats were not replaced. The data in this report is for six goats that survived and the one that died of trypanosomes infection, before its death.

3.2 Clinical determination

3.2.0 Body weight and temperature determination

During the acclimatization period, the goats were weighed thrice a week to determine their pre-infection body weight (kg) and to accustom them to the weighing process. The rectal temperature (°C) of each goat was also taken thrice a week. Body weight and rectal temperature measurement after the acclimatization period were done once a week. During the weighing process, each goat was placed into a weighing bag which was then attached to a weighing balance. The bag weight zeroed on the weighing scale. For rectal temperatures, a clinical thermometer with liquid oil applied to its tip to facilitate entry was

introduced into the rectum of the animals and temperature reading taken when the rectal temperature was stable. This was generally after 2-3 minutes.

3.2.1 Haematology and parasitology

During the fourth week of the acclimatization period, a 1 ml blood sample from the jugular vein was taken from each goat and then used to determine the following blood parameters: Red Blood Cell (RBC) count, White Blood Cell (WBC) count, and Packed Cell Volume (PCV) by the standard microhaematocrit method and by Coulter counter (Coulter Electronic Ltd., England). The haemoglobin (Hb) level for each goat was also determined with a haemoglobinometer. After the acclimatization period, haematology measurements were carried out on the goats fortnightly for the duration of the study.

Parasitaemia level in each goat was monitored weekly by buffy coat method as described by Paris *et. al.* (1982).

3.3 Hormonal analysis

3.3.0 Blood sampling and experimental design

Three ml blood samples were collected from the jugular vein of the goats every day between 8:00 and 10:00 am for five consecutive days after the acclimatization period and thereafter every other day except around the oestrus period when sampling was again done for three consecutive days. The sampling was done for 8 weeks in the pre-infection phase of the study and the blood samples collected during this period were used to determine the basal LH plasma levels. After the 8 weeks, the goats were infected with *T. congolense* and the blood sampling continued for another period of 10 weeks. Immediately after collection in phosphate buffered saline containing EDTA, the blood samples were centrifuged at 1500 g

for 10 min. The plasma was decanted and aliquoted into three tubes and stored at -20 °C for hormonal analysis.

The animals were challenged with clonidine (Catapres, Boehimger) 7 days before and 28 and 69 days after infection with *T. congolense* parasites. The GnRH challenge was given 23 and 60 days after infection. During the GnRH and clonidine challenge blood samples were collected with an indwelling jugular cannula (i.d 1.85mm, o.d 1.95mm, B. Braun Melsungen A.G., West Germany). Each goat was tied to a peg to immobilise it and then cannulated. Blood samples (3 ml) were collected at intervals of 10 and then 20 minutes for a period of 45 min before giving the GnRH-agonist ([D-Ala^6]-Luteinizing Releasing Hormone, L 1898; Sigma, St Louis). The GnRH-agonist was weighed and dissolved in sterile saline to make a concentration of 20 μg per ml. 1 ml of the solution was then given to each animal through the cannula and then further blood samples were collected at 10, 20, 40, 60, 80, 100, 120 and 140 min. In sheep this dose of GnRH-agonist provides a LH response lasting 2-3 hours (Siddall and Crighton, 1977). One week after the GnRH-agonist challenge, the goats were challenged with clonidine (Catapres, Boehimger). The goats were once again immobilised, cannulated and blood samples (3 ml) from the jugular vein collected at 10 min intervals for 40 min before the clonidine injection (25 μg, dissolved in 5ml of deionized water for injection) and then at 10 min intervals for the next 80 min after the injection. The clonidine injection was given through a micro-pore filter (0.05 millipores, Millipore Corporation, Bedford, Massachusetts).

3.3.1 Plasma LH concentration

Plasma LH concentration was determined by an improved *in vitro* bioassay method using mouse Leydig cell preparation (Van Damme *et. al.*, 1974). Mice between 6-10 weeks

were sacrificed with cervical dislocation, the testis decapsulated and placed in Eagle's Medium with 2% calf serum. The testis were than cut into small pieces and Eagle's Medium added to make a final a solution containing 2 testis/10 ml. The solution was then stirred and filtered through a fine nylon mesh. The filtrate was pre-incubated at 34 °C for 1 hr in O_2/CO_2 (93.5:6.5) atmosphere in a metabolic shaker (New Brunswick Scientific) at 80 rpm. After the pre-incubation, the cell suspension was centrifuged (130 g) at 4 °C for 15 min. The supernatant was then discarded and the cells resuspended in a fresh Eagle's Medium containing 2% calf serum. Assay tubes were prepared containing 25 x 10^4 cells to which LH standard and goat plasma was added. The tubes were incubated at 34 °C for 3 hr under a O_2/CO_2 atmosphere in a metabolic shaker set at 80 rpm. At the end of 3 hr, the tubes were placed on ice and the testosterone concentration in the tubes determined by radioimmunoassay using the materials provided by WHO and procedures as described in the WHO Matched Reagent Programme (1985).

The intra-assay coefficient of variation (CV) was 6.1 $\pm$ 0.4% at 3.76 $\pm$ 0.33 IU/ml. The inter-assay CV for 21 assays was 7.1 $\pm$ 0.06%. The detection limit of the assay, defined at 90% confidence limit was 0.44 IU/ml.

3. 4 Determination of oestrus cycles

The day of oestrus was taken as the highest plasma LH level (LH surge) measured over a period of two months. The days between the LH surge was taken as the period of the oestrus cycle. In the absence of infection, the duration of the oestrus cycle was between 20-24 days. Hence every 20-24 days after an LH surge another LH surge occurred (Fig. 10A,B). After infection the LH surge was not readily discernable. In these goats the highest plasma LH level occurring after 20-24 days after the previous LH surge was taken to

represent the LH surge (Fig 10B,C,D,E,F). The oestrus cycles were represented by synchronizing the plasma LH levels around the LH surge or the level on the expected day of the LH surge (Fig. 10A-F).

3.5 Statistical Analysis

All values are expressed as the mean $\pm$ standard error of the mean (SEM). Significance tests were carried out using the Student's t-test and expressed as the probability. The area under the curve was determined by the trapezoid rule.

CHAPTER 4

RESULTS

4.1 Clinical observations

All the infected goats became parasitaemic within 5-9 days after infection with *Trypanosoma congolense*. The first parasitaemic peak ($8 \times 10^4 \pm 2.3$ tryps/ml) occurred around 20 days after infection. The goat with the highest parasitaemic score (1.5×10^5 tryps/ml) on that day died within 24 hr. It had become lethargic, weak, anaemic, and showed reduced locomotory activity and severe respiratory distress. The remaining infected goats showed less severe symptoms of trypanosomosis. They remained parasitaemic for the duration of the study (69 days) though the parasitaemia levels in the blood fluctuated (Fig. 3). Trypanosomes were not detectable in the blood 30 days after infection; they reappeared 5 days later. In the control goats no trypanosomes were detected in the blood for the duration of the study.

Rectal temperature (°C) before and after infection was recorded at 5 day intervals in all the goats. The control goats maintained a temperature range of 36.7 - 38.9 °C throughout the study period while the infected goats had significantly elevated ($P<0.001$) temperature following the onset of parasitaemia. The mean rectal temperature in the infected goats, 20 days after infection, was 39.5 ± 0.32 °C compared to the rectal temperature in the control goats of 37.4 ± 0.65 °C. This rise in the rectal temperature in the infected goats coincided with the first peak of the parasitaemia wave. By day 30 post infection, the rectal temperature

Figure 3. Parasitaemia level (mean ± SEM) in *T. congolense* infected female goats. On day 0 the goats were infected with 2.4×10^4 trypanosomes.

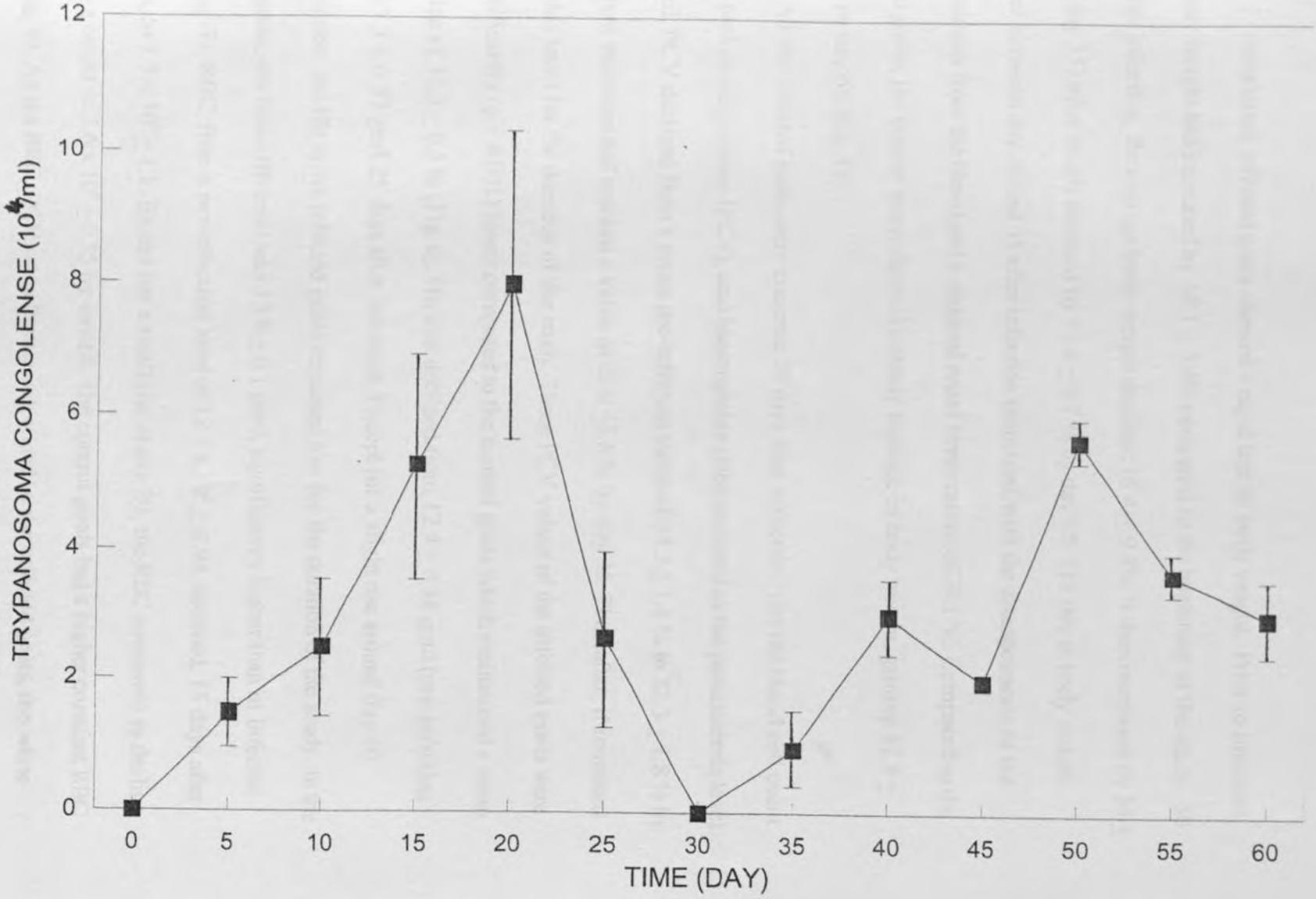

TRYPANOSOMA CONGOLENSE (10⁴/ml)
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
45
50
55
60
TIME (DAY)

Fig. 3

in the infected goats had reached 40 ± 0 °C and thereafter fluctuated between 39.1 and 40 °C (Fig. 4).

T. congolense infected goats showed a rapid loss in body weight. Prior to infection, their body weight had increased by 34.1 ± 5.4% compared to the beginning of the study. 30 days after infection, the average body weight declined 16.4 ± 9.8%. It then increased by 24 ± 8.8 % (day 35) after which declined by 15.4 ± 8.7 % by day 65. The rise in body weight observed between day 30 and 35 after infection coincided with the disappearance of the trypanosomes from the blood and a reduced rectal temperature of 39.1 °C. Compared to the infected goats, the control goats showed a steady increase in body weight, gaining 82.4 ± 0.55 % by day 65 (Fig. 5).

All the infected goats were anaemic 20 days after infection. The red blood cell count (RBC), packed cell volume (PCV), and haemoglobin (Hb) declined as the parasitaemia level increased. PCV declined from a mean pre-infection value of 34.3 ± 1.4 % to 22.3 ± 1.8 % by day 20 post infection and reached a value of 20.0 ±3.6 % by day 35. Thereafter, it remained around this level for the duration of the study. These PCV values of the infected goats were also significantly ($p < 0.001$) lower compared to the control goats which maintained a mean PCV value of 31.5 ± 0.3 % (Fig. 6). Hb also declined from 12.4 ± 0.38 gm/l (pre-infection level) to 7.3 ± 0.57 gm/l 25 days after infection. Except for a slight rise around day 40 post-infection, the Hb in the infected goats remained low for the duration of the study. In the control goats, the mean Hb level was 13.6 ± 0.1 gm/l, significantly higher than in infected goats (Fig. 7). RBC, from a pre-infection level of 12.1 x 10^6 ± 0.98, declined, 15 days after infection, to 7.7 x 10^6 ± 1.1. Except for a small rise at day 20, the RBC continued to decline reaching a count of 5.6 x 10^6 ± 0.55 by day 60. The control goats had a higher constant RBC count (Fig. 8). As the RBC, PCV, and Hb values declined in the infected goats, the white

Figure 4. Rectal temperature (mean ± SEM) in control (●) and *T. congolense* infected female goats (□) 65 days before and 60 days after infection. Goats infected on day 0.

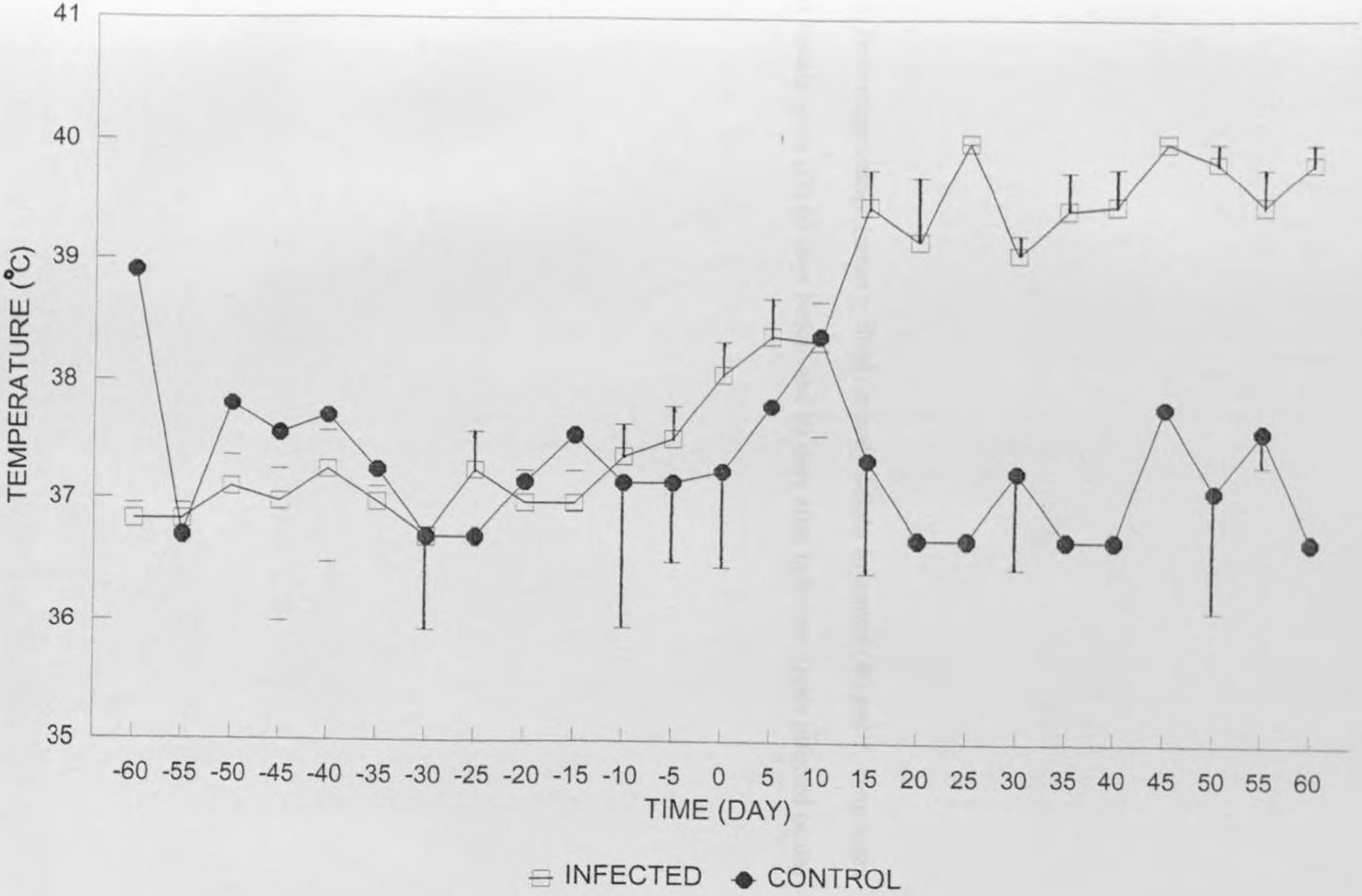

41
40
39
38
37
36
35
TEMPERATURE (°C)
-60 -55 -50 -45 -40 -35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
TIME (DAY)
INFECTED
CONTROL

Fig. 4

Figure 5. Percentage change (mean ± SEM) in body weight in control (●) and *T. congolense* infected female goats (□) 65 days before and 60 days after infection. Goats infected on day zero.

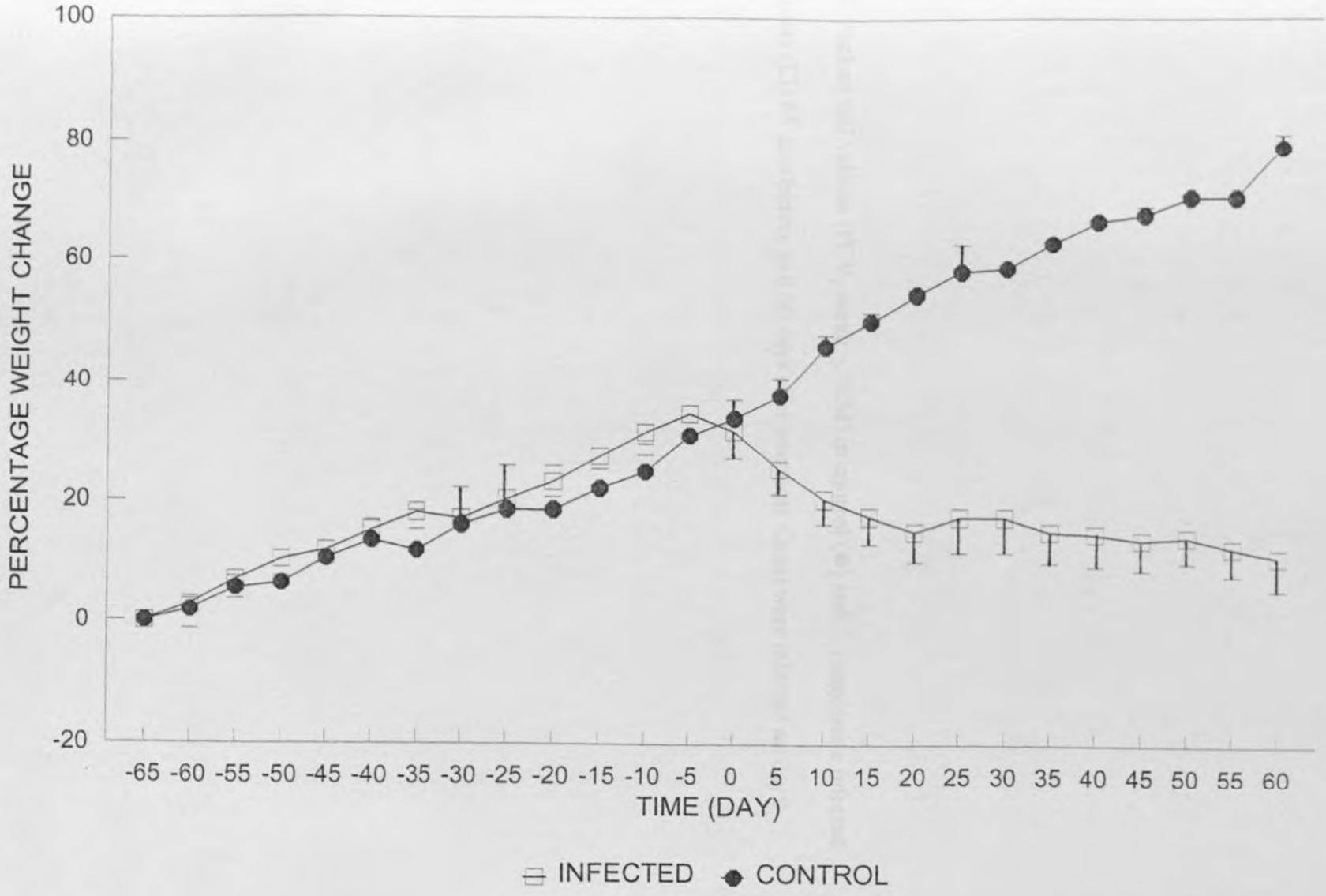

100
80
60
40
20
0
-20
PERCENTAGE WEIGHT CHANGE
-65 -60 -55 -50 -45 -40 -35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
TIME (DAY)
INFECTED
CONTROL

Fig. 5

Figure 6. Packed cell volume (PCV; mean ± SEM) in control (●) and *T. congolense* infected female goats (□) 65 days before and 60 days after infection. Goats were infected on day 0.

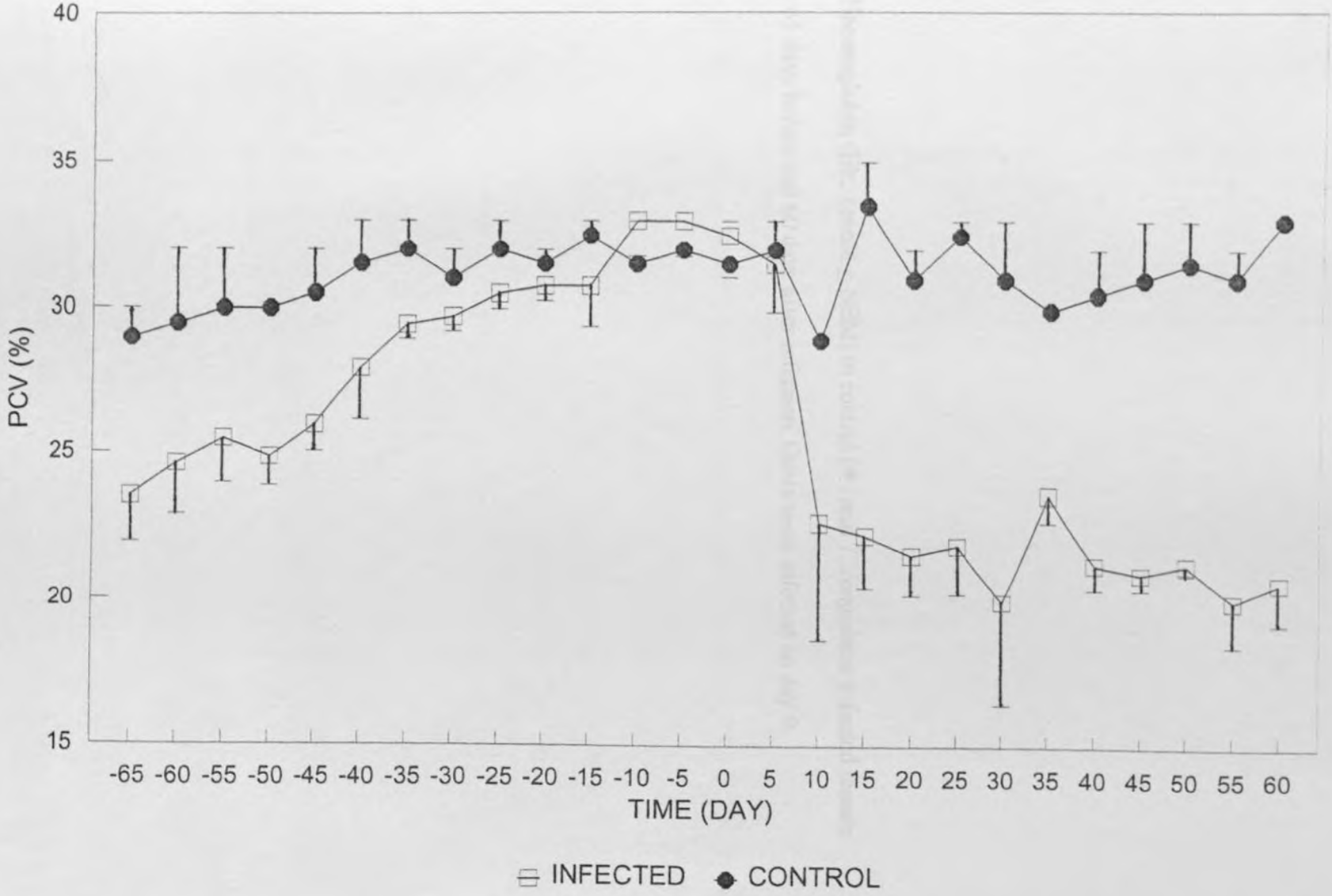

40
35
30
25
20
15
PCV (%)
-65 -60 -55 -50 -45 -40 -35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
TIME (DAY)
INFECTED
CONTROL

Fig. 6

Figure 7. Haemoglobin (Hb; (mean ± SEM) in control (●) and *T. congolense* infected female goats (□) 65 days before and 60 days after infection. Goats were infected on day 0.

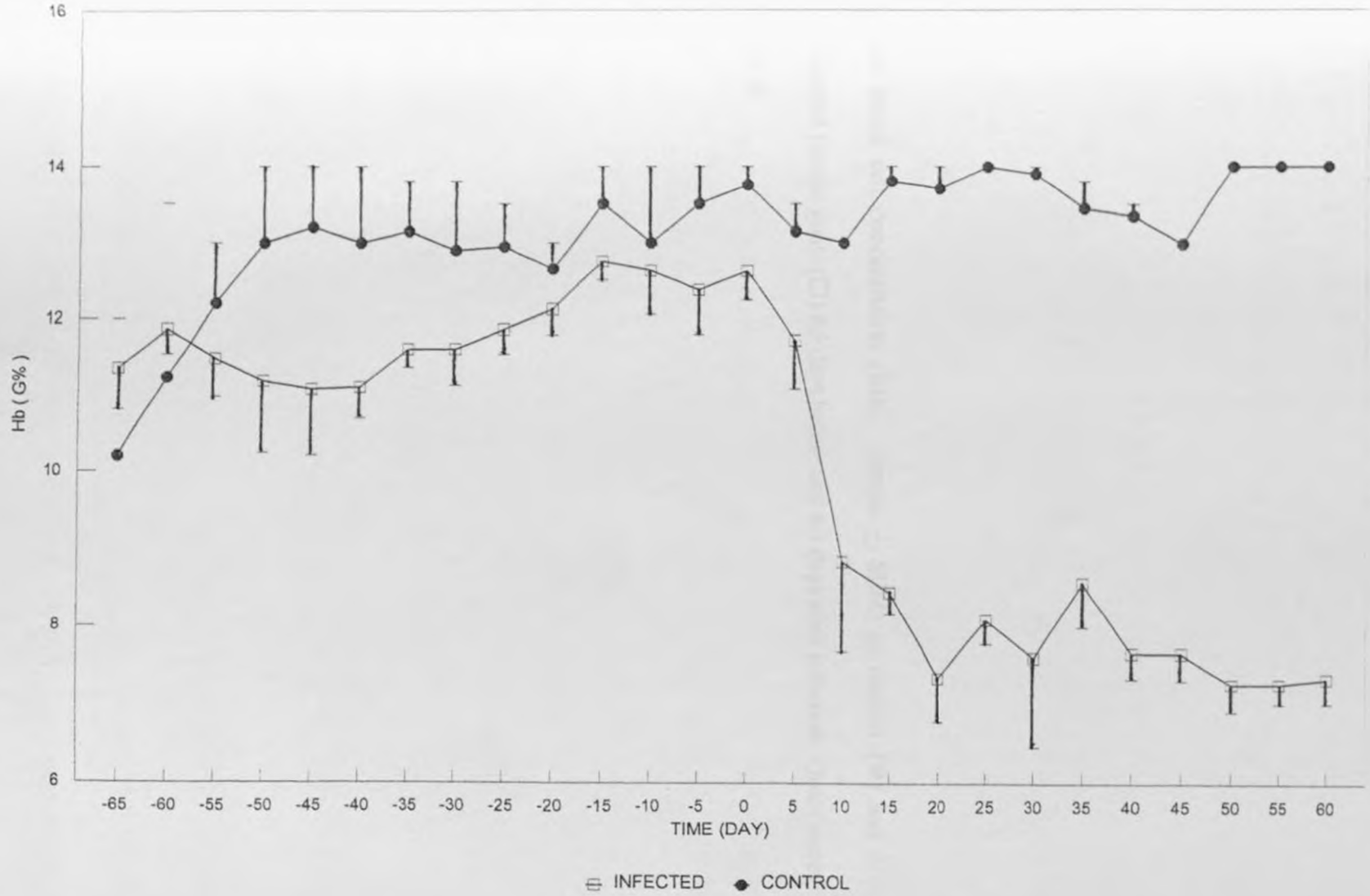

16
14
12
10
8
6
Hb (G%)
-65 -60 -55 -50 -45 -40 -35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
TIME (DAY)
INFECTED
CONTROL

Fig. 7

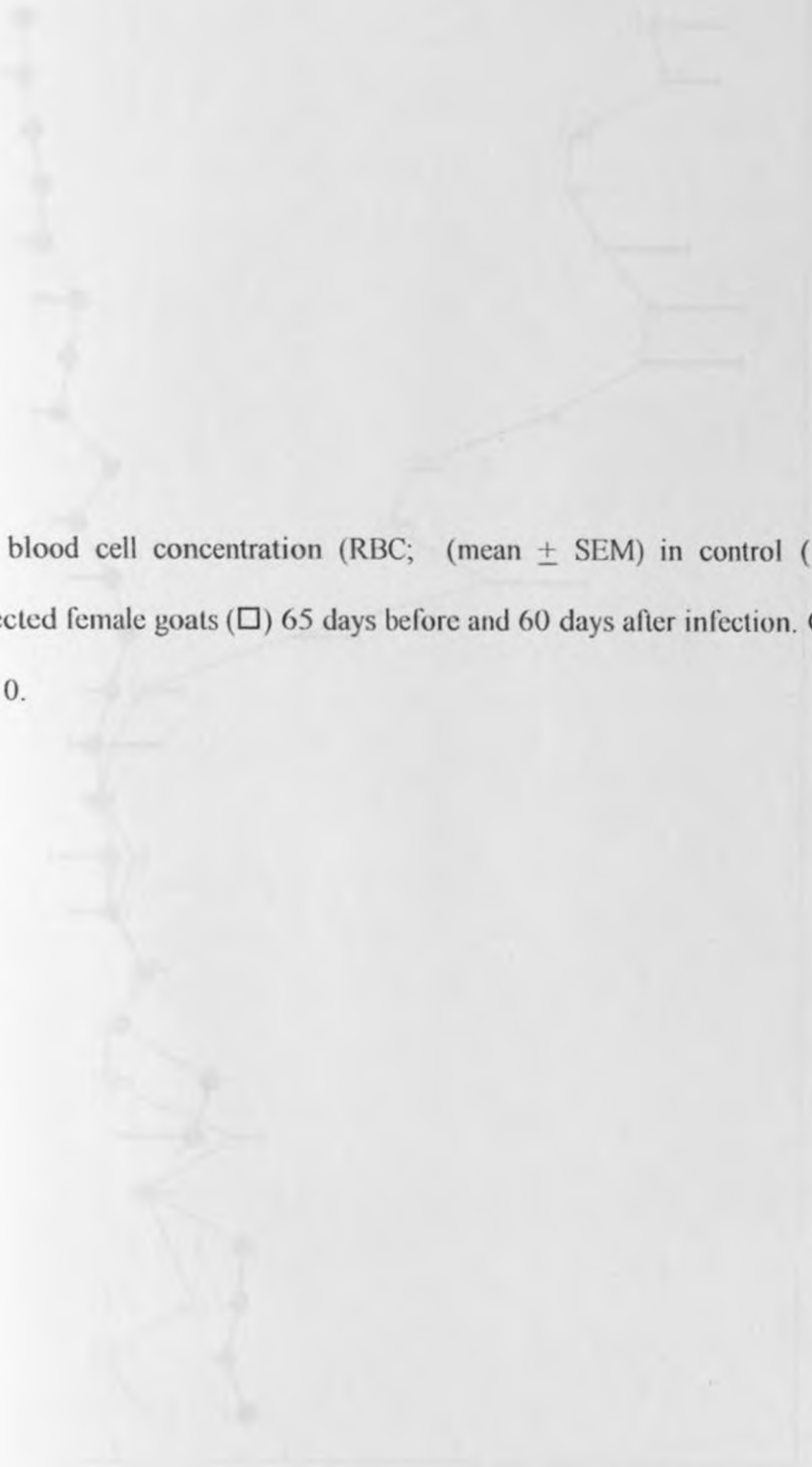


Figure 8. Red blood cell concentration (RBC; (mean ± SEM) in control (●) and *T. congolense* infected female goats (□) 65 days before and 60 days after infection. Goats were infected on day 0.

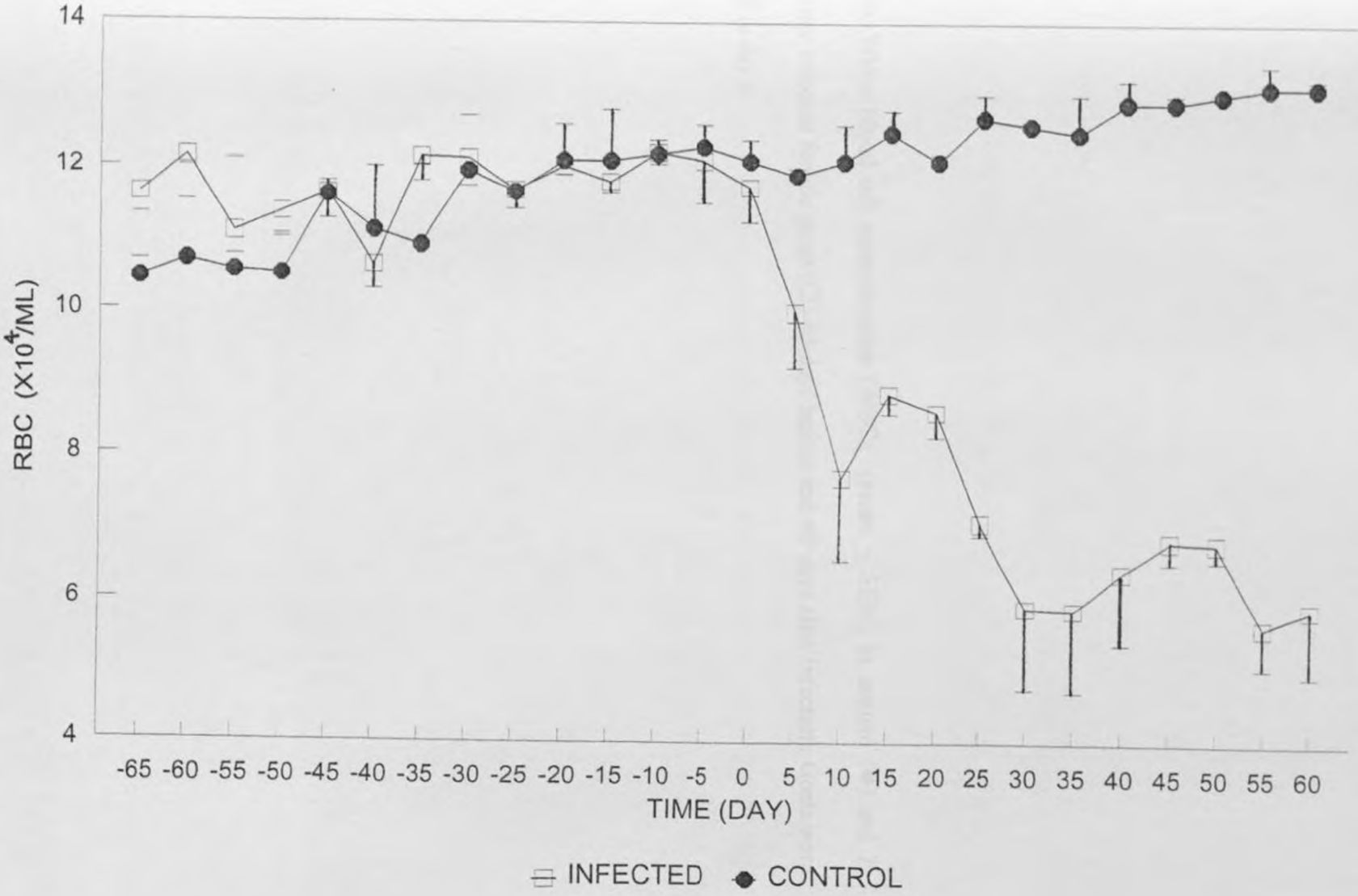

14
12
10
8
6
4
RBC (X10⁴/ML)
-65 -60 -55 -50 -45 -40 -35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
TIME (DAY)
INFECTED
CONTROL

Fig. 8

Figure 9. White blood cell concentration (WBC; (mean ± SEM) in control (●) and *T. congolense* infected female goats (□) 65 days before and 60 days after infection. Goats were infected in day 0.

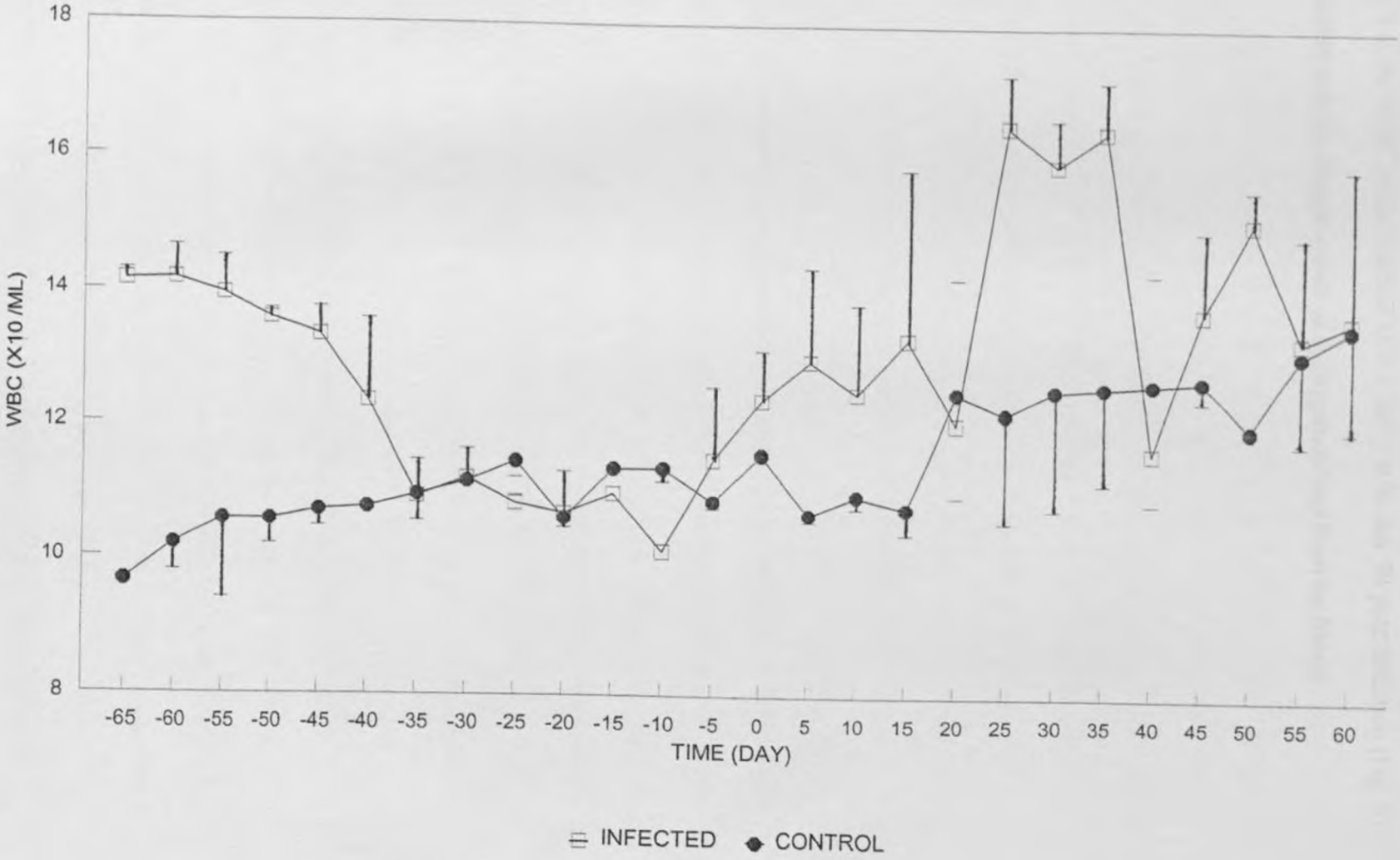

18
16
14
12
10
8
WBC (X10 /ML)
-65 -60 -55 -50 -45 -40 -35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
TIME (DAY)
INFECTED
CONTROL

Fig. 9

blood cell count (WBC) increased significantly ($p < 0.001$). From a pre-infection level of $11.53 \times 10^3 \pm 1.1$, the WBC count reached $16.5 \times 10^3 \pm 0.76$ day 30 post infection (Fig. 9). This rise coincided with the disappearance of the trypanosomes from the blood .

4.2 PLASMA LUTEINIZING HORMONE (LH) CONCENTRATION AND OESTRUS CYCLES

Plasma luteinizing hormone (LH) concentration, before and after *T. congolense* infection, was measured over a period of 4 months at one day intervals and daily for a period of 3 days around the oestrus period in 6 female goats. The onset of oestrus period in a female goat was evident by the behavioural changes exhibited by the female goat in the presence of a male goat: increased frequency of non-specific bleating, mounting of other female goats, and immobilization accompanied by tail deviation and turned back head. In addition, during the oestrus, the female goat rubbed its neck and back against other female goats and stood still as if ready for mounting. These behaviour changes associated with oestrus period lasted 2-3 days.

Prior to trypanosome infection, the plasma LH levels showed prominent periodic rises or peaks at intervals of 20-24 days (Figs 10A, B) which coincided with appearance of oestrus behaviour. Taking the interval between LH peaks as the length of the oestrus cycle, the average length of the oestrus cycle before infection was 23.2 ± 1.3 days. After infection with *T. congolense*, this prominent surge in plasma LH level was not observable (Fig 10C, D, E, F). Instead there appeared to be rises in the LH concentration occuring at irregular intervals ranging from 14-18 days. These rises in LH concentration were associated with oestrus behaviour patterns suggesting a muted increase in LH levels. During the first month of infection in two goats (no. 63 & 64, Fig 10D,E), this oestrus behaviour was particularly evident and lasted 4-5 days compared to the 2-3 days seen in non-infected goats. In another infected goat (no. 66, Fig. 10C) there was only a weak sign of oestrus behaviour lasting one day. In the second month of infection, all the infected goats were weak and showed no response to mounting behaviour by non-infected female goats in oestrus as was the case

Figure 10A,B,C,D,E,F. Pre- and post-infection plasma luteinizing hormone (LH) levels in individual control (A,B) and *T. congolense* infected female goats (C,D,E,F). Upper part of each figure shows the plasma LH levels at various intervals over a period of 70-121 days. Lower part of each figure shows the plasma LH levels of oestrus cycles synchronised around the highest LH level at periods of 20-24 days (see *Materials and Methods*). Asterisks represent plasma LH level taken as the LH surge.

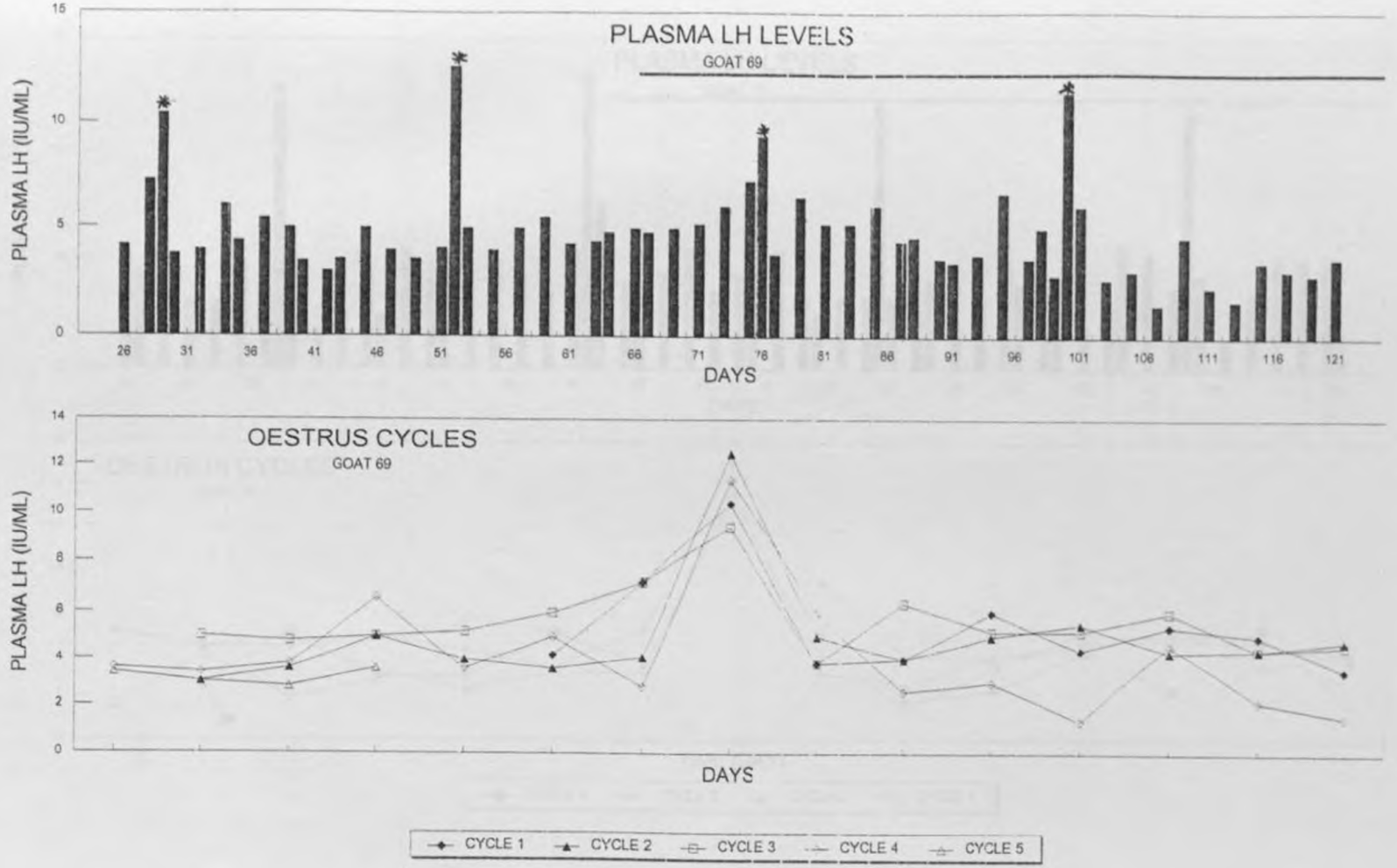

PLASMA LH LEVELS
GOAT 69
PLASMA LH (IU/ML)
DAYS
OESTRUS CYCLES
GOAT 69
PLASMA LH (IU/ML)
DAYS
CYCLE 1
CYCLE 2
CYCLE 3
CYCLE 4
CYCLE 5

Fig. 10A

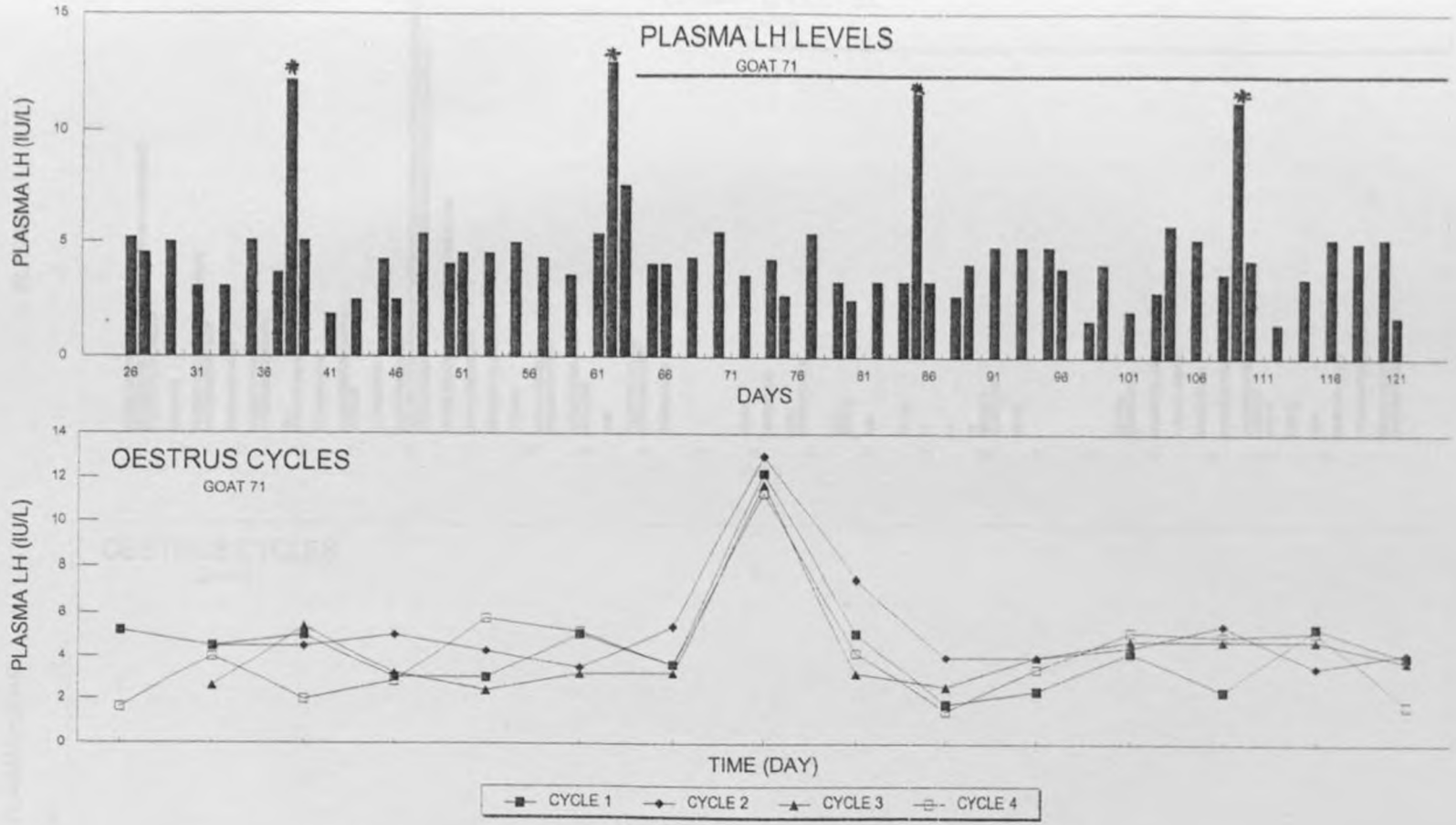

PLASMA LH LEVELS
GOAT 71
PLASMA LH (IU/L)
DAYS
OESTRUS CYCLES
GOAT 71
PLASMA LH (IU/L)
TIME (DAY)
CYCLE 1
CYCLE 2
CYCLE 3
CYCLE 4

Fig. 10B

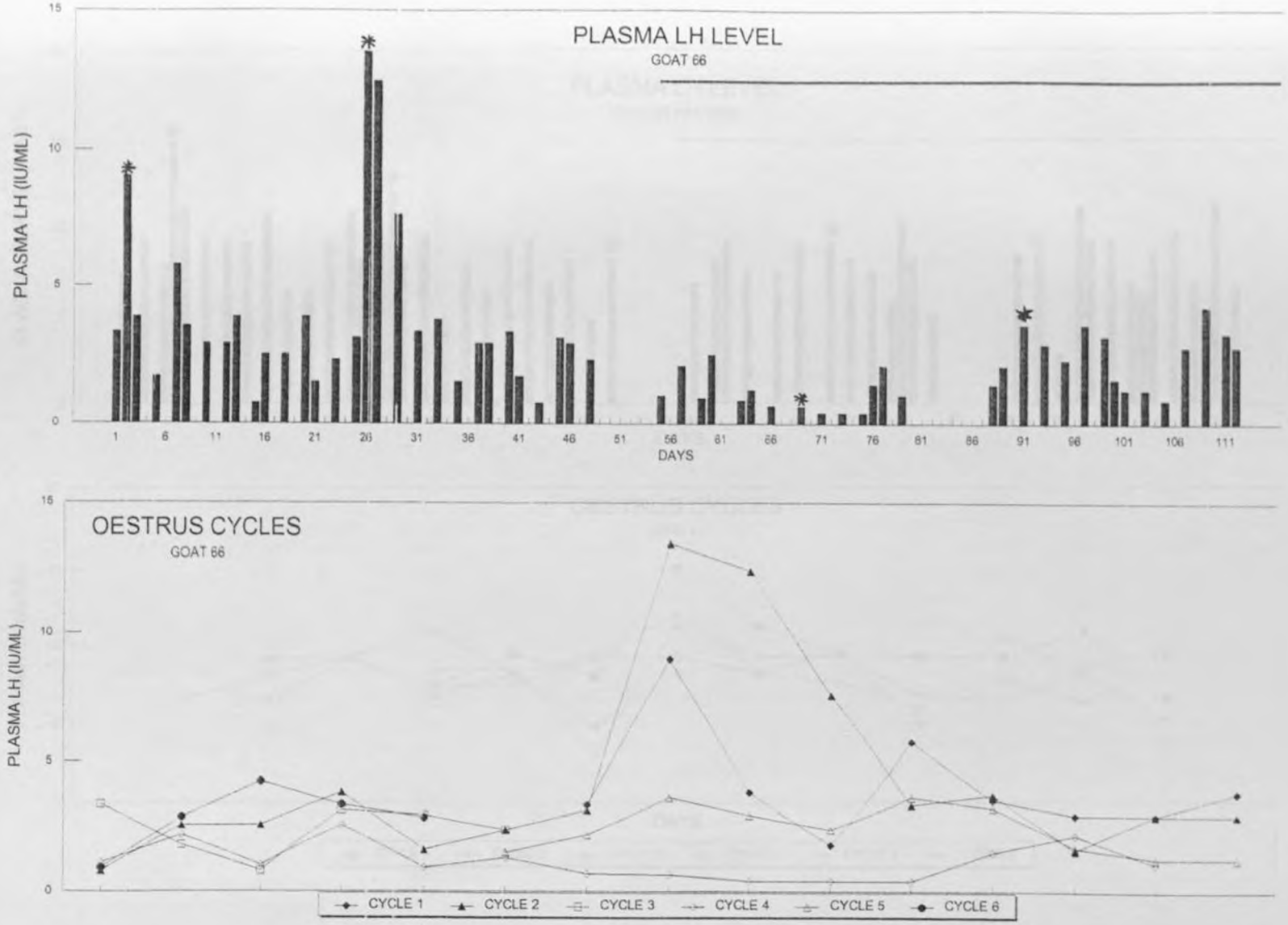

PLASMA LH LEVEL
GOAT 66
PLASMA LH (IU/ML)
DAYS
OESTRUS CYCLES
GOAT 66
PLASMA LH (IU/ML)
CYCLE 1
CYCLE 2
CYCLE 3
CYCLE 4
CYCLE 5
CYCLE 6

Fig. 10C

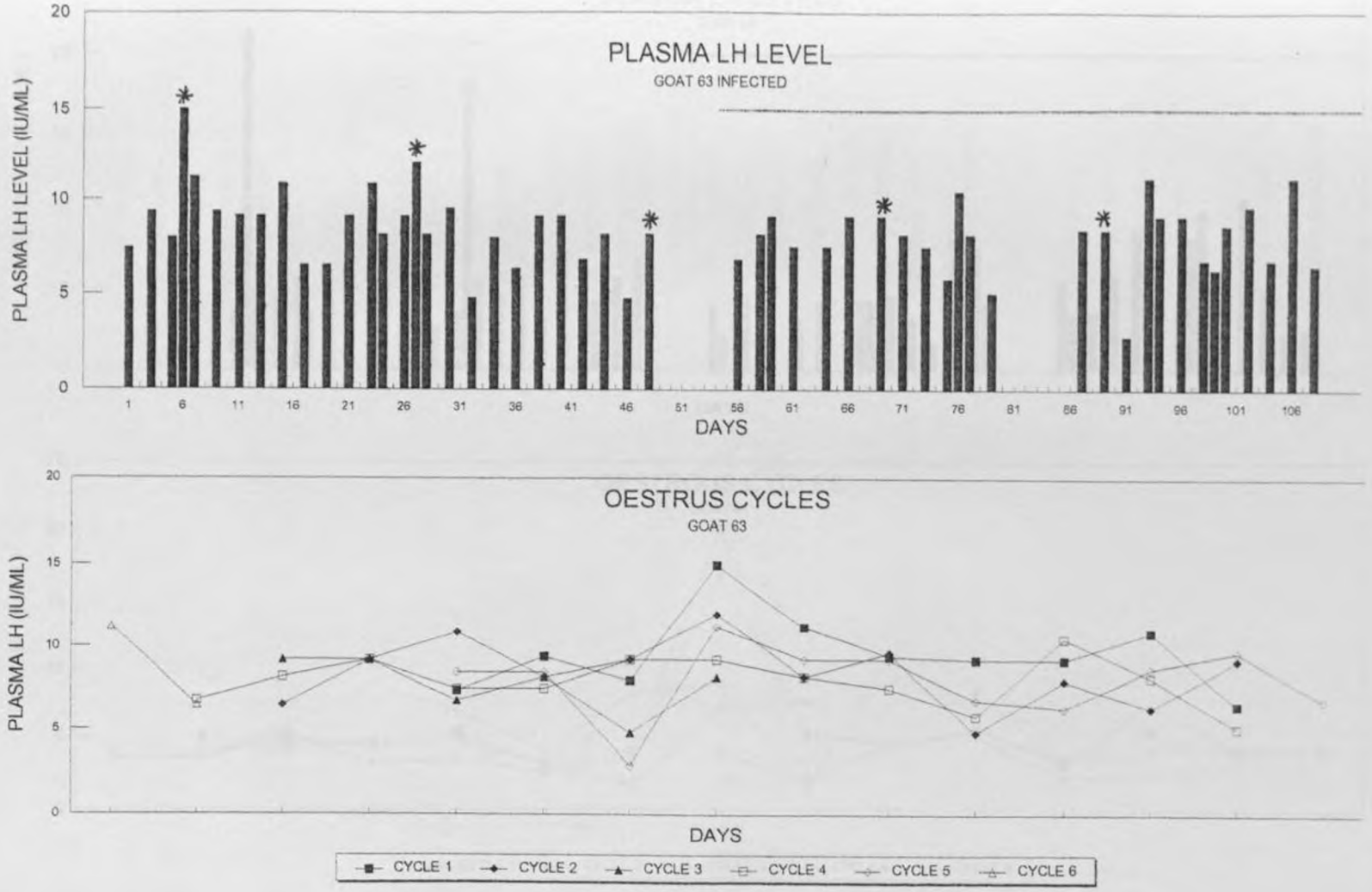

PLASMA LH LEVEL
GOAT 63 INFECTED
PLASMA LH LEVEL (IU/ML)
20
15
10
5
0
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
76
81
86
91
96
101
106
DAYS
OESTRUS CYCLES
GOAT 63
PLASMA LH (IU/ML)
20
15
10
5
0
DAYS
CYCLE 1
CYCLE 2
CYCLE 3
CYCLE 4
CYCLE 5
CYCLE 6

Fig. 10D

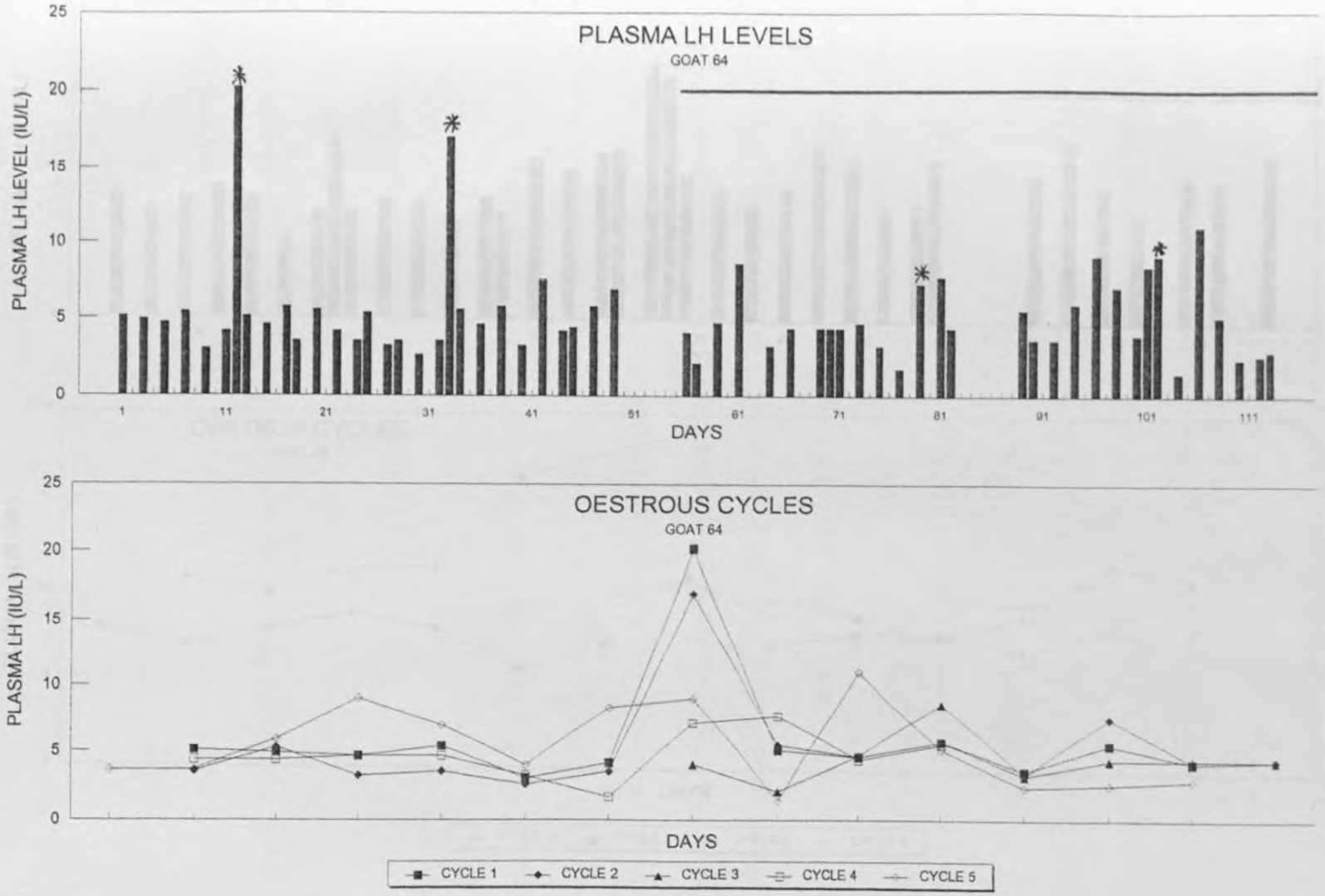

PLASMA LH LEVELS
GOAT 64
PLASMA LH LEVEL (IU/L)
25
20
15
10
5
0
1
11
21
31
41
51
61
71
81
91
101
111
DAYS
OESTROUS CYCLES
GOAT 64
PLASMA LH (IU/L)
25
20
15
10
5
0
DAYS
CYCLE 1
CYCLE 2
CYCLE 3
CYCLE 4
CYCLE 5

Fig. 10E

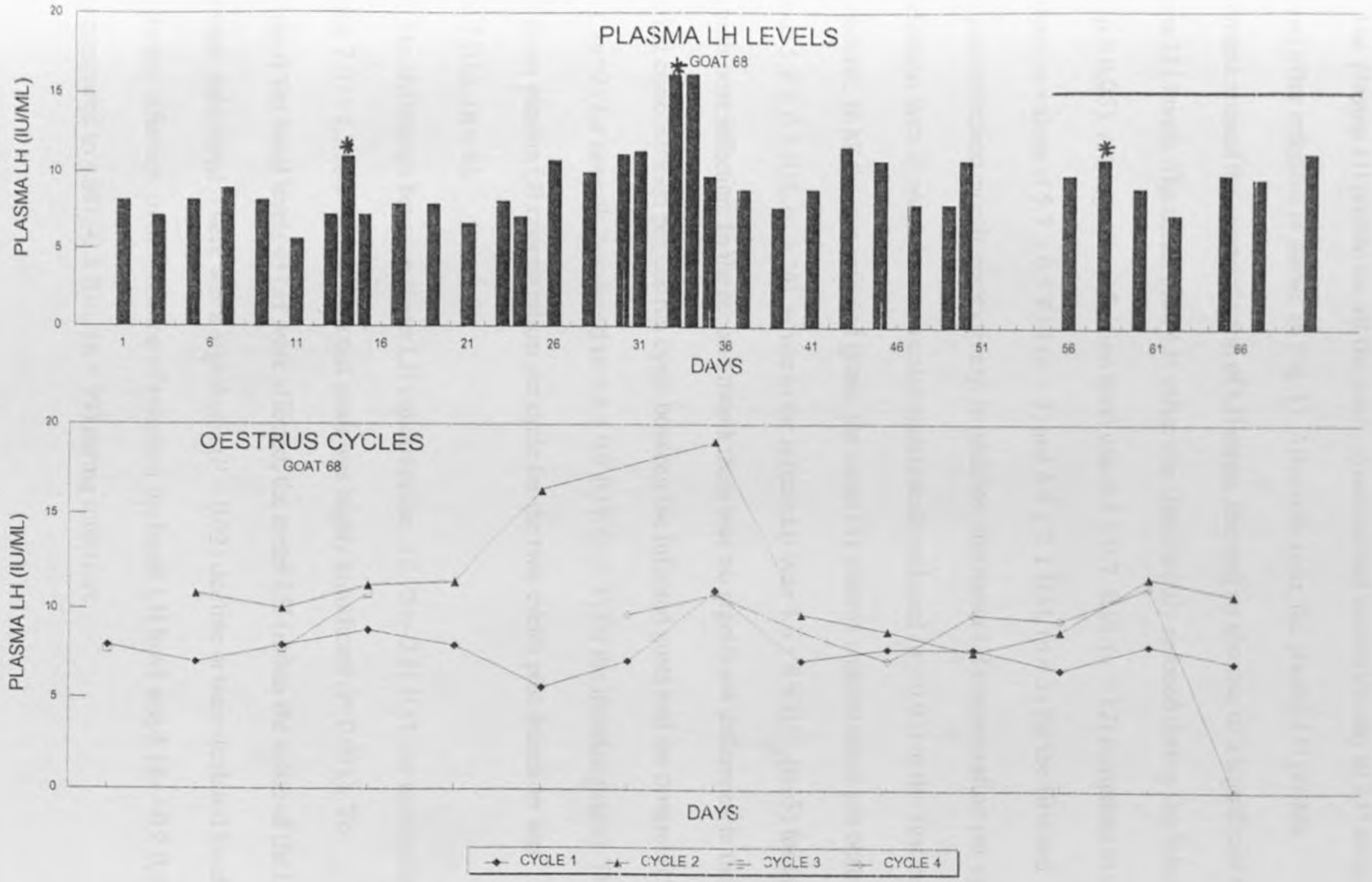

PLASMA LH LEVELS
GOAT 68
PLASMA LH (IU/ML)
DAYS
OESTRUS CYCLES
GOAT 68
PLASMA LH (IU/ML)
DAYS
CYCLE 1
CYCLE 2
CYCLE 3
CYCLE 4

Fig. 10F

before infection.

The plasma LH profile for all the goats, synchronised around the day of LH surge, before and after infection is shown in Fig. 11. After infection, the plasma LH profile synchronised around the expected time of LH surge, showed an absence of a significant rise in plasma LH levels. The mean peak LH value was significantly reduced during the infection period ($p < 0.05$). Average pre-infection level was 8.5 ± 0.7 IU/L ($n = 12$) compared to the post-infection values of 5.7 ± 0.5 IU/l ($n = 3$) and 4.9 ± 2.1 IU/L ($n = 3$) for the first and second post-infection month, respectively. In addition, the mean LH concentration per cycle after infection with *T. congolense* was also significantly reduced ($p < 0.01$) in the first month post infection. In all the non-infected goats, the mean LH plasma concentration per oestrus cycle was 5.8 ± 0.5 IU/L ($n = 16$), while in the infected it was 4.6 ± 0.9 IU/L ($n=3$) for the first month post infection. In the second month there was no significant difference in the plasma LH concentration per oestrus cycle between the infected goats and the controls (5.8 ± 0.5 IU/L ($n=2$) for controls compared to 5.4 ± 0.9 IU/L ($n = 3$) for the infected goats). The overall mean plasma LH concentration per cycle for the two cycles post-infection was 5.0+/-0.7 IU/L ($n = 6$).

The difference between mean LH concentration, 12.75+/-0.81 IU/L for non-infected goats and 7.0+/-1.3 IU/L for the infected goats was highly significant ($P<0.001$). To determine if the basal levels of LH were affected, the mean LH (minus the value of the LH surge) were calculated. There was a significant ($P < 0.02$) decline in the calculated basal LH levels during infection. In the absence of infection the basal LH level was 5.18+/-0.2 IU/L ($n = 183$) compared to 4.88+/-0.3 IU/L ($n = 99$) during infection.

Figure 11. Pre- (■) and post infection (□) mean plasma LH levels (mean ± SEM) during oestrus cycles in control (upper figure,n=2) and *T. congolense* infected female goats (lower figure,n=3). Day 0 representing the day of LH surge.

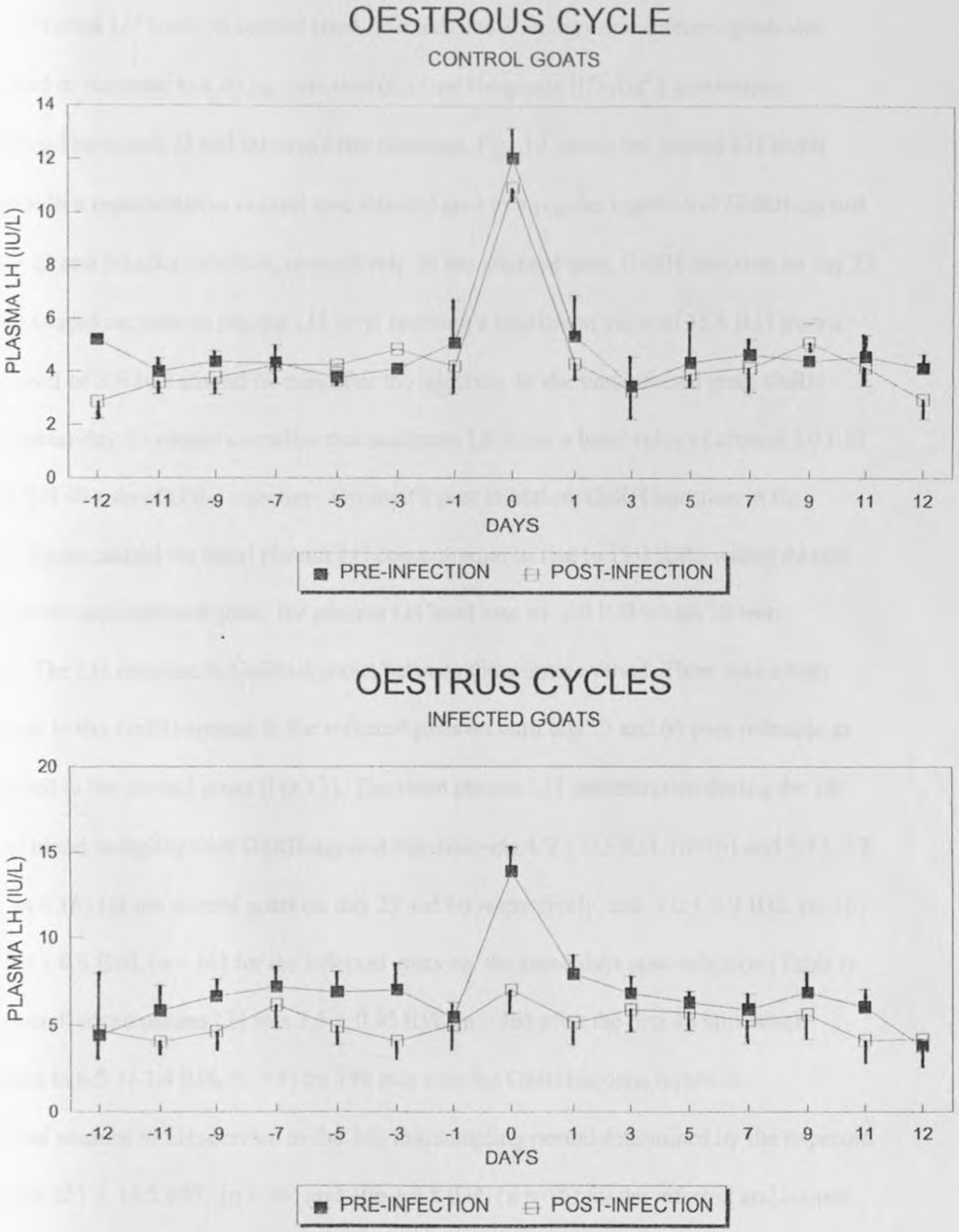

OESTROUS CYCLE
CONTROL GOATS
PLASMA LH (IU/L)
14
12
10
8
6
4
2
0
-12 -11 -9 -7 -5 -3 -1 0 1 3 5 7 9 11 12
DAYS
PRE-INFECTION
POST-INFECTION
OESTRUS CYCLES
INFECTED GOATS
PLASMA LH (IU/L)
20
15
10
5
0
-12 -11 -9 -7 -5 -3 -1 0 1 3 5 7 9 11 12
DAYS
PRE-INFECTION
POST-INFECTION

Fig. 11

4.3 PLASMA LH LEVELS IN RESPONSE TO GnRH INJECTION

Plasma LH levels in control (non-infected) and *T. congolense* infected goats was measured in response to a 20 μg injection of a GnRH-agonist ([D-Ala^6]-Luteinizing Releasing Hormone) 23 and 60 days after infection. Figs 12 shows the plasma LH levels response in a representative control and infected goat to a jugular injection of GnRH-agonist on day 23 and 60 after infection, respectively. In the infected goat, GnRH injection on day 23 caused a rapid increase in plasma LH level reaching a maximum value of 15.4 IU/l from a basal level of 3.9 IU/l around 60 min after the injection. In the non-infected goat, GnRH injection on day 23 caused a smaller rise in plasma LH from a basal value of around 3.0 IU/l to 5.8 IU/l 40 min after the injection. On day 60 post infection, GnRH injection in the infected goat caused the basal plasma LH concentration to rise to 15.0 IU/L within 40 min, while in the non-infected goats, the plasma LH level rose to 8.0 IU/l within 20 min..

The LH response to GnRH-agonist between the animals varied. There was a high response to the GnRH-agonist in the infected goats on both day 23 and 60 post-infection as compared to the control goats (Fig 13). The mean plasma LH concentration during the 140 min of blood sampling after GnRH-agonist injection was 4.7 $\pm$ 0.3 IU/L (n=16) and 5.7 $\pm$ 0.7 IU/L (n = 16) for the control goats on day 23 and 60 respectively, and, 7.0 $\pm$ 0.9 IU/L (n=16) and 7.0 $\pm$ 0.6 IU/L (n = 16) for the infected goats on the same days post-infection (Table I). The overall mean plasma LH was 7.5 $\pm$ 0.45 IU/L (n = 16) after the first 40 min which declined to 6.5 +/-1.4 IU/L (n = 4) by 140 min after the GnRH-agonist injection.
The total amount of LH secreted in the 140 min sampling period determined by the trapezoid rule was 151 $\pm$ 14.5 IU/L (n = 16) and 106 $\pm$ 8.8 IU/L (n = 16) for the infected and control goats respectively and this difference was highly significant ($P < 0.001$).

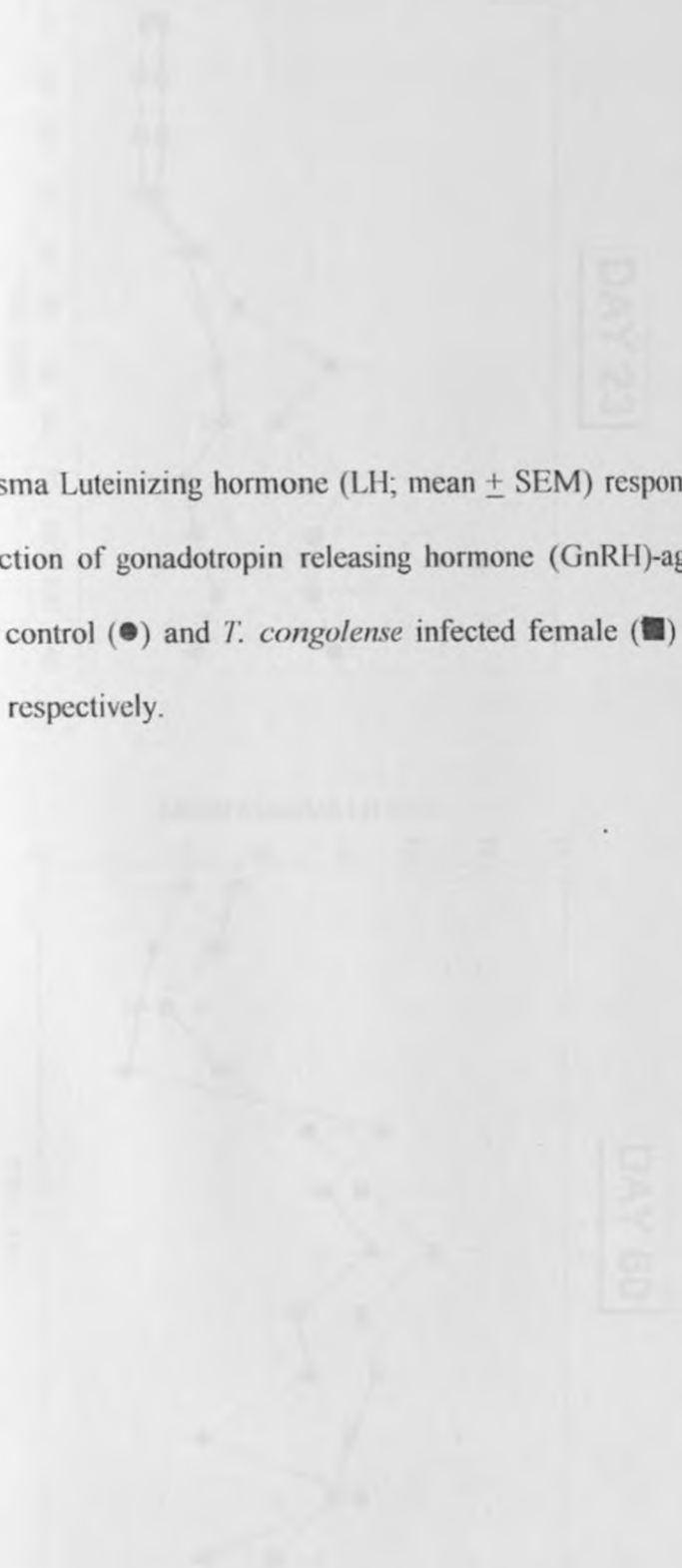


Figure 12. Plasma Luteinizing hormone (LH; mean ± SEM) response over a period of 140 min after injection of gonadotropin releasing hormone (GnRH)-agonist at time zero in a representative control (●) and *T. congolense* infected female (■) goat on day 23 and 60 post-infection, respectively.

GnRH CHALLENGE

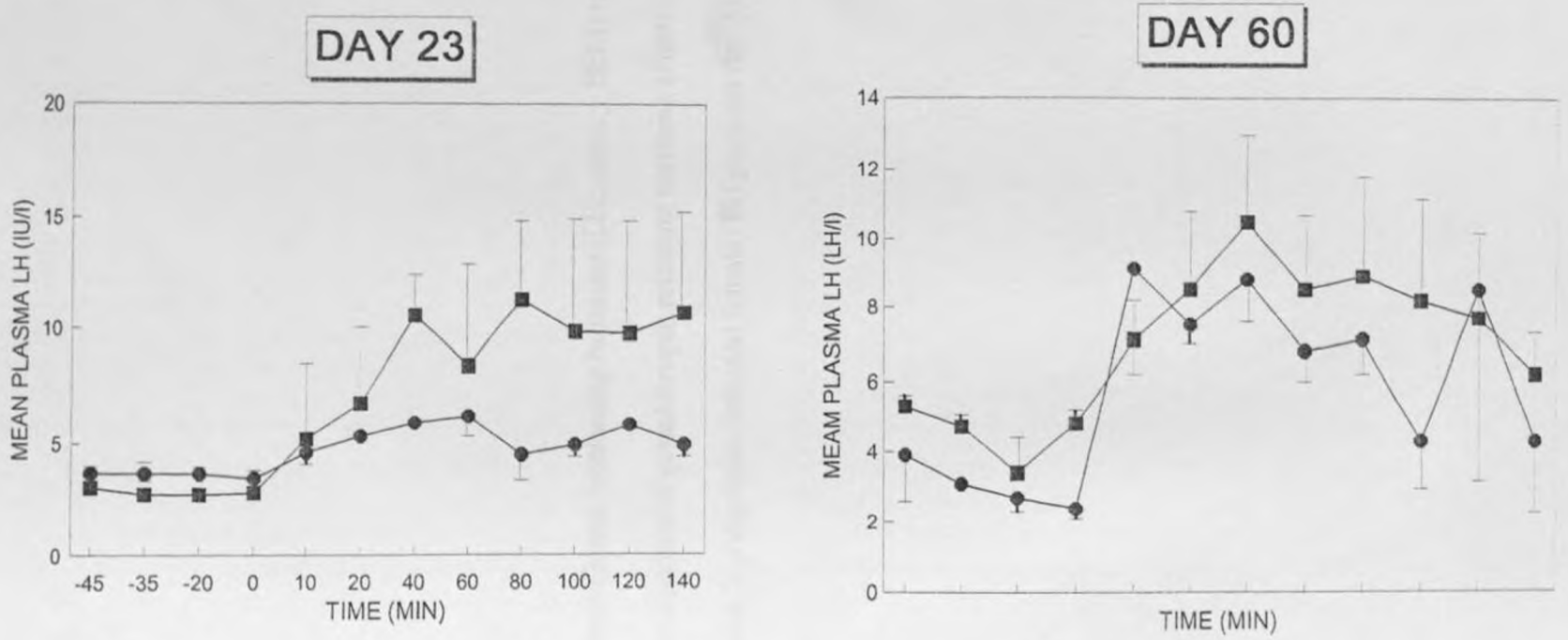


Fig. 12

Figure 13. Mean plasma luteinizing hormone (LH; mean ± SEM) response over a period of 140 min after injection of gonadotropin releasing hormone (GnRH)-agonist at time zero in control (●) and *T. congolense* infected female (■) goats on day 23 and 60 post-infection, respectively.

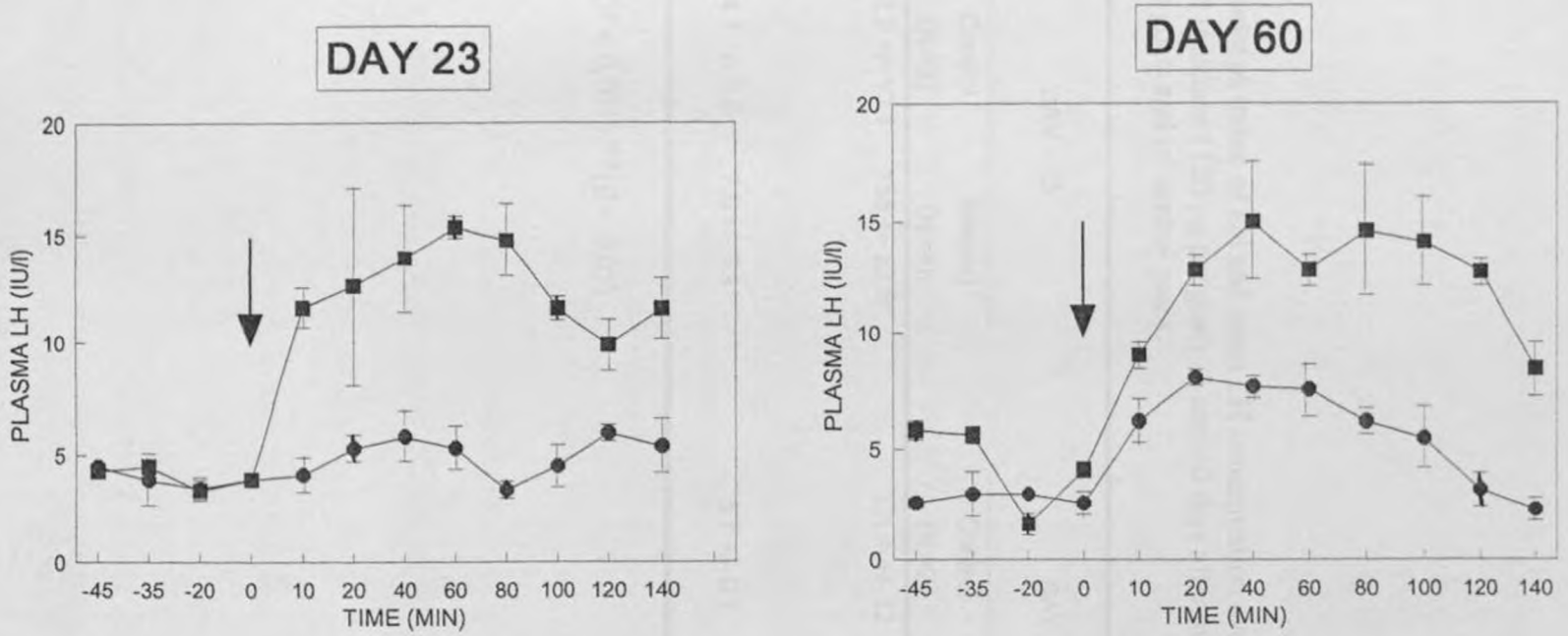

GnRH CHALLENGE
ALL GOATS
DAY 23
DAY 60
PLASMA LH (IU/l)
TIME (MIN)
0
5
10
15
20
-45
-35
-20
0
10
20
40
60
80
100
120
140

Fig. 13

TABLE I. Area under the curve as index of LH and mean LH concentration over a 140 min period in response to GnRH injection (120 μg per goat) 23 and 60 days after infection in *T. congolense* infected female goats and in control goats.

	DAY 23		DAY 60	
	Control (N =2)	Infected (N =4)	Control (N =2)	Infected (N =4)
Area under curve (iu/l in 140 min) after injection	92.3 +/- 10.1	158 +/- 22.6*	121.1 +/- 12	145 +/- 16.7*
Mean (iu/l)	4.7 +/- 0.3	7.0 +/- 0.9**	5.7 +/- 0.7	7.0 +/- 0.6**

Value of mean +/- SEM: *($P < 0.001$) **($P < 0.05$)

4.4 PLASMA LUTEINIZING HORMONE CONCENTRATION IN RESPONSE TO CLONIDINE INJECTION

Clonidine, a central acting alpha-2 adrenergeric agonist, was injected via the jugular vein into female goats before and after *T. congolense* infection to stimulate the hypothalamus. In a representative female goat, clonidine (25 μg) injection, seven days before infection, caused the appearance of a pulsatile pattern in plasma [LH] (Fig 14). A hormonal pulse (peak) was concidered present if a rise exceeded the previous basal value by at least three standard deviations (3 SDS) and when that peak value was followed by either a decline or no significant increament both of the latter values being significantly different from mean basal levels. Over the 80 min of observation after the injection, 3 LH peaks were detected. The first peak appeared 10 min after the injection with the plasma LH concentration rising to 7.5 $\pm$ 0.3 IU/l from 5.1 $\pm$ 0.17 IU/l, the second peak appeared at 40 min (8.6 $\pm$ 0.53 IU/l), and the third peak appeared at 70 min (8.6 $\pm$ IU/l). Clonidine injection in the same goat on day 28 and 69 after infection did not cause any detectable rise or appearance of LH peak though there was some indication of some changes in plasma LH levels. This was in contrast to the effect of clonidine injection in the representative control goat which showed appearance of peaks in plasma LH levels on both days 28 and 69 of the post-infection period (Fig.15). This absence of detectable rises in plasma LH level was observed in all the infected goats (Fig. 16) injected with clonidine on days 28 and 69 after infection while the non-infected goats showed rises and peaks in plasma LH level.

The frequency of LH pulses, the mean plasma LH concentration, the mean LH peak amplitude, and the total LH released (area under the curve after injection) over the 80 min after clonidine injection were all significantly reduced in the infected goats compared to

controls (Table II). In the infected goats the total LH release and the mean plasma LH were significantly higher on day 69 compared to day 28 after infection (Table II).

Figure 14. Plasma luteinizing hormone (LH; mean ± SEM) response to clonidine injection over a period of 80 min given at time zero in a representative *T. congolense* infected female goat on day 7 (□) before infection and day 28 (●) and 69 (■) after infection.

CLONIDINE CHALLENGE

INFECTED GOAT 66

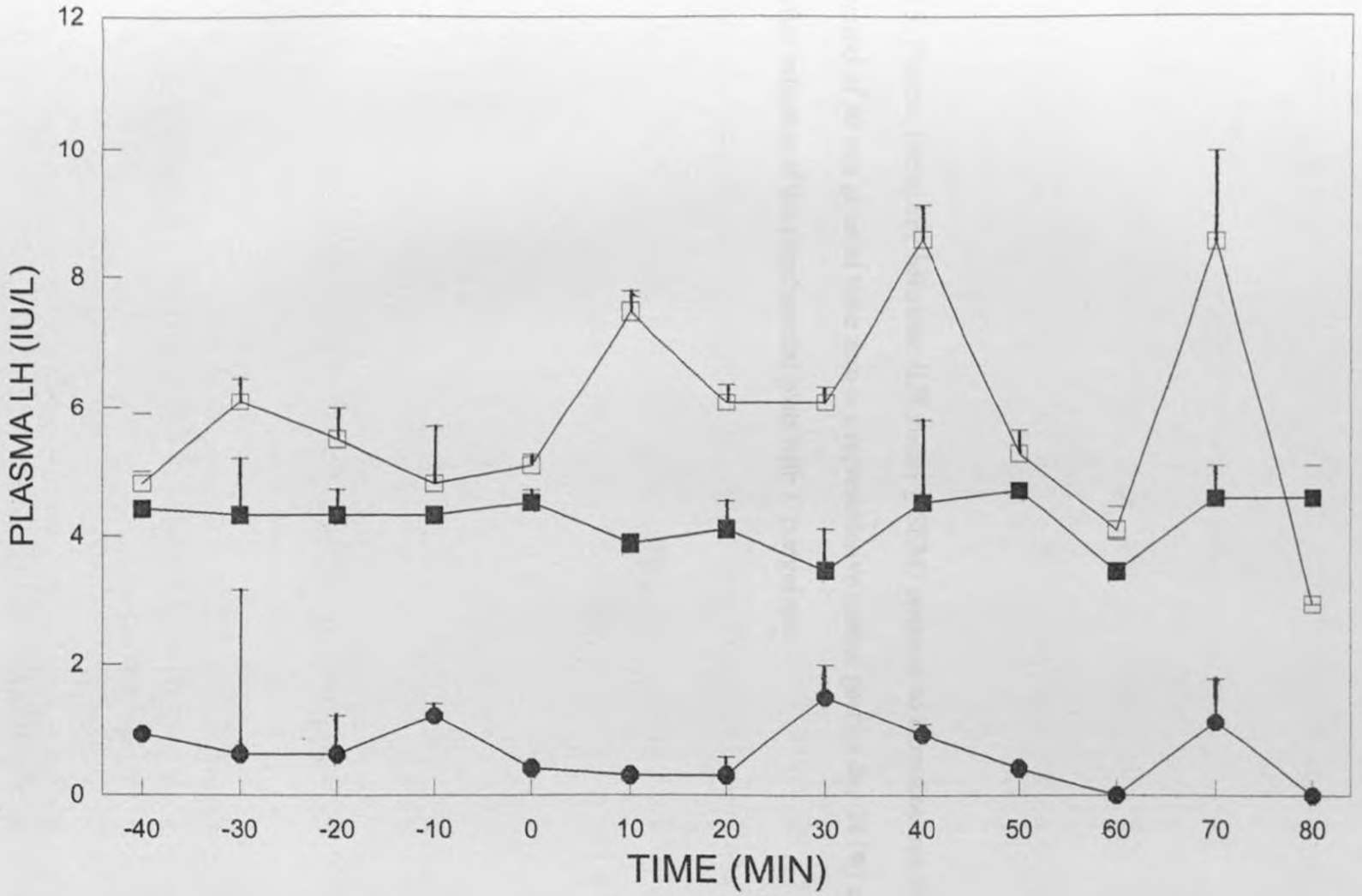


Fig. 14

Figure 15. Plasma luteinizing hormone (LH; mean ± SEM) response to clonidine injection over a period of 80 min given at time zero in a representative control goat on day 28 (●) and 69 (■) after infection of the experimental goats with *T. congolense*.

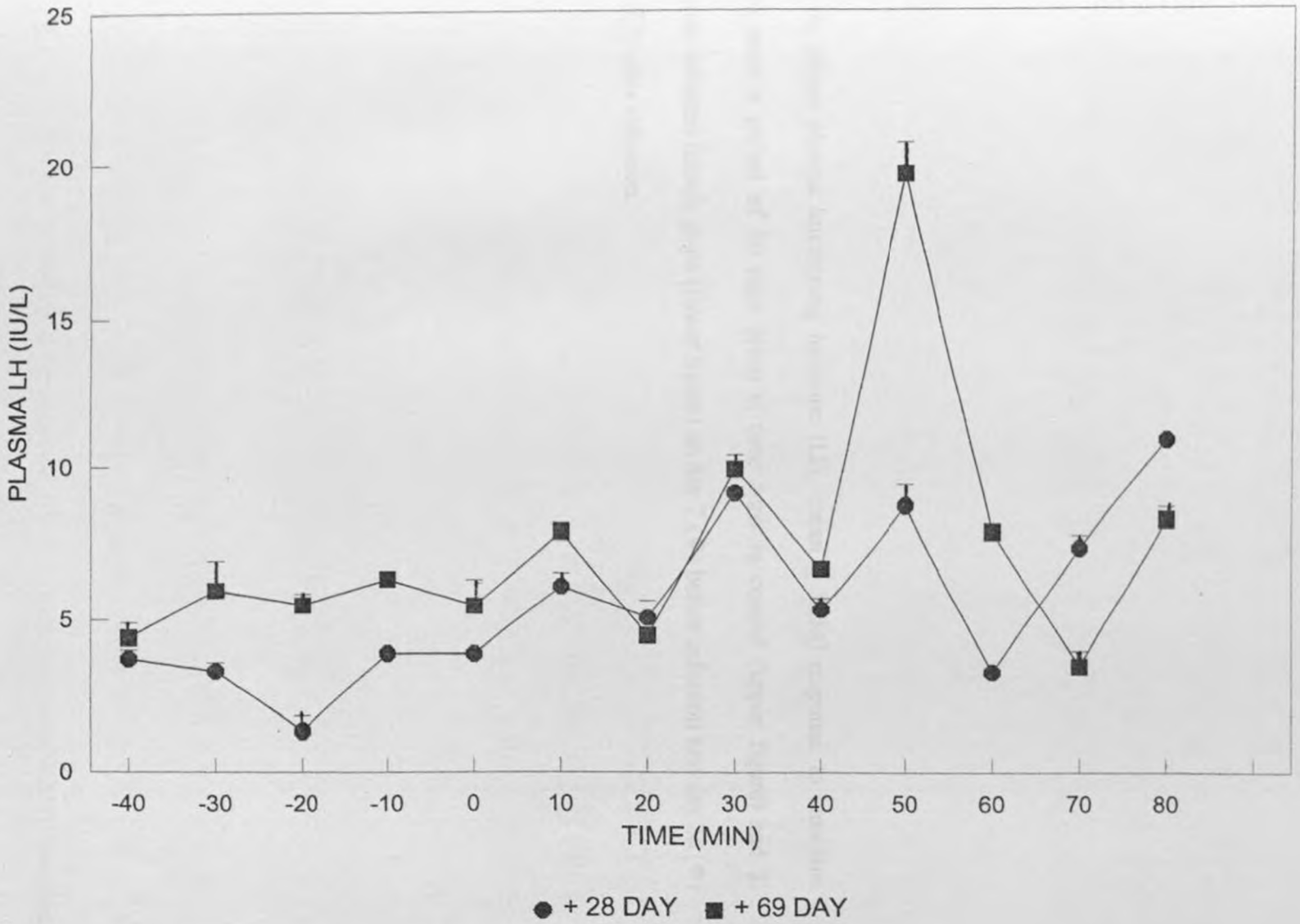

CLONIDINE CHALLENGE
CONTROL GOAT 69
PLASMA LH (IU/L)
25
20
15
10
5
0
-40
-30
-20
-10
0
10
20
30
40
50
60
70
80
TIME (MIN)
+ 28 DAY
+ 69 DAY

Fig. 15

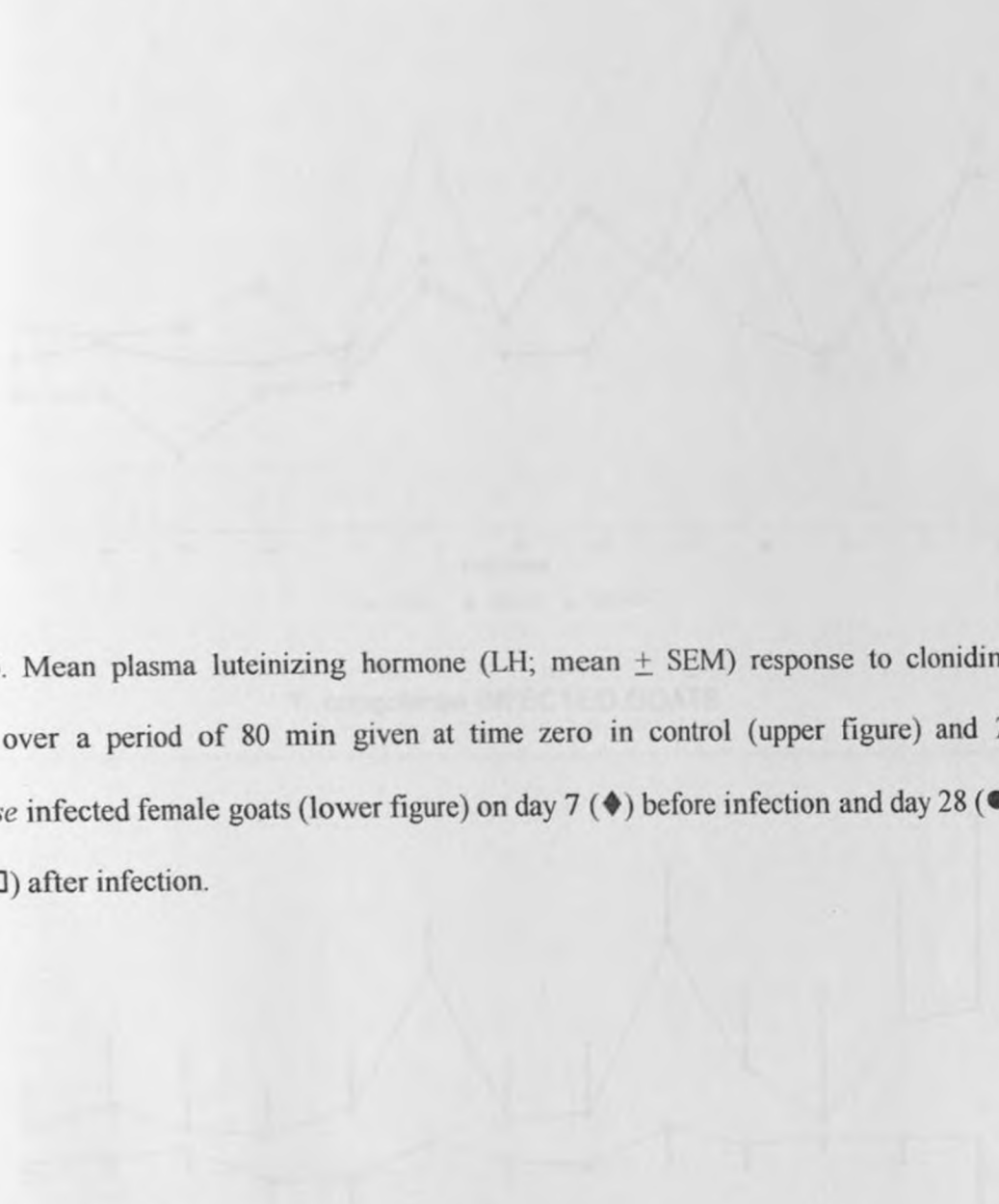


Figure 16. Mean plasma luteinizing hormone (LH; mean ± SEM) response to clonidine injection over a period of 80 min given at time zero in control (upper figure) and *T. congolense* infected female goats (lower figure) on day 7 (♦) before infection and day 28 (●) and 69 (□) after infection.

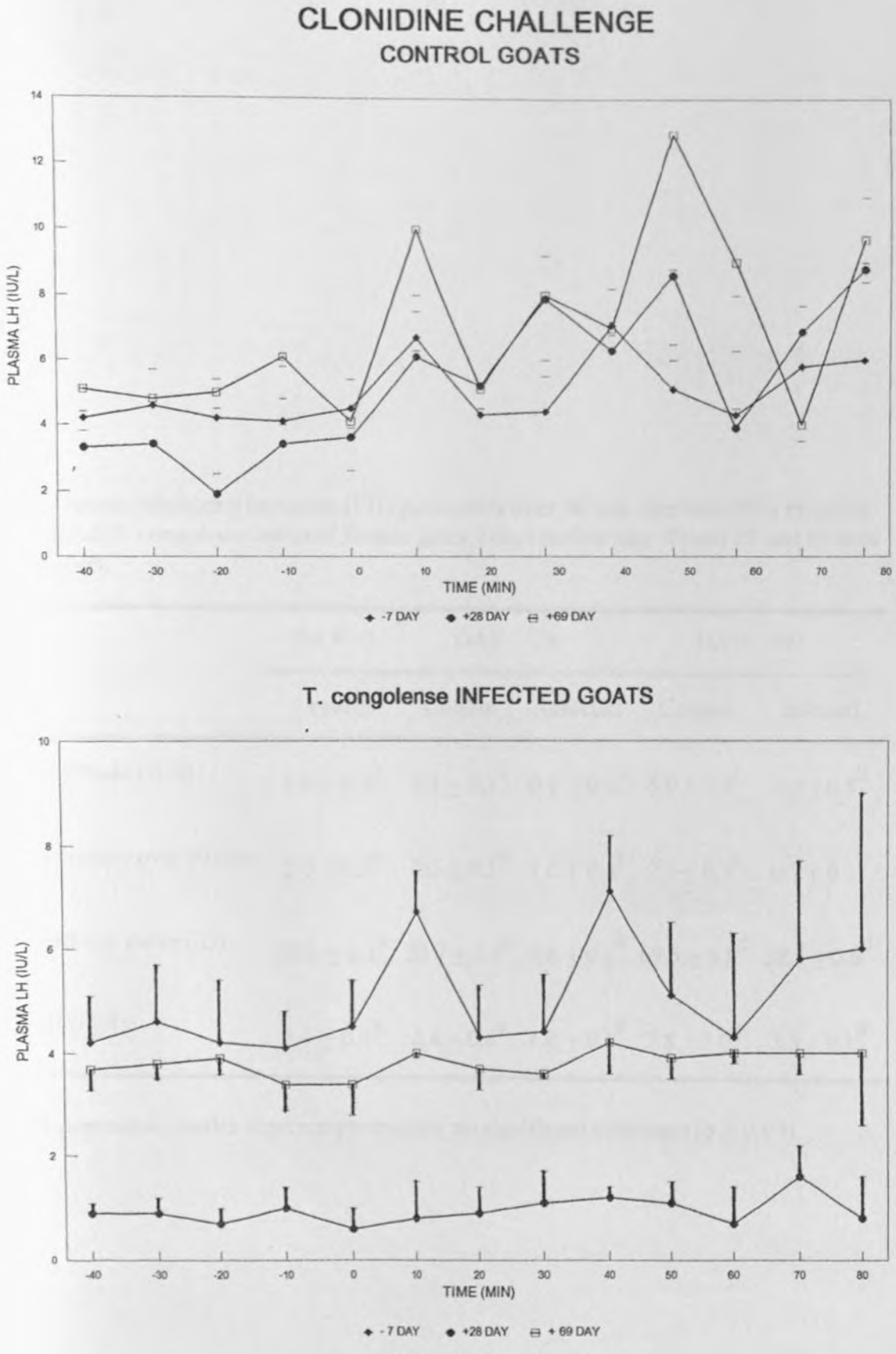

CLONIDINE CHALLENGE
CONTROL GOATS
PLASMA LH (IU/L)
TIME (MIN)
-7 DAY
+28 DAY
+69 DAY
T. congolense INFECTED GOATS
PLASMA LH (IU/L)
TIME (MIN)
- 7 DAY
+28 DAY
+ 69 DAY

Fig. 16

Table II. Plasma luteinizing hormone (LH) parameters over 80 min after clonidine injection in control and *T. congolense infected* female goats 7 days before (day -7) and 28 and 69 days after infection.

	DAY -7	DAY 28		DAY 69	
	Control	Control	Infected	Control	Infected
Pulse amplitude (IU/l)	3.0 ± 0.3^{a}	3.1 ± 0.3^{a}	0.9 ± 0.3^{b}	6.9 ± 1.9^{c}	0.9 ± 0.5^{b}
Pulse frequency over 80 min	2.3 ± 0.3^{a}	2.5 ± 0.5^{a}	1.0 ± 0.6^{b}	2.5 ± 0.5^{a}	0.7 ± 0.3^{b}
Area under the curve (IU)	50.2 ± 2.1^{a}	52.7 ± 5.4^{a}	9.6 ± 0.5^{b}	69.5 ± 5.5^{c}	38.1 ± 0.6^{d}
Mean LH (IU/l)	5.4 ± 0.4^{a}	6.4 ± 0.6^{a}	1.0 ± 0.1^{b}	7.8 ± 1.0^{c}	3.9 ± 0.1^{d}

For each parameter, similar superscripts indicate no significant difference ($p > 0.05$)

CHAPTER 5

DISCUSSION

The present study showed that chronic trypanosomosis causes disruption of the normal pattern of LH release by the pituitary. Trypanosomosis caused irregular, and ultimately, abolished the normally expected periodic surge in plasma LH level during oestrus cycle. Both mean LH concentration per oestrus cycle and LH peak amplitude in all infected goats were reduced soon after the infection. One infected goat was acyclic (characterised by absence of LH rise and reduced basal LH concentration) throughout the post-infection period. The other infected goats had irregular and shortened oestrus cycles with a significant ($p<0.02$) decline in basal LH concentration.

Trypanosomosis causes menstrual disorders and sterility in both humans (Apted, 1970; Kimata *et al.*, 1994) and domestic animals (O'Hara *et al.*, 1985; Luckins *et al.*, 1986). This reproductive disorder has been suggested to be due to failure of the pre-ovulatory LH surge, particularly as in trypanosomosis, there is ovarian dysfunction and decline in the steroid hormone levels. Consequently, the positive feedback action of estradiol necessary for the pre-ovulatory LH surge is absent. Mutayoba *et al* (1988b) found that the changes occurring in oestrus cycles of *T. congolense* infected goats were accompanied by a decline in the monthly, and peak, progesterone and oestradiol levels. Similarly, in rams, trypanosomosis causes alterations in LH pulsatile secretion and resultant decline in plasma LH concentration (Mutayoba *et al,*. 1994). Therefore, since under normal circumstances, the LH surge is expected to occur once every oestrus or menstrual cycle, it's failure to occur or, a reduction in its amplitude, may account for the decline in the total amount of LH secreted per oestrus cycle and the trypanosomosis associated disruption in the oestrus or menstrual cycle.

The LH secretory pattern throughout the reproductive cycle is influenced by estradiol-negative and -positive feedback loops. The pre-ovulatory LH surge is the consequence primarily of a positive feedback action of estradiol. The effective stimulus depends on the strength-duration pattern of serum estradiol concentration which accompanies follicular maturation in the later phase of the reproductive cycle (Keye and Jaffe, 1975; Young and Jaffe, 1976). Several lines of experimental evidence support the involvement of estradiol. Antibodies to estradiol administered to rats on the morning of diestrus-2 prevent the expected ovulation in the afternoon (Ferin *et. al.*, 1964). Ovariectomy on the morning of diestrus-2 also result in the absence of the pre-ovulatory LH surge (Schwartz, 1969). While ovariectomy, followed immediately by implantation of estradiol-containing silastic capsules, restore the pre-ovulatory LH surge (Legan and Karsch, 1975). This estradiol-induced LH surge has been demonstrated in the monkeys (Knobil,1974) and humans (Young and Jaffe, 1976). Implantation of estradiol-containing silastic capsules early in the menstrual cycle, when estradiol levels are normally low, result in a LH surge. The full LH response depends on the duration of the estradiol treatment and the positive feedback action takes place when the increase in serum estradiol is sustained for approximately 36 hr (Knobil, 1974). Hence the reduction or absence of the LH surge observed in the *T. congolense* infected goats in this study could be due to the absence of serum estradiol levels for sufficient duration and level to produce a positive feedback action on the LH release by the pituitary.

The hypothalamus releases GnRH which stimulates the secretion of LH from the pituitary gonadotrophs. Arimula *et al* (1974) showed, in rats, that injection of antibodies to GnRH on the morning of proestrus prevented the LH surge and blocks ovulation that normally occurs in the afternoon. Abrupt reduction, following a single i.v injection of GnRH antiserum, in serum LH concentration also occurs in ovariectomized rhesus monkeys (Knobil

and Plant, 1978). Using Althensin as an anaesthetic agent, Sarkar *et al* (1976) found that in rats the concentration of GnRH in pituitary stalk plasma is low throughout the cycle, but rises dramatically in the early afternoon of proestrus just preceding and coincident with the LH surge. In monkeys, Neill *et al* (1977) found LH in peripheral plasma and GnRH in pituitary stalk plasma to be low during the early follicular phase of the oestrus cycle. Injection of estradiol during the mid-follicular phase of the cycle caused the levels of both LH in the peripheral plasma and GnRH in the pituitary stalk plasma to increase. These findings demonstrate the role of GnRH in the pre-ovulatory LH surge. They also suggest that the stimulation of LH secretion by estradiol is accomplished, in part, by stimulation of hypothalamic GnRH secretion.

GnRH is a decapeptide that regulates the production and release of both LH and follicle simulating hormone (FSH) in the pituitary gonadotrophic cells located in the adenohypophysis. As the GnRH producing cells release GnRH rhythmically in short bursts, they are also called the GnRH pulse generator. Only when GnRH is released in pulses do the gonadotrophs respond by synthesis and rhythmic release of LH and FSH in the amounts and ratios necessary for adequate gonadal function in sexually mature males and females (Knobil, 1980).

The negative feedback action of estradiol on LH secretion could be by either direct action on the pituitary gonadotrophs or by an indirect action through the hypothalamus. Studies on ovariectomized rat where the influence of oestradiol is absent, show that the magnitude and frequency of pulsatile LH activity increases during the period of normally low LH levels in an oestrus cycle (Arendash and Gallo, 1978; Gallo, 1980b). In fact ovariectomy is routinely carried out in studies on the neuroendocrine regulation of pulsatile LH secretion to cause experimental elevation of plasma LH and make the pulsatile pattern of LH readily

discernible. From the reports of decline in plasma estradiol and progesterone in trypanosomosis it would be expected, as the estradiol feedback inhibition is weak or absent, an increase in plasma LH to be observed in the *T. congiense* infected goats used in this study. However a reduction in plasma LH level and peak LH amplitude per oestrus cycle were observed in the infected goats. This suggests there is disruption or failure of the both the negative and positive estradiol feedback mechanisms in trypanosomosis.

The results of the present study lend support to the proposed hypothesis that trypanosomosis related reproductive disorders could be due in part to the absence of the LH surge (Luckins *et al.*, 1986). The effects of trypanosomosis on LH surge regularity and peak amplitudes in the oestrus cycle, observed in this study, indicate that the failure of the normal LH release could be associated with a disruption of the release of hypothalamic GnRH. The possible absence of the positive feedback action by estradiol on LH secretion in tryponosomosis could occur as previous studies have measured low estradiol levels in the trypanosomes infected female goats (Mutayoba *et al.*, 1988b). Trypanosomosis could cause desensitisation of oestradiol receptors in the extrahypothalamic areas (which influence the hypothalamic activity) or in the hypothalamic neurones or in the pituitary. An inhibitory or competitive binding may occur between the endogenous receptor agonists and trypanosome or trpanosome-induced material. Such effects could disrupt or abolish synchronised GnRH/LH release.

The LH surge is also influenced by inputs from more anterior brain regions: the preoptic area, and suprachiasmatic nuclei (SCN) (Barry and Barette, 1975; Silverman *et al.*, 1987; Standish *et al.*, 1987), and is coupled to the light dark/cycle (Meijer and Rietreld, 1989). The SCN which appears to have control or influence over the LH surge is a hypothalamic area which plays an integral role in circadian rhythms. In mammals, the SCN

controls circadian rhythms by synchronising or entraining the endogenous body rhythms to external environmental cues such as the light/dark cycle (Meijer and Rietveld, 1989). Reversal of c-fos gene expression in the SCN in relation to the sleep/wake cycle has been observed in trypanosome infected mice (Bentivoglio *et al.*, 1994). Hence the absence of the LH surge observed in these *T. congolense* infected goats could be due to the disruption in the normal activity of the SCN which in turn could lead to a lack of synchrony in the hypothalamo-pituitary co-ordination with the resultant impairment of GnRH and LH secretion.

To investigate if the cause of disruption of normal plasma LH levels was due to the failure of the pituitary gonadotrophs to respond to GnRH, the LH response to GnRH challenge was measured. The results showed that GnRH injection caused plasma LH levels to increase in both infected and non-infected goats. This indicates that the GnRH receptors in the pituitary gonadotrophic cells are expressed and functional in trypanosomosis. However the stronger LH response in the infected goats suggests that LH is being synthesised by the pituitary gonadotrophs but that its release is not taking place. Similar findings (stronger LH response in infected animals) have been reported using immunohistochemical and endocrine function tests (Boly *et al.*, 1994; Mutayoba *et al.*, 1994 and 1996). Hublart *et al.* (1990) found in rats infected with *T. brucei* significant increase in the pituitary LH but stable plasma LH levels. Hence it appears that in trypanosomosis, pituitary integrity and response to its endogenous agonist, GnRH, is not affected but rather the release of LH is affected.

GnRH, a neuropeptide, is synthesised in the hypothalamic neuronal cell bodies whose activities are influenced by neurotransmitters and sex steroids (Fuxe *et al.*, 1978; Weiner and Ganong, 1978). Since in trypanosomosis, release of proteases has been observed (Hublart *et al.*, 1990; Huet *et al.*, 1992), GnRH could be degraded before it reaches the pituitary.

Although GnRH specific peptidases have not been looked for in trypanosomosis, stimulation of their release could occur which would affect the action of GnRH on the pituitary gonadotrophs. Proteases originating from trypanosomes have been shown to reduce serum concentration of testosterone and increase pituitary LH content (Hublart *et al.*, 1990; Huet *et al.*, 1992). Similar degradation of GnRH could occur since GnRH is known to be susceptible to degradation in plasma (Swift and Crighton, 1979).

GnRH secretion depends on the input of a variety of peptidergic and monoaminergic neurones to the GnRH neurones (Weiner and Ganong, 1978). Monoamines shown to effect GnRH release include noradrenaline, dopamine, and 5-hydroxytryptamine (5HT) (Donoso *et al.*, 1971; Iversen, 1975). Chronic infection of mice (Amole *et al.*, 1989) and rabbits (Stibbs, 1984) with *T. b. brucei* and mice (Stibbs and Curtis, 1987) with *T. b. gambianse* cause marked disturbances in the concentration of several monoamine neurotransmitters including dopamine, 5HT and noradrenaline in the midbrain, thalamus, and hypothalamus. In several studies, clonidine, a central alpha-2 adrenergic or imidazoline receptor agonist, has been shown to stimulate the release from the pituitary of LH and growth hormone (GH) by inducing the release of their respective hypothalamic releasing hormones - GnRH and growth hormone releasing hormone (GHRH). Clonidine induces a surge in plasma GH levels (Becker *et al.*, 1995; Lanzi *et al.*, 1994; Ryna *et al.*, 1994; Thomas *et al.*, 1994 Margnan *et al.*, 1994) as well as potentiating the GH response to GHRH challenge in hypothalamic GHRH deficient rats (Arce *et al.*, 1995). In middle aged and old (constant oestrus) rats, clonidine restores normal pulsatile LH secretion (Kerry *et al.*, 1982; Estes *et al.*, 1982; Gallo, 1980; Gallo, 1981). In present study, plasma LH levels in the control goats, in response to clonidine, showed an increase in the characteristic pulsatile LH release on day 28 and 69 of the post-infection period. In contrast, the plasma LH level, pulse amplitude, and frequency in the

infected goat showed hardly any response to clonidine given on the same days. Area under LH response curve and the mean LH concentration during the 80 min sampling period after clonidine challenge were also significantly lower in the infected goats than control goats. Compared to day 28, the area under LH response curve and mean LH concentration in control goats on day 69 post-infection, were higher. This could have been due to the repeated stimulation of the hypothalamic neurones with clonidine. Similar effects on a smaller scale were observed in the infected goats. These results indicate that either the synthesis or secretion or both of GnRH from the hypothalamic neurones appears to be affected in trypanosomosis. This could be due to an inhibition of the α_2-adrenergic receptors in the hypothalamus by either an endogenous antagonist or trypanosome released material. There could also be a down regulation of the central adrenergic receptors. Besides its action on the alpha2 adrenergic receptor, clonidine also binds to a class of recently discovered receptors - the imidazoline receptors. An endogenous ligand called the clonidine-displacing substance (CDS) has been isolated from various tissues including the brain. It is possible that in trypanosomosis, there is increased levels of CDS which could effect the action of clonidine.

Localisation of large numbers of trypanosomes in the brain microvasculature has been shown to occur during the infection and this leads to vasodilatation of brain capillaries and development of pressure on the surrounding brain parenchyma (Mwambu & Losos, 1978). Though not measured in this study, high numbers of trypanosomes in the hypothalamus-pituitary blood vessels could interfere with the GnRH reaching the pituitary gonadotrophs.

GnRH release into the portal venous blood is characterised by intermittent pulses superimposed on a continuous basal secretion. This pattern reflects the pulsatile LH release from the pituitary. Since GnRH regulates the release of LH, it has been suggested that a

reduction in plasma LH is most likely to be associated with reduced GnRH secretion (Jeffcoate *et al.*, 1992). Studies in monkeys have shown that lesions in the arcuate nucleus abolish the pulsatile pattern of LH secretion while lesions sparing the arcuate have less devastating effects (Plant *et al.*, 1989). Arcuate nucleus and periventricular area, and the anterior hypothalamus preoptic area are the two sites where GnRH cell bodies have been located (Barry and Barette, 1975; Silverman *et al.*, 1987; Standish *et al.*, 1987; Moore, 1978). The frequency of GnRH secretion from these regions is determined by the firing rate of these neurones. The GnRH pulses amplitude, however is not only determined by stimuli acting at the neuronal cell body, but also by stimuli affecting the nerve terminals in the median eminence (Barry and Barette, 1975; Silverman *et al.*, 1987; Standish *et al.*, 1987). In turn the LH response to the GnRH pulses depends on the sensitivity of the pituitary gonadotrophs to the GnRH. Since from the present and other studies no pituitary impairment has been observed, the decline in LH pulse frequency and amplitude seen could be as a result of reduced release of GnRH by the hypothalamic neurones in trypanosomosis.

The initiation of normal pulsatile LH secretion, typical of young ovariectomized rats, in middle-aged rats after a single dose of clonidine indicates that the initial loss of pulsatile LH secretion involves a deficiency in the central adrenergic system (Kerry *et al.*, 1982). This deficiency which may be secondary to a decline in noradrenaline (NE) activity which may render the hypothalamo-hypophyseal-LH secretory mechanism refractory to centrally mediated stimuli for LH secretion (Kerry *et al.*, 1982). In rats, as in other animals, the episodic discharge of LH is initiated by pulsatile release of GnRH from the basal hypothalamus (Levine and Ramirez, 1980; Blake and Sawyer, 1974; Soper and Weick, 1980). With the onset of the constant oestrus (CE) state the frequency of GnRH signals from the hypothalamus decrease to an extent that hypersecretion of LH does not occur (Kerry *et al.*,

1982). This age related decline in LH pulse frequency is due to a decline in the rate of GnRH discharge from the medial basal hypothalamus, whereas the dampened LH pulse amplitude is due to central neurones system or gonadotrophs alterations or both (Kerry *et al.*, 1982). As the results from this study indicate that pituitary gonadotroph function is not affected in trypanosomosis, the disease probably causes a disruption of the central neurones activity that regulates GnRH release. In young rats several neuronal systems may participate in pulsatile release of LH after ovariectomy (Gallo, 1980a and 1980b). Primary among these is the central noradrenergic system. NE synthesis inhibition (Honma and Wuttke, 1980; Drouva and Gallo, 1976; Estes *et al.*, 1982) as well as α-adrenergic blockade (Gnodde and Schuiling, 1976) severely diminishes pulsatile LH release. The central adrenergic system therefore appears to play a permissive role in episodic LH release and a disease induced impairment of central adrenergic neurotransmission may deprive the GnRH neurones of inputs normally required for pulsatile GnRH release. However, as a single bolus of clonidine restores pulsatile LH secretion (Gallo, 1980; Kerry *et al.*, 1982), the pulsatile GnRH release may not be tightly coupled with or driven by regular episodic α–adrenergic discharge. The lack of response to clonidine in the trypanosomes infected female goats seen in the present study may therefore reflect a decrease in number or sensitivity, or both, of hypothalamic adrenergic receptors. However, as the level of GnRH in the hypothalamus or in the hypophyseal blood was not measured, it is possible the lack of LH response to clonidine in trypanosomosis may be due to diminished GnRH stores. In old rats where α-adrenergic stimulation has apparently been ineffective in enhancing the frequency of GnRH discharge from the hypothalamus, it has been shown that the extrahypothalamic adrenergic receptors are reduced (Greenberg and Weiss, 1978; Misra *et al.*, 1980), and there is also a decline in available GnRH stores (Wise and Ratner, 1980; Barnea *et al.*, 1980; Steger *et al.*, 1979).

Studies show that the brain releases an endogenous ligand designated clonidine-displacing substance (CDS) which is biologically active in the kidney, stomach and on platelets (Meeley *et al.*, 1992). CDS has a hypertensive action upon injection into the rostral ventrolateral area of the brain stem and antagonises clonidine binding sites (Atlas *et al.*, 1992; Abdel-Rahman, 1994). CDS also inhibits in a dose-dependent manner the binding of [3H]-clonidine to rat brain membranes (Synetos *et al.*, 1991). The lack of response to clonidine in *T. congolense* infected goats suggests that trypanosomosis may stimulate the release of CDS. The infection may also induce the release of α_2–adrenoceptor antagonists which would lead to block of central α_2–adrenoceptors. Although the present study did not investigate pharmacologically active substance that may be involved in the impairment of GnRH release, evidence suggests block of adrenergic inputs. GnRH pulse generator activities and associated LH pulses are subject to neuromodulation, and a block of α-adrenergic inputs by phentolamine as well as dopaminergic activity by metaclopromide have been shown to inhibit the frequency of pulse generation or arrests it altogether (Knobil, 1980). Morphine or opioids also have an inhibitory effect (Knobil, 1989) although endogenous opioids have failed to exhibit this effect in monkeys (Medhamurthy *et al.*, 1990) and humans (Petraglia *et al.*, 1986). Instead, evidence suggests that the onset of puberty, but not prepubertal gonadotropin nadir, is associated with increased opioidergic tone (Petraglia *et al.*, 1986) and enhanced pro-opiomelanocortin (POMC) gene expression in arcuate nucleus (Wiemann *et al.*, 1989). There have been no studies on opioid or opiod-related substances in trypanosomosis and, hence, what role, if any, they play in affecting GnRH release is not known .

Trypanosomes do not cross the blood-brain barrier of the infected animals, but tend to localise in regions such as the choroid plexus, trigerminal and dorsal root ganglia and the circumventricular organs including pineal gland, median eminence and area postrema

(Schultzberg *et al.*, 1988). This localization suggests that effects on the brain activity are caused by either molecules released by the trypanosome parasite or as a reaction by the host cells in response to the infection. The paraventricular and supraoptic hypothalamic nuclei are affected during trypanosomosis (Bentivoglio *et al.*, 1994), and this may explain, in part, the lack of hypothalamic response to clonidine in the *T. congolense* infected goats.

There are also reports of central and peripheral demyelination in chronic cases of human trypanosomosis (Dumas and Bao, 1988). CNS damage in mice has been shown to occur due to production of autoantibodies to the myelin basic proteins - galactocerebrosides and gangliosides (Hunter *et al.*, 1992b). This indicates that part of neuropathology of trypanosomosis could be associated with demyelination. There is also reactive gliosis in trypanosomosis and astrocytes are stimulated to produce cytokine, in particular, IL-1α, IL-6, FN-γ, TNFα and prostaglandins (Adams *et al.*, 1986; Eddlestone and Mucke, 1993; Stevens and Moulton, 1977; Anthoons *et al.*, 1989). These substances enhance the neuropathologic effects of trypanosomosis (Alifiatoyo *et al.*, 1994) and their actions may account for the changes observed in LH secretion during the hypothalamic challenge with clonidine, particularly, if they are involved in demyelination of adrenergic nerve fibres.

The clinical features of *T.congolense* infected goats showed a sharp increase in WBC count between 20 and 30 days post-infection which thereafter fluctuated. Following the onset of parasitaemia, 5-9 days post-infection, the goats became anaemic, emaciated, and showed elevated rectal temperature findings similar to other studies (Murray *et al.*, 1981; Mutayoba *et al.*, 1989 and 1995). In this study, WBC differential count was not done thus, it is not possible to know whether there was a difference in the ratios of basophils, eosinophils, lymphocytes, mononuclear and polymorphonuclear cells found in the blood. The immune response is evident by the rise in WBC count observed 25-35 and 50 days post-infection

which followed parasitaemia peaks seen on day 20, 40 and 50 post-infection. The first decline in parasitaemia, 30 days post-infection, was preceded by a rise in WBC count 5 days earlier. The resurgence in parasitaemia, 35-40 days after infection, is probably due to surviving parasites producing and presenting new antigenic variants by which to evade the immune surveillance of the host. Several studies have reported that trypanosomes are capable of changing their antigen conformations (Blum *et al.*, 1993; Pentreath, 1991). Pathologic effects of trypanosomosis is therefore enhanced by the parasite's ability to change its surface glycoproteins. The parasitaemia curve showed peaks on days 20, 40, and 50 post-infection which could be associated with three different antigenic variants, each appearing after the elimination of the majority of the previous antigenic population.

The parasite escape from the host immune response leads to immunosuppression (Borowy *et al.*, 1990). Bakhiet *et. al.* (1993), showed that *T. b. brucei* release a Trypanosome Lymphocyte Triggering Factor (TLTF) which stimulates interferon-γ (IFN-γ) production from CD8+ T cells which simulate trypanosome growth (Bentivoglio *et al.*, 1994). Thus part of the host's response to the parasite aids the parasites' survival, particularly in the host's lymphoid tissues from where the parasites enter the blood stream. The rise in the temperature seen during the trypanosome infection was probably due to the pyrogenic substances released by the parasites.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER STUDIES

The results of the present study show that in *T. congolense* infected female goats, oestrus cycles are disrupted. This is characterized by a decline in the mean LH levels per oestrus cycle and a reducted LH surge. Since the pattern of LH levels is important for normal reproductive cycles in females, the changes observed in this study may undrely the infertility observed in trypanosomosis. The reduced LH levels lead to reduced cellular activity in the cells that are normally stimulated by the hormone. Failure of the LH surge to occur or a reduction in the surge leads to failure of ovulation and hence infertility.

The problem in LH secretion could be due to the pituitary gland dysfunction. However the results of the present study together with other published reports(see chapter 2 and 5) indicate that the changes in LH levels are not due to pituitary dysfunctions. The trpanosomes infection did not affect the response of female goats to GnRH change. This observation indicates that LH synthesis, secretion and release by the gonadotrophes in the pituitary gland is not affected. In addition to the GnRH receptors on the gonadotrophes are being expressed and are receptive to GnRH. Thus it is probable that there could be lack of stimulation of the gonadotrophes due to low levels of GnRH reaching the pituitary from the hypothalamus. The above observations therefore point to a hypothalamicdys function as the cause of disruption in the LH secretion in trypanosome-infected goats.

From the clonidine experiment, trypanosomosis appears to disrupt the hypothalamic function. Clonidine challenge did not cause changes in the LH secretion pattern during the infection as was observed before the infection. This could be an indication tha the a-adrenergic receptors through which clonidine acts are probably affected by trypanosomes infection.

In conclusion the present study has found that in T. congolense infected female goats the oestrus cycles were disrupted, LH surge value reduced and there was a decline in the mean LH concentration per oestrus cycle. The LH response to exogenous GnRH challenge in the infected female goats was normal while LH response to clonidine challenge was absent in infected goats. These observations therefore suggest that the trypanosomosis-related reproductive disordes may be due in part to a hypothalamic or high CNS dysfunctions. However further investigations should be carried out in infected and non-infected female goats inorder to elucidate the mechanisms underlying these disorders.

Topics for further studies:

I Determination of LH secretion pattern over several hours in infected and non-infected female goats before and after challenge with alpha-2-adrenergic-agonist.

The hypothalamus releases GnRH into the portal venous blood in intermittent pulses which are reflected in the pulsatile pattern of LH secretion from the pituitary in normal females. The activities of these two hormones are depedent on the delicate synchrony of their secretion in pulses. Determining the LH secretion pattern over several hours will help to establish whether the naturally expected pattern is being affected by the trypanosomes infection. The present study has shown lack of stimulation of the hypothalamus by clonodine and therefore determination of LH secretion pattern in response to alpha-2-adrenergic-agonist would help to confirm the implied lack of stimulation ti the pituitary.

II Determination of GnRH levels in the hypophyseal portal system and the hypothalamus

GnRH is the hypothalamic hormone responsible for the regulation of LH release by the pituitary gland. Because the results of the present study are pointing to a hypothalamic dysfunction as the primary cause of reproductive dysfunctions in trypanosomosis , its important that the effects of the disease on the GnRH levels be measured directly in the

hypophyseal portal system blood. Findings from this study may reveal whether the disease affectssyntheseis, release or secretionog GnRH. The measurement of GnRH levels should be done both at the hypophyseal portal system and the hypothalamus.

III Histological study of the hypothalamic neuroendocrine system

GnRH is secreted by neuroendocrine cells of the hypothalamus. A study of the cell structures before and after or in infected and in no-infeted female goats can help to establish whether the cells are affected during trypanosomosis or not. Immunohistochemical study techniques can reveal which regions of the hypothalamus are affected by the disease.

IV Determination of plasma oestrogoen levels and oestrogen receptor presence in the brain.

Oestrogen hormone is required for the development of follicles and also feedback mechanism in the release of GnRH and LH. The determination of plasma oestrogen levels will confirm the reduced level which have been reported in some previous studies. DEcline in the normal oestrogen levels would indicate lack of sufficient oestrogen to facilitate the process of follocle development and also the feedback mechanism. DEtermination oif oestrogen receptor levels and status will show their presence and availability to oestrogen stimulation.

V Determination of proteases and peptidases levels in the brain

Proteases and peptidases are enzymes that breakdown or degrade peptide hormones. Determining the levels of these enzymes in infected and non-infected or before and after infection can help to establish whether trypanosomosis triggers an increase in their levels. Incase the levels of these enzymes are elevated in the brain they may cause degradation of GnRH leading to insufficient quantities to stimulate active secretion of LH. Therefore in

addition to determining the levels of GnRH in the hypothalamus and the hypophyseal portal system the levels of proteases and peptidases should also be determined in these areas.

REFERENCES

Abdel-Rahman, A. A. (1994). Alcohol abolishes the hypotensive effect of clonidine in spontaneously hypertensive rats. *J. Hypertens.* 24(6):802-807.

Abebe, G. and Eley, R. M. (1992) : Hypothalamic-pituitary-adrenal (HPA) axis responsiveness to insulin induced hypoglycaemia is modified by trpanosome infection in Boran (*Bos indicus*) cattle. *Res. Vet. Sci.* 53:68-73.

Abebe, G., Eley, R. M. and Ole-Moiyoi. O. K.(1993) : Reduced responsiveness of the hypothalamus-pituitary-adrenal axis in Boran (*Bos indicus*) cattle infected with *Trypanosoma congolense.Acta Endocrinol.* 129:75-88.

Adams, J.H., Doua, F., Dago-Akribi, Bao, Y. and Haller, L. (1986). Human trypanosomiasis (*T.b. gambianse*): A study of 16 fatal cases of sleeping sickness with some observation on acute reactive arsenica encephalopathy. *Neuropath. Appl. Neurobiol.* 12:390-404.

Alafiatayo, R.A., Cookson, M.R., and Pentreath, V.W. (1994). Production of prostaglandins D2 and E2 by mouse fibroblasts and astrocytes in culture caused by *Trypanosoma brucei brucei* products and endotoxin. *Parasitol. Res.* 80:223-229.

REFERENCES

Abdel-Rahman, A. A. (1994). Alcohol abolishes the hypotensive effect of clonidine in spontaneously hypertensive rats. *J. Hypertens.* 24(6):802-807.

Abebe, G. and Eley, R. M. (1992) : Hypothalamic-pituitary-adrenal (HPA) axis responsiveness to insulin induced hypoglycaemia is modified by trpanosome infection in Boran (*Bos indicus*) cattle. *Res. Vet. Sci.* 53:68-73.

Abebe, G., Eley, R. M. and Ole-Moiyoi. O. K.(1993) : Reduced responsiveness of the hypothalamus-pituitary-adrenal axis in Boran (*Bos indicus*) cattle infected with *Trypanosoma congolense.Acta Endocrinol.* 129:75-88.

Adams, J.H., Doua, F., Dago-Akribi, Bao, Y. and Haller, L. (1986). Human trypanosomiasis (*T.b. gambianse*): A study of 16 fatal cases of sleeping sickness with some observation on acute reactive arsenica encephalopathy. *Neuropath. Appl. Neurobiol.* 12:390-404.

Alafiatayo, R.A., Cookson, M.R., and Pentreath, V.W. (1994). Production of prostaglandins D2 and E2 by mouse fibroblasts and astrocytes in culture caused by *Trypanosoma brucei brucei* products and endotoxin. *Parasitol. Res.* 80:223-229.

Amole, B., Sharpless, N., Wittner, M. and Tanowitz, H.B. (1989). Neurochemical measurements in the brain of mice infected with *Trypanosoma brucei brucei*. *Ann. Trop. Med. Parasitol.* 83:223-232.

Anosa, V. O. and Isoun, T. T. (1980) : Further observations of testicular pathology in *Trypanosoma vivax* infection in sheep and goats. *Res. Vet. Sci.* 28:151-160.

Anthoons, J.A.M.S., Van Marck, E.A.E., Gigase, P.J.J. and Stevens, W.J. (1989). Immunohistochemical characterization of the mononuclear cells in the brain of the rat with experimental chronic *Trypanosoma brucei gambianse* infection. *Parasitol. Res.* 75:251-256.

Apted, P.I.C. (1970). Clinical manifestation and diagnosis of sleeping sickness. In : *The African Trypanosomiasis*, Ed. by H.W. Mulligan and W.H. Potts, P681-683. Allen and Unwin, London.

Arce, V., Garscia, Baros, M., Vara, E., Lima, L., Tresguerres, J.A. and Devesa, J. (1995). Clonidine potentiates growth hormone response to a growth hormone releasing hormone challenge in hypothalamic growth hormone deficient rats. *J. Neuroendocrinol.* 61(5):552-558.

Arendash, G.W., and Gallo, R.V. (1978). A characterization of the effects of pentobarbital on episodic LH release in ovariectomized rats. *Neuroendocrinology.* 27: 204-215

Arimura, A., Ddebeljuk, L. &Schally, A. (1974). Blockade of the pre-ovulatory surge of LH and FSH and of ovulation by anti-LHRH serum in rats. *Endocrinology.* 95:23-325

Aronin, N., Sagar, S.M., Sharp, F.R. and Schwartz, W.J. (1990). Light regulates expression of a Fos-related protein in rat suprachiasmatic nuclues. *Proc. Natl. Acad. Sci.* USA 87:5959-5962

Atlas, D., Diamant, S. and Zonnenschein, R. (1992). Is imidazoline a unique receptor? A correlation with clonidine-displacing substance activity. *J. Hypertens.* 5(4pt2):83S-90S.

Bakhiet, M. , Olosson, T. , Ljungdahl, A. , Hojeberg, B. , Van der Meide, P. , Kristensson, K. (1995): Induction of interferone-γ, transforming growth factor-β and interleukin-4 in mouse strains with different susceptibility to *Trypanosoma brucei. (In press).*

Barnea, A., Cho., G. and Porter, J.C. (1980). Effects of aging on the subneuronal distribution of LH releasing hormone in the hypothalamus. *Endocrinology.*106: 1980.

Barry, J. and Barnette, J. Barette, B. (1975). Immunolourescence study of LRF neurones in primates. *Cell Tissue Res.* 164:163.

Becker, K., Stegenga, S, and Conway, S. (1995). Role of insulin-like growth factor-1 in regulating growth hormone release and feedback in the male rat. *J. Neuroendocrinol.* 61(5):573-583.

Bentivoglio, M., Grassi-Zucconi, G. and Kristensson, K. (1994) : From trypanosome to the nervous system, from molecules to behaviour: A survey on the occassion of the 90th anniversary of Castellan's discovery of the parasite in sleeping sickness.*Ital. J. Neurol. Sci.* 15: 77-89.

Bentivoglio, M., Balercia, G. and Krujer, L. (1991). The specificity of the non-specific thalamus: The midline nuclei. In: G. Hostege Ed. Progess in Brain Research Elsevier, Amsterdam 87:53-58.

Blake, C.A and Sawyer, C.H. (1974). Effects of hypthalamic deafferentation on the pusatile rhythm in plasma concentrations of LH in ovariectomized rats. *Endocrinology.* 94:730-736.

Blum, M.L., Down, J.A., Gurnett, A.M, Carrington, M., Turner, M.J. and Wiley, D.C. (1993). A structural motif in the variant surface glycoproteins of typanosoma brucei. *Nature* 362:603-608.

Boly H. Hunblot P. Tillet Y. Thibier M. (1994). Effects of *Trypanosoma congolense* infection on pituitary gland of Baoule bulls: Immunohistochemistry of LH- and

FSH-secreting cells and response to plasma LH and testosterone of combined dexamethosone and GnRH treatment. *J. Reprod. Fert.* 100:157-62.

Borowy, N.K., Sternberg, J.M., Schrieber, D. Nonnengasser, C. and Overath, P. (1990). Suppressive macrophage occuring in murine Trypanosma brucei infection inhibit T-cell responses in in vivo and in vitro. *Parasit. Immunol.* 12: 233-246.

Borst, P. (1991). Molecular genetics of antigenic variation.. *Immunol. Today* 12: 29-33.

Canny, B.J. Clarker, J. and Funder, W. (1990). Adrenocorticotropin response to endogenous and exogenous secretagogues in the sheep: Specificity of glucocorticoid action. *Neuroendocrinology.*51:181-189

Chicoteau, P. Bassinga, A. Sidibe, I. , Pobel, T. ,Richard, X. and Clausen, P. (1990) : Influence of high risk exposure to *Trypanosoma* infection on the reproduction of the Baoule' cattle in Burkina Faso. *Rev. Elev. Med. Vet. Pays Trop.* 43(4) : 473-477 *(Eng. Abstr.).*

Donoso, A.O., Bishop, W. Fawcett, C.P., Kruilch, L., and McCann, S.M. (1977). Effects of drugs that modify brain monoamines concentrations on plasma gonadotropin and prolactin levels in the rat. *Endocrinol.* 89: 774-784.

Drouva, S.V. and Gallo. R.V. (1976). Catecholamine involvement in episodic LH release in adult overiectomized rats. *Endocrinology.* 99: 651-658.

Dumas, M., and Bao, F.Y. (1988). Human African trypanosomiasis. In *Handbook of Clincial Neurology*, 8, edited by P.J. Vinken, G.W. Bruyn and H.L. Klawens. Amsterdam: Elsevier: 339-344.

Eakin, A.E., Mills, A.A., Harth, G., Mckerrow, J.H. and Craik, C.S. (1992). The sequence, organisation and expression of the major cysteine protease (cruzain) from *Trypanosoma cruzi. J. Biol. Chem.* 267: 7411-7420.

Eddlestone, E. and Mucke, L. (1993). Molecular profile of reactive astroytes-implications for their role in neurologic disease. *Neuroscience*. 54: 15-36.

Edgar, D.m>, Dement, W.C., Fuller, C.A. (1993). Effect of SCN lesions on sleep in squirrel monkeys: Evidence for opponent processes in sleep-wake regulation. *J. Neurosci.*, 13: 1065-1079.

Estes, K.S., Simpkins, J.W. and Kalra, S.P. (1982). Resumption with clonidine of pulsatile LH release following acute norepinephrine depletion in overiectomized rats. *Neuroendocrinology*. 35: 56.

Estes, K.S. , Simpkins, J.W. and Chen, C.L. (1980). Alteration in pulsatile release of LH in aging rats. *Proc. Soc. Exp. Biol. Med.* 163:384.

Ferin, M., Tempone, A., Zimmerign, P. and VandeWiele, R. (1964). Effect of antibody to 17β-estradiol and progesterone on the estrous cycle of the rat. *Endocrinology.* 85:1070-1078.

Fiennes, R.N.T.W. (1950). The cattle trypanosomiasis, some considerations of pathology and immunity. *Ann. Trop. Med. Parasitol.* 44: 42-54.

Fuxe, K.A. Loftstrom, T. Hokfelt, L. Ferland, K. Andersson, L., Agnati, P. Eneroth, J.A. Gustafsson and P. Skett (1978). Influence of central catecholamines on LHRH-containing pathways. *Clin. Obs. Gyn.* 5: 251-269.

Gallo, B. Robert (1981). Pulsatile LH release during the ovulatory LH suge on proestrus in the rat. *Biol. Reprod.* 24: 100-104.

Gallo, R.V. (1980a). Effects of manipulation of brain dopaminergic and serotoningergic systems on basal pulsatile release and perisuprachiasmatic-induced suppression of pulsatile LH release in ovariectomized rats. *Neuroendocrinology.* 31: 161-167.

Gallo, R. (1980b). Neuroendocrine regulation of pulsatile luteinizing hormone release in the rat. *Neuroendocrinology.* 30: 122-131.

Gea, Ordonez, P. Cerban, F. Losa, D., Chizzolini, C. and Vottero-cima, E. (1993). Chagas' disease cardioneuropathy: association of anti-*Trypanosoma cruzi* and anti-sciatic nerve antibodies. *Am. J. Trop. Med. Hyg.* 49: 481-588.

Gnodde, H.P and Schuiling, G.A. (1976). Involvement of catecholaminergic and cholinergic mechanisms in the pulsatile release of LH in the long term overiectomized rats. *Neuroendocrinology*. 20: 212.

Goodwin, J.S. and Webb, D.R. (1980). Regulation of the immune response by prostaglandins. *Clin. Immunol. Immunopathol*. 15: 106-122.

Grassi-Zucconi G., Bentivoglio, M., Harris, J., Mohammed, A.H and Kristensson, K. (1995). Changes in circadian time keeping and sleep structure in experimental tryapanosomiasis in the rat. (*In Press*).

Grassi-Zucconi, g., Menegazzi, M., Carcereri De Prati, A., Bassetti, A., Montagnese, P., Mandile, P., Cosi., C. and Bentivoglio, M. (1993). C-fos mRNA is spontaneously induced in the rat brain during the activity period of the circadian cycle. *Euro. J. Neurosci.* 5: 1071-1078.

Gray, A.R. and Luckins, A.G. (1980). The initial stages of infection with cyclically transmitted *Trypanosoma* in rabbits, calves and sheep. *J. Comp. Pathol.* 90: 499-512.

Greenberg, L.H. and Weiss, B. (1978). β-adrenergic receptors in aged rat brain; reduced number and capacity of pineal gland to develop supersensitivity. *Science.*201: 61-63.

Greenwood, B.M. (1974). Possible role of a B-cell mitogen in hypergammaglobinaemia in malaria and trypanosomiasis. *Lancet* 16: March 435-436.

Hawking, F., and Greenfield, J.G. (1941). Two autopsies on Rhodesian sleeping sickness, visceral lesions and significance of changes in the CSF. *Trans. Roy. Soc. Trop. Med. Hyg.* 35: 155-164.

Hayaishi, O. (1991). Molecular mechanisms of sleep-wake regulation. Role of prostaglandin D2 and E2. *FASEB J.* 5: 2575-2581.

Honma, K. and Wuttke, W. (1980). Norepinephrine and dopamine turnover rates in the medical preoptic area and the media basal hypothalamus in the rat brain after various endocrinological manipulations. *Endocrinology.* 106: 1848-1853.

Hontebeyerie-Joskowicz, M., Said, G. Milton, G., Marchal, G. and Eisen, H. (1987). L3T4$^+$ T cells able to mediate parasite-specific delay-type hypersensitivity play a role in the pathology of experimental Chagas' disease. *Euro. J. Immunol.* 17: 1027-1033.

Hublart, B., Tetaert, D., Croix, D., Boutignon, F., Degand, P. and Boersma, A. (1990). Gondadotrpic dysfunction by *Trypanosoma brucei brucei* in the rat. *Acta Tropica.* 47: 177-184.

Huet, G., Richet, C., Demeyer, D., Biisiau, H., Soudan, B., Teteart, D., Han, K.K. and Degand P. (1992). Characteristic of different proteolytic activities in *Trypanosoma brucei brucei. Biochimica Biophysica Acta* 38: 213-221.

Hunter, C.A. Jennings, F.W., Kennedy, P.G.E. and Murray, M. (1992a). Astrocyte activation correlates with cytokine production in central nervous system of *Trypanosoma brucei brucei* infected mice. *Lab. Investig.* 67: 635-642.

Hunter, C.A. , Jnning, F.W., Tierney, J.F., Murray, M. and Kennedy, P.G.E. (1992b). Correlation of autoantibody titres with central nervous system patholoty in experimental African trypanosomiasis. *J. Neuroimmunol.* 41: 143-148.

Hunter, C.A., Gow, J.W., Kennedy, P.G.E., Jennings, F.W. and Murray, M. (1991). Immunopathology of experimental African sleeping sickness: detection of cytokine mRNA in brains of *Trypanosoma brucei brucei* infected mice. *Infect. Immunol.* 59: 4636-4646.

Ibuka, N. and Kawamura, H. (1975). Loss of circadian rythmn in sleep-wakefulness cycle in rat by suprachiasmatic nucleus lesions. *Brain Res.* 96: 76-81.

Ikede, B. O. and Losos, G. F. (1975) :Pathogenesis of *Trypanosoma brucei* infection in sheep. III hypophyseal and other endocrine lesions. *J. Comp. Path. 33:85.*

Ikede, B. O. ,Elizabeth, E. and Akpane, S. O. (1988) . Reproductive diaorders in African trypanosomosis : A review. *Acta Tropica 45: 5-10* .

Ikede, B.O., Akpokodje, J.U., Hill, D.H and Ajidagba, P.O. (1977). Clinical haematological and pathological studies in donkeys experimentally infected with *Trypanosoma brucei*. *Trop. Anim. Hlth Prod.* 9: 93-98.

Imura, H., (1958). ACTH and releated peptides: Molecular Biology, Biochemistry and regulation of secretion. *J. Clin. Endocrinol. Metabol.* 14: 845-866.

Isoun, T. T. and Anosa, B. O. (1974). Lesions in reproductive organs of sheep and goats experimentally infected with *Trypanosoma vivax*. *Trop. Parasit.* 25:269.

Iversen, L.L. (1975). Dopamine receptors in the brain. *Science* 188: 1084-1089.

Jeffcoate, I.A. (1992). Concentrations of luteinizing hormone and oestradiol in plasma and in response to injection of gonadotropin- releasing hormone analogue at selected stages of anoestrus in domestic bitches. *J. Reprod. Fertil.* 94: 423-429.

Kaaya, G. P. and Oduor-Okelo, D. (1980). The effects of *Trypanosoma congolense* on the testes and epididymis of the goats . *Bull. Anim. Hlth Prod. Africa.* 28: 1-5.

Kalsbeek, A., Teclemariam-Mesbah, R. and Pevet, P. (1993). Efferent projections of suprachiasmatic nucleus in golden hamter (*Masecricetus auratus*). *J. Comp. Neurol.* 332: 293-314.

Kanyari, P.W.N., Allonby, E.W., Wilson, A.J. and Munyua, W.K. (1983). Some economic effects of trypanosomiasis in goats. *Trop. Anim. Hlth. Prod.* 15: 153-160.

Kerry, S., Estes, K.S. and Simpkins, J.W. (1982). Resumption of pulsatile luteinizing hormone release after α-adrenergic stimulation in ageing, constant oestrus rats. *Endocrinology.* 111: 1778-1784.

Keye, W. and R. Jaffe (1975). Strength-duration characteristics of estrogen effects on gonadotropin response to GnRH in women. I. Effects of varying duration of estradiol administration. *J. Clin. Endocrinol. Metab.* 41: 1003-1008.

Kierszenbaum, F. and Budzko, D.B. (1973). Immunological properties of trypanosomal lipopolysaccharides and its effects on the infection with *Trypanosoma (Schizotrypanum) cruzi. Immunolog. Comm.* 2: 265-276.

Kimata, D.M., Makawiti, D.W., Tengekyon, K.M., Dadzie, S. and Waindi, E.N. (1994). Delayed recovery of adrenocortical and testicular function after chemotherapy of human trypanosomosis. *Acta Tropica* 57: 69-74.

Kimeto, B.A. Mugera, G.M. and Nyaga, P.N. (1990). Haemorhagic pancarditis in cattle infected with *Trypanosoma vivax. Vet. Parasitol.* 43: 295-301.

Kinoshita, F., Nakai, Y., Katakami, H., Imura, H., Shimizu, T. and Hayaishi, O. (1982). Suppressive effects of prostaglandin (PG) D2 on pulsatile luteinizing hormone release in conscious castrated rats. *Endocrinology.* 110: 2207-2209.

Knobil, E. and T. Plant (1978). Neuroendocrine control of gonadotropin secretion in the female rhesus monkey. In: *Frontiers in Neuroendocrinology*, W. Ganong and L. Martini, eds., Raven Press. N.Y., 5: 249-264.

Knobil, E. (1980). Neuroendocrine control of the menstrual cycle. *Rec. Prog. Horm. Res.*36:53

Knobil, E. (1974). On the control of gonadotropin secretion in the rhesus monkey. *Rec. Prog. Horm. Res.* 30: 1-46.

Knobil, E. (1989). The electrophysiology of the GnRH pulse generator. *J. Steroid Biochem.* 33: 669.

Kornhauser, J.M. Nelson, D.E. Mayo, K.E. and Takahashi, J.S. (1990). Photic and circadian regulation of c-fos gene expression in the hamster suprachiasmatic nucleus. *Neuron* 5: 127-134.

Lambert, P.H., Berney, M. and Kazyumba, G. (1981). Immune complexes in serum and cerebrospinal fluid in African trypanosomiasis. Correlation with polyclonal B. cell

activation and with intracerebral immunoglobuli synthesis. *J. Clin. Investig.* 67: 77-85.

Lanzi, R., Lapointe, M., Gurd, W. and Tannenbaum, G.S. (1994). Evidence of a primary involvement of somatostatin in clonidine-induced growth horomone release in conscious rats. *J. Endocrinol.* 144(2): 259-266.

Le, J. and Vilcek, J. (1987). : Biology of diseases. Tumor necrosis factor and interleukin-1: cytokines with multiple overlapping biological activities. *Lab. Investig.* 56 :234-248.

Legasn., S. and F. Karsch (1975). A daily signal for the LH surge in rats. *Endocriology.* 96: 57-62.

Levine, J.E. and Ramires, V.D. (1980). In vivo release of LH releasing hormone estimated with push-pull cannulae from the media basal hypothalami of overiectomized, steroid-primed rats. *Endocrionology.* 107: 1782.

Losos, G.J. and Ikede, B.O. (1970). Pathology of experimental trypanosomiasis in the albino rat, rabbit, goat and sheep. A preliminary report. *Canad, J. Comp. Med.* 34: 209-212.

Losos, G.J. and Ikede, B.O. (1972). Review of pathology of the disease of domestic and laboratory animals caused by *Trypanosoma congolense, T, vivax, T. brucei, T rhodensiense and T. gambianse. Vet. Pathol. Supp.* 9: 1-71.

Luckins, A. G. ,Llewelyn, C. , Munro, C. D. and Murray, M. (1986). : Effects of pathogenic trypanosomes on the mammalian reproductive system. *IAEA-SM-* 292/23 :351-363.

Margnan, E., Cataldi, M., Guillaume, V., Massocchi, L., Dutour, a., Razafindraibe, H. Sauze, N, Renard, M. and Oliver, C. (1994). Role of growth hormone (GH)-releasing hormone and somatostatin in the mediation of clonidine-induced GH release in sheep. *J. Endocrinol.* 134(2): 562-567.

Malmberg, A.B. and Yaksh, T.L. (1992). Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. *Science* 257: 1276-1279

Mc Arther, A.J. Gillettee, M.U. and Prosser, R.A. (1991). Melatonin directly resets the rat suprachiasmatic circadian clock in vitro. *Brian Res.* 565: 158-161.

Medhamuthy, R., Gay, V.L. and Plant, T.M., (1990). The prepubertal hiatus in gonadotropin secretion in the male rhesus monkey (Macaca mulatta) does not appear to involve endogenous opioids peptide restraint of hypothamic gonadotropin releasing hormone release. *Endocrinology.* 126: 1036.

Meely, M.P., Hensley, M.L., Ernsberger, P., Felsen, D. and Reis, D.J. (1992). Evidence for bioactive clonidine-displacing substance in peripheral tissues and serum. *J. Biochem. Pharmacol.* 44(4): 733-740.

Meijer, J.H. and Rietveld, W.J. (1989). Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. *Physiol. Rev.* 69: 671-707.

Misra, C.H. Shelat, H.S. and Smith, R.C. (1980). Effects of age on adrenergic and dopaminergic receptor binding in rat brain. *Life Sci.* 27: 521.

Mistlberger, R.E. Bergmann, B.M., Waldenar, W. and Reschtschaffen, A. (1983). Recovery sleep following sleep deprivation in intact and suprachiasmatic nuclei-lesioned rats. *Sleep* 6: 217-233.

Moberg, G.P. (1985). Biological response to stress: Key to assessment of animal well-being. In *Animal Stress*. Ed P.M. Moberg. Bethesda, Maryland, American Physilogical Society: pp 22-49.

Moore, R.Y. (1978). Neuroendocrine regulation of reproduction. In: *Reproductive Endocrinology*. Yen, S. and R. Jaffe, eds., W.B. Saunders Co., Philadelphia, pp 3-33.

Morgan, J.I. and Curran, T. (1991). Stimulus-transciption coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Ann. Rev. Neurosci.* 14: 421-451.

Morgan, J.I. and Curran, T. (1989). Stimulus-transciption coupling in neurones: role of cellular immediate-early genes. *Trends Neurosci.* 12: 459-462.

Morrison, W.I. Murray, M., Sawyer P.D. and Preston, J.M. (1981). The pathogenesis of experimentally induced *Trypanosoma brucei* infection in the dog. I. Tissue and organ damage. *Am. J. Pathol.* 102: 168-181.

Mouton, J.E. and Sollod, A.E. (1976). Clinical, serologic and patologic changes in calves with experimentally induced *Trypanosoma brucei* infection. *Am. J. Vet. Res.* 37: 791-802.

Murray, M., Clifford, D.J. Gettinby, G. Snow, F. and McIntyre, W.I.M. (1981). Susceptibility to African trypanosomiasis of N'dama and Zebu cattle in areas of *Glossina morsitans submorsitans*. *Vet. Rec.* 109: 503-510.

Mutayoba, B. M. and Gombe, S. (1989). Effects of African trypanosomiasis on plasma cortisol and thyroxine concentration in goats. *Res. Vet. Sci.* 47:315-318.

Mutayoba, B. M. ,Gombe, S. and Waindi, E. N. (1988b). Trypanosome induced ovarian dysfunction. *Acta Tropica.* 45 :235-237.

Mutayoba, B. M. , Meyer, H.H.D., Osaso, J. and Gombe, S. (1989). Trypanosome induced increase in prostaglandin F2α and it's relationship with corpus luteum function in the goat. *Theriogenology.* 32:545-555.

Mutayoba, B. M. , Eckersall, P. D. , Jeffcoate, I. A. , Gray, C. E. and Holmes, P. H. (1995). : Effects of *Trypanosoma congolense* on pituitary and adrenocortical function in sheep: Changes in adrenal gland and cortisol secretion . *Res. Vet. Sci.* 58 :174-179.

Mutayoba, B. M. ,Eekersall, P. D. , Jeffcoate, I. A. , Cestnik, V. and Holmes, P. H. (1994). Effects of *Trypanosoma congolense* infection in rams on the pulsetile secretion of LH and testosterone and responses to injection of GnRH. *J. Reprod. Fert.* 102 (2):425-431.

Mutayoba, B. M. , Gombe, S. , Kaaya, G. P. and Waindi, E. N. (1988a). : Effects of chronic experimental *Trypanosoma congolense* infection on the ovaries, pituitary, thyroid and adrenal glands in female goats. *Res. Vet. Sci.* 44: 140-146

Mutayuba B. M., Eckersall, P. D., Cestnik, V., Jeffcoate, I. A., Gray, C. E., and Holmes, P. H. Effects of *Trypanosoma congolense* on pitutary and adrenocortical function in sheep: changes in the adrenal gland and cortisol secretion. *Res. Vet. Sci.* 1995:58:174-9.

Mutayuba, B. M., Eckersall, P. D., Seely, C., Gray, C. E., Cestnik, V., and Jeffcoate, I. A. Effects of *Trypanosoma congolense* on pitutary and adrenocortical function in sheep: response to exogenous corticotropin-releasing hormone. *Res. Vet. Sci.* 1995:58:180-5.

Mutayuba, B. M., Eckersall, P. D., Jeffcoate, I. A., Cestnik, V., and Holmes, P. H. (1994) Effects of *Trypanosoma congolense* infections in rams on the pulsatile secretion of LH and testosterone and respones to infection of GnRH. *J. Reprod. Fert.* 102:425-31.

Mwanbu, P.M. and Losos, G.J. (1978). Ultrastuctural changes in blood vessel and tissues of cattle experimentally infected with *Trypanosoma congolense* and *T. vivax:* A preliminary report . In *Pathogenicity of Trypanosomes* pp 184-185 Eds G. Losos and A Chouinard. International Development Research Centre. Ottawa.

Nieill, J.J. Patton, R., Dailey,R., and Tsou, G. Tindal (1977). LHRH in pituitary stalk blood of rhesus monkeys: Relationship to level of LH release. *Endocrinology.* 101: 430-434.

Norton, W.T., Aquino, D.A., Hozumi, I., Chiu, F.C. and Broznzn, C.F. (1992). Quantitive aspect of reactive gliosis: A review. *Neurochem. Res.* 17: 877-885.

O'Callaghan, J.P. and Seidler, F.J. (1992). Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced astrogliosis does not require activation of ornithine decarboxylase. *Neuorsci. Lett.* 148: 105-108.

O'Hara, H. B. ,Gombe, S. and King, G. P. (1985). Responsiveness of pituitary to CRH and adrenal gland to ACTH following *Trypanosoma congolense* infection in female goats. *7th International Congress of Protozoology, Nairobi , Kenya.*

O'Hara, H. B. and Gombe, S. (1989). Fertility of small East African goats following prepubertal infection with *Trypanosoma congolense. Isotope aided studies on sheep and goats production in the tropics.* 195-201.

Olsson, T. , Bakhiet, M. , Bojeberg, B., Ljungdahl, A., Edlund, C., Andersson, G., Ekre, H. P., Fung Lueng, W. P., Mark, T., Wigzell, H., Fiszer, U. and Kristensson, K. (1993). CD8 is critically involved in lympocyte activation by a *Trypanosoma brucei brucei* released molecule. *Cell.* 72 :715-728.

Ouaissi, a., Cornette, J., Velge, P. and Capron, A. (1988). Identification of anti-acetylcholinestrase and anti-idiotype antiboides in human and experimental Chafas' disease pathological implicaions. *Europ. J. Immunol.* 18: 1889-1994.

Paris, J., Murray, M., and McOdimba, (1982). A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. *Acta Tropica.* 39: 307-316.

Parkne, D.J. and Dhake, R.P. (1972). Abortions due to *Trypanosoma evansi* in the buffalo, (*Bubalus bubalis*). *Ind. Vet. J.* 49: 1091-1092.

Pentreath, V.W. (1991). The search of primary events causing the pathology in African sleeping sickness. *Trans.Roy. Soc. Trop. Med. Hyg.* 85: 145-147.

Pentreath, V.W., Rees, K., Owolabi, O.A. Philip, K.A. and Doua, F. (1990). The somnogenic T-lymphocyte suppressor prostaglandin, D2 is selectively elevated in cerebrospinal fluid of advanced sleeping sickness patients. *Trans. Roy. Soc. Trop. Med. Hyg.* 84: 795-799.

Pentreath, V.W. (1989). Neurobiology of sleeping sickness. *Parasitol. Today* 5: 215-218.

Pentreath, V.W. (1995). Trypanosomiasis and the nervous system: Pathology and immunology. *Trans. Roy. Soc. Trop. Med. and Hyg.* (*In press*).

Petraglia, F., Bernsconi, s., Lughett, L., Loche, S., Romanin, f., Facchinetti, F., Marcellini, C. and Genazzani, A.R. (1986). Naloxone-induced luteininzing hormone secretion in normal, precocious and delayed puberty. *J. Clin. Endocrinol. Metab* 63: 1112.

Petry, K., and Van Voohis, W.C. (1991). Antigens of *Trypanosoma cruzi* that mimic mammalian tissues: Investigations of their role in the autoimmune pathophysiology of chronic chagas' disease. *Res. Immunol.* 142: 151-156.

Petry, K., and Eisen, H. (1989). Chagas' disease: a model for the study of autoimmune disease. *Parasitol. Today* 5: 111-116.

Philip, K.A. Dascombe, M.J., Fraser, P. A. and Pentreath, V. (1995). Blood-brain barrier pathology in experimental trypanosomiais. *Ann. Trop. Med. Parasitol.*(1995) (in press)

Plant, T.M. Gay, V.L., Marshall, G.R. and Arslan, M. (1989). Puberty in monkeys is trigeered by chemical stimulation of the hypothalamus. *Proc. Natl. Acad. Sci. USA* 86: 2506.

Poltera, A.A. Hochmann, a., Rudin, W. and Lambert, P.H. (1980). *Trypanosoma brucei brucei:* a model for cerebral trypanosomiasis in mice-an immunological, histological and electrimicroscopic study. *Clin. Exp. Immunol.* 40: 496-507.

Rusak, B., Robertson, H.A., Wisden, w. and Hunt, S.P., (1990). Light pulses that shift rhythms induce gene expression in the suprachiasmatic nucleus. *Science* 248: 1237-1240.

Ryna, N.D. Dah, R.E., Birmaher, B. Williamson, D.F. Lyengar, S. Nelson, b., Puig-Antich, J. and Perel, J.M. (1994). Stimulatory test of growth hormone secretion in prepubertal major depressions: depressed versus normalchidren. *J. Am Acad Child Adolesc. Psy.* 33(6) 824-833.

Said, G., Jaskowicz, M., Barreira, A.A. and Eisen, H. (1985). Neuropathy associate with experimental chagas' disease. *Ann. Neurol.* 18: 676-683.

Sarkar, D.S. Chiappa, G. Fink and N. Sherwood (1976). GnRH surge in proestrous rats. *Nature* 264: 461-463.

Schultzberg, M., Olsson, T., Samuelsson, E.B. Machlen, J. and Kristensson, K. (1989). Early major histocompatibility complex (MHC) class 1 antigen inductio in hypthalamic supraoptic and paraventricular nuclei in trypanosome-infected rats. *J. Neuroimmunol.* 24: 105-112.

Schulzberg, M. , Ambatsis, M. , Samuelsson, E. B. , Lristensson, K. , and Van Meirvenne, N. (1988). Spread of *Trypanosoma brucei* to the nervous system ; early attack on circumventricular organs and sensory ganglia. *J. Neurosci. Re.* 21 : 56-61.

Schwartz, N.B. (1969). A model for the regulation of ovulation the rat. *Rec. prog. Horm. Res.* 25: 1-55.

Sekoni VO, Njoku C, Saror D, Sannusi A, Oyejola B, Kumi-Diaka J. (1990). Effects of chemotherapy on elevated ejaculation time and deteriorated semen characteristics consequent to bovine trypamisomiasis. *Br. Vet. J.* 1990;146:368-73.

Sekoni VO, Kumi-Diaka J, Saror D, Njoku C. (1988). The effect of *Trypansoma vivax* and *Trypanosoma congolense* infections on the reaction time and semen characteristicts in the Zebu bull. *Br.Vet. J.* 1988; 144:388-44.

Sekoni, V. O. (1992). : Effects of *Trypanosoma vivax* infection on semen characteristics of Yankasa rams. *Br. Vet. J.* 148(6) : 501-506.

Siddal, B. and Crighton, B.D. (1977). Effects of certain analogs of synthetic LHRH on the release of LH and FSH. *J. Endocrinol.* 75: 49-57.

Silenghen, M. R., Flynn, S. R., and Williams, D. H. (1993). Secretion of costimulatory cytokines by monocytes and macrophages during infection with *Trypanosoma congolense* in susceptible and tolerant cattle. *Vet. Immunol.Immunopathol.* 37:123-34.

Silverman, A.J., Hamandas,J. and Renaud, L.P. (1987). Localization of LH releasing hormone neurones that project to the median eminence. *J. Neurosci* 7: 2312.

Soper, B.D. and Weick, R.F. (1980). Hypothalamic and extrohypothalamic mediation of the pulsatile discharge of LH in the ovariectomized rats. Endocrinology 106: 348.

Soudan, B. , Boersma, A. , Degand, P. and Tetaert, D. (1993). Hypogonadism induced by African trypanosomes in humans and animals.*Comp. Biochem. Physiol. Comp. Physiol.* 104(4) 757-763.

Soudan, B. , Tetaert, D. , Racadot, A. , Degand, P. and Boersman, A. (1992). Decrease of testosterone level during an experimental African trypanosomiasis: Involvement of a testicular LH receptor desentitization. *Acta Endocrinol-Coepenh,* 127(1) :86-92.

Soulsby, E. J. L. (1966). : Biology of parasites (pathogenesis of blood protozoa). *Academic Press. New York.* 237-254.

Standish, L.J. , Adamsn, L.A., Vician, L., Clifton, D.K. and Steiner, R.A. (1987). Neuroanatomical localisation of cells containing gonadotropin releasing hormone messenger ribonucleic acid in the primate brain by in-situ hybridization histochemistry. *Mol. Endocrinol* 1: 371.

Steger, R.W., Huang, H.H. and Meits, J. (1979). Relation of aging to hypothalamic LHRH content and serum gonadal steroid in female rats. *Pro. Soc. Exp. Biol. Med.* 161: 251.

Sternberg, J. and McGuigan, F. (1991). Nitric oxide mediates supression if T cell respoonses in murine *Trypanosoma brucei* infection. *Eur. J. Immunol.* 22: 2741-2744.

Sternberg, J. , and McGuigan, F. (1992). Nitric oxide mediates suppression if T cell responses in murine *Trypanosoma brucei* infection. *Eur. J. Immunol.* 22 :2741-2744.

Stevens, R..R and Moulton, J.E. (1977). Experimental meningoencephalitis in *Trypanosoma brucei* infection in deer mice (Peromyscus maniculatus). A light in immunofluorescent, and electron microsocopic study. *Acta Neuropathologica* (Berlin) 38: 173-180.

Stibbs, H.H. (1984). Effects of African trypanosomiasis on brain levels of dopamine, serotonin, hydorxyindoacetic acid and homovaliic acid in the rabbit. *Neuorchem.* 48: 1252-1256.

Stibbs, H.H. and Curtis, D.S. (1987). Neurochemical changes in experimental African trypanosomiasis in voles and mice. *Ann. Trop. Med.Parasitol.*. 81: 673-679.

Swift, A.D. and Crighton, D.B. (1979). Relative activity, plasma elimination and tissue degradation of synthetic GnRH and certain if its analogues. *J. Endocrinol.* 80: 141-152.

Synetos, D., Manolopopulos, V.G., Atlas, D. and Pipili-Synetos, E. (1991). Human plasma material with clonidine displacing substance (CDS)-like properties contracts the isolated rat aorta. *J. Auton. Pharmacol.* 11(6): 343-351.

Thomas, G.B., Scott, C.J., Cummins, J.T. and Clarke, I.J. (1994). Adrenergic regulation of growth hormone secretion in the ewe. *J. Domest. Anim. Endocrinol.* 11(2): 187-95.

Van Damme, M.P. Robertson, D.M. and Diczyfalusy, E. (1974). An improved *in vitro* bioassay mehtod for measuring Luteininzing Hormone (LH) activities using mouse Leydig cell preparations. *Acta Endocrinologica* 77: 655-671.

Vickerman, K. (1985). Development cycles and biology of pathogenic trypanosomes. *Br. Med. Bull.* 41(2): 105-114.

Waindi, E. N. , Gombe, S. , and Oduor-Okelo, D. (1986). Plasma testosterone in *Trypanosoma congolense* infected Toggenburg goats. *Arch.Androl.* 17: 7-17.

Walker, P.J. (1970). The African Trypanosomiases. Eds. H.W. Mulligan and W.H. Potts. London George Allen and Unwin p91.

Watts, A.G., Swanson, L.W. and Sanchez-Watts, G. (1987). Efferent projections of the suprachiasmatic nucleus: I. Studies anterograde transport of *Phaseolus vulgaris* leucosagglutinin in the rat. *J. Comp. Neurol.* 258: 204-229.

Weiner, R. and W. Ganong (1978). Role of brain monamines and histamine in regulation of anterior pituitary secretion. *Physiol. Rev.* 58: 905-976.

Whitelaw, D.D., Gardiner, P.R. and Murray M. (1988). Extravascular foci of *Trypanosoma vivax* in goats, the central nervous system and acqueous humour of the eye as potential sources of relapse infections after chemotherapy. *Parasitology*. 80: 51-61.

Wiemann, J.N. Clifton, D.K. and Steiner, R.A. (1989). Pubertal changes in gonadotropin releasing hormone and proopimelancocortin gene expression in the brain of the male rat. *Endocrinology* 124: 1760.

Wise, P.M. and RaGtner, A. (1980). LHRH-induced LH and FSH response in aged female rat *J. Gerontol* 35: 506.

Young , J. and Jaffe, R. (1976). Strength-duration characteristic of estrogen effects on gonadotropin response to GnRH in women. 11 Effects of varying concentrations of estradiol. *J. Clin. Endocrinol. Metab*. 42: 432-442.

Zoli, M., Zini, I., Grimaldi, R., Biagini, G. and Agnati, L.F. (1993). Effects of polyamine synthesis blockade on neuronal loss and astrological reaction after transient forebrain ischemia. *Intl. J. Dev. Neurosci.* 11: 175-187.