EFFECTS OF *SCHISTOSOMA MANSONI* INFECTION ON SOME REPRODUCTVE PARAMETERS IN RABBITS.

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ABSTRACT

Schistosomiasis is a major public health problem in tropical and subtropical countries. An estimated 200 million people are infected and 600 million are at risk of infection. In this study, the effect of *S. mansoni* on bioactive luteinizing hormone (LH), plasma levels of testosterone, histology of testes, epididymides and livers in the rabbit model were investigated. Male Newzealand rabbits were divided into four groups; Group I and III had 5 animals each, while group II and IV had 6 animals each. Animals in Group I, II, and III were exposed to 1000, 100 and 1000 *S. mansoni* cercariae respectively, while the fourth group remained as uninfected controls. Five weeks after infection, animals in Group III were treated orally with praziquantel (PZQ) at dose rate of 1000 mg/Kg body weight. Blood samples from the lateral ear vein were collected at 20 minute intervals for 4 hours on day 14 before infection (day -14) and also on days 14, 42 and 70 after infection. *S. mansoni* infection significantly decreased the pulse frequence ($P<0.05$), amplitude ($P< 0.05$), area under LH curve ($P<0.05$) and mean plasma bioactive LH concentration ($P<0.05$) on day 42 and 70 in Group I and Group II animals. Pulsatility pattern of LH was observed in control rabbits (Group IV) on the entire period.

Area under the response curve for plasma testosterone levels decreased significantly ($P<0.05$) by day 42 post infection in Group I and Group II animals compared to day -14 values. In rabbits which were infected with 1000 or 100 cercariae,

young round spermatids with pathological condensed nuclei were observed. There was also a reduction in the epithelial thickness and depletion of mature spermatozoa. Cauda epididymides of infected animals showed increased population of inflammtory cells. In the liver, the major lesions included periportal fibrosis, lymphocytic infiltration, blood vessels and bile duct proliferation. Early treatment with praziquantel inhibited endocrine changes but hepatic fibrosis was still observed. However, rectal temperature remained within normal range (38.2-39.5°C) in the course of the experiment and haematological parameters (i.e. PCV, Hb, and RBC) showed no significance difference ($P>0.05$) between the infected and control animals. It is concluded that, the observed reproductive endocrine changes was due to a pituitary or hypothalamic hypofunction, and a lack of LH may play a role in the pathological transformation of the testicular tissue.

DECLARATION.

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

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DEDICATION

This work is dedicated to my father the late Ta Sospater Kasilima, who did not live long enough to witness my final year of BVM, and to my mother Ma Evangelina Mugizi. This work is also dedicated to my wife Julliet, our sons; Niwagira and Niwamanya, my sibling Sisters; Owonyesiga, Owokusima, Owemilembe, Murungi, Owekisha and Akanganyira.

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CHAPTER 1

1.0. INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

Schistosomiasis (or bilharziasis) is a chronic debilitating parasitic disease which affects both humans and animals. It is caused by digenetic trematodes in the genus Schistosoma which inhabit the blood vessels of their definitive host and are commonly known as schistosome or blood flukes. The disease is a major public health problem in much of Africa, Latin America, Asia and the Caribbean. There are now 79 countries in which Schistosomiasis is endemic with more than 600 million people at risk of infection and some 250 million infected (Cummings and Nyame, 1996).The major schistosoma species causing diseases in humans are *Schistosoma mansoni, Schistosoma haematobium* and *Schistosoma japonicum. Schistosoma mansoni* occurs in Africa, parts of America and the Caribbean region, *S. haematobium* is confined to Africa and parts of middle East and *S. japonicum* occurs in South-East Asia (WHO, 1987).

All species have developmental stages in fresh-water snail intermediate hosts, from which free swimming cercariae are shed to penetrate the skin of the definitive human or animal hosts when in contact with the water. After successful penetration of a host's skin, the cercariae transform to Schistosomula, which migrate through veins and

lymph vessels to the lungs. From there, they migrate to the liver, developing into young male and female worms in the portal blood vessels. After 4-6 weeks, mating takes place and the worm pairs move to their final destination, which in *S. haematobium* infection is the vessels of the bladder and in *S. mansoni/ S. japonicum*, the vessels of the intestines. Some eggs, produced by the female worm, work their way out through the vessel walls and are shed in urine or feaces thereby completing the cycle if they reach water where they hatch into miracidium ready to infect the snail intermediate host. Many other eggs are retained in the tissues where they provoke an inflammatory reaction. The granulomatous inflammatory response induced by Schistosoma eggs entrapped in the microvasculature of host tissues is considered responsible for much of the manifestations of schistosomiasis (Doenhoff *et al.*, 1986).

Several studies have shown that schistosomes impair reproductive capacity of their snail host (Dejong-Brink and Bergamin 1989; Sluiters, 1981; McCLelland and Bourns, 1969) When the snail (*Lymnae stagnalis)* is infected soon after hatching, development of both the male and female part of the reproductive tract is nearly completely inhibited but when infected at maturity stage (i.e at a size of 10-11mm), the development of the male and female parts of the reproductive tract is retarded (Dejong-Brink, 1995). The mechanism by which schistosomes induce failure in reproductive function in their invertebrate host has been studied. McClelland and Bourns (1969) suggested that the parasites produce a substance which exerts a hormonal effect resulting

in lack of maturation of the reproductive system. Hurd (1990) suggested that host-parasite competition for energy-rich and other essential nutrients may change the hosts' food intake, digestion, assimilation, metabolic conversion efficiency and other nutritionally related energy consuming physiological processes such as growth and reproduction. The parasite also may interfere with the neuroendocrine system of their invertebrate host leading to alteration of host endocrine function. A neuropeptide factor, called schistosomin has been isolated from schistosome infected snails. This factor seems to antagonize gonadotropic hormones in fresh water snails (Hordijk. *et al.*, 1991).

While there is some information on schistosome mediated castration of invertebrates by metazoan parasites, little information exists on the effects of the parasite on vertebrate reproduction. Results of some recent studies are contradictory. Earlier studies indicated a decrease in plasma levels of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in *S. mansoni* infected rats and hamsters (Lansoud-Soukate *et al.*, 1991) and in mice (Isseroff *et al.*, 1986), but a more recent study has shown that schistosome infection causes an increase in plasma levels of testosterone, progesterone and 17β-estradiol in infected mice (Abdalla *et al.*, 1994).

A reduced reproductive efficiency in parasitized vertebrates has also been reported, in which pregnant mice infected with *S. mansoni* were reported to produce

substantially fewer viable litters than uninfected control (Amano *et al.*, 1990). This was caused by high numbers of abortions, maternal deaths and infanticides.

The mechanism by which schistosomes induce failure in reproductive function of their mammalian host is not yet fully understood. One report by Isseroff *et al.* (1986) has suggested that schistosomes induce the activation of the host's endogenous opiate system which may lead to decreased release of the hypophyseal luteinizing hormone (LH), which in turn results in a low rate of testosterone synthesis.

Hence there is lack of adequate knowledge on schistosome-induced reproductive dysfunction in vertebrate host. Further studies on the effects of schistosomiasis on reproductive dysfunction of mammalian hosts are therefore required. This study was therefore designed to explore the effects of schistosomiasis on male reproductive system by using a Newzealand rabbit model.

1.2. LITERATURE REVIEW

1.2.1. Pathological Effects of Schistosome on the Vertebrate Hosts

Many rodents, rabbits, dogs, primates and variety of ungulates are susceptible to one or more of the human schistosomes. Disease caused by *S. mansoni* results from a granulomatous response to ova retained in the tissues and subsequent intrahepatic venous obstruction (Farah *et al.*, 1997). *S. haematobium* infection induce chronic inflammation and irritation in the urinary bladder and is associated with increased incidence of cancer at this site (Rosin *et al.*, 1994). In general the disease is induced by both *S. mansoni* and *S. haematobium* by host immunological reactions (granulomata) around eggs trapped during passage through the organ walls or after being carried by the blood flow to the liver or lungs (Boros and Warren, 1970). Subsequent fibrosis impedes blood or urine flow, thereby resulting in chronic disease that can ultimately be fatal.

On a worldwide scale hepatosplenic schistosomiasis is one of the most prevalent causes of portal hypertension in man and is characterized by a presinusoidal portal block and a well-preserved liver parenchyma (Da Silva and Carrilho, 1992). The eggs carried continuously through the portal circulation produce inflammation and gross amputation of the intrahepatic veins, portal and periportal granulomas, and eventually, a coarse

perilobular fibrosis ("pipe-stem") (Da Siliva and Carrilho, 1992). This may subsequently result in portal hypertension, esophageal varices and hepatosplenomegaly.

In their recent study, Farah and Mramba, (1996) reported intestinal pathology in acute baboon *S. mansoni* infection characterized by smooth muscle hypertrophy, goblet cell hyperplasia, eroded crypts of lieberkuhn and deformed, flattened villi. The intestinal pathology observed in acute *S. mansoni* infection may compromise the host's digestion and nutrient absorption abilities. Anaemia has also been reported to be a common feature in schistosomiasis, and this could be due to the combination of hemodilution, hemorrhage and dyshaemopoiesis (Preston and Dargie, 1974).

1.2.2. Impact of Schistosomiasis on Mammalian Reproductive System

1.2.2.1. Effects on Female Reproductive System

Female genital schistosomiasis is associated with a wide range of pathological manifestations in the lower genital tract such as tumours and ulcers (Richter *et al.*, 1995). There is also some evidence that it is associated with complications such as infertility, abortion, pre-term delivery and extra-uterine pregnancy (Okunufua *et al.*, 1990; Ville *et al.*, 1991;). Infertility or sterility are believed to be a possible sequelae of schistosome

egg deposition in physiologically vital areas of the female genital tract (Harouny and Pedersen, 1988). Interestingly, deposition of parasite ova in the tissue of female genital tract does not always provoke an inflammatory reaction (Gelfand *et al.*, 1971). The eggs can be deposited in any part of the female genital tract. For example, schistosoma ova have been found in 2.5% of various genital tissue taken for histopathological examination in Gabon (Ville *et al.*, 1991).

Genital schistosomiasis manifests as a generalized pelvic disease involving the bladder, ureters and rectum, as well as the external and internal reproductive organs (Hermann and Kratz, 1993). Wright *et al.* (1982) observed *S. haematobium* infection throughout the genital tract of 176 cases of gynaecological schistosomiasis with 60% of the cases involving the cervix. This indicated that *S. haematobium* might be a significant cause of gynaecological morbidity, particularly when infection involved the genital tract. A study performed on 138 African patients with infertility and 42 patients with incomplete abortion showed that there was a significant association between primary infertility and bilharziasis due to *S. haematobium* (Bullough, 1976).

Fallopian and ovarian schistosomiasis may lead to infertility if secondary bacterial infection causes adhesion of epithelial cells. In additional granuloma formation in the pelvic-peritoneal area can cause anatomical obstruction and thus interfere with physiological flexibility of the ovaries and the fallopian tubes (Harouny and Pedersen,

1988). It has been observed that even after anti-schitosomal treatment, and correction of ovulatory defects, only 40% of previously infected infertile women later became pregnant (El-Mahgoub, 1982). The patho-physiological mechanisms involved in this phenomenon however are not precisely known. More interesting is the fact that 63% of the infertile women with cervicovaginal schistosomiasis were found to have antispermatozoal antibodies (El-Mahgoub, 1972.)

Cervical schistosomiasis is the most common form of genital schistosomiasis. It assumes medical importance because clinically it may be mistaken for cervical malignancy (Schwartz, 1984). It has been proposed that cervical schistosomiasis may lead to the development of cervical carcinoma (Njagi, 1978). The localisations of the genital schistosomiasis may be responsible for many dysfunctional sequelae such as pelvic aches, menstrual troubles, aberrant nidations, spontaneous abortions, and permanent sterilities (Gouzouv *et al.*, 1984).

Existing literature indicates that schistosomiasis may give rise to complications in pregnancy. Three types of complications are known to be associated with the disease namely, ectopic pregnancy, complication during normal pregnancy and placental involvement eventually leading to damage of the foetus (Okunufua *et al.*, 1990; Renaud *et al.*, 1972). Granulomatous endometritis and salpingitis observed by Vuong *et al.* (1996) in experimental mice infected with *S. haematobium* may result in ectopic

pregnancy and infertility. A further study by Ville *et al.* (1991) showed three cases of tubal pregnancies in which the histopathological examination showed a bilharzial disease of the tube. A study performed on pregnanct women suffering from *S. haematobium* in Ghana also showed that there was a high rate of pre-term deliveries, and significant lowering of birthweight of children from such mothers (Siegrist and Siegrist-Obimpeh, 1992). Bindseil *et al.* (1989), reported a reduced reproductive performance in infected mice which was associated with a significant decline in peripheral levels of Alpha-fetoprotein (AFP) in pregnant infected mice.

1.2.2.2. Effect on Male Genital Organs

Schistosomiasis may cause anatomic anomalies of the male genital organs which may be responsible for permanent or reversible infertility (Lansoud-Soukate *et al.*, 1991). Male genital schistosomiasis is commonly caused by *S. haematobium* and it presents in different pathological manifestation, with the testis and epididymis frequently showing tumoral features (Mikhail *et al.*, 1988; Githac, 1992; Ricosse *et al.*, 1980). Histopathological examination of tissue obtained from 50 cadavers in Zambia indicated that 58% had schistosomiasis of the prostate while less than 50% were found with schistosomiasis of the seminal vesicles (Patil and Elem, 1988). In some cases, humans with schistosomiasis have been observed to have small testicles in postadolescent stage (Zaki *et al.*, 1971). Also there have been references to possible reduction of libido in

advanced schistosomiasis, when there is significant deposition of eggs in the sympathetic ganglia and nerve related to the seminal vesicles (Gelfand *et al.*, 1970). The muscular wall of the latter may be destroyed and its contractions abolished at the height of an orgasm.

Schistosoma eggs have been found in the prostate, seminal vesicles, testis, vas deference and epididymis in humans (Gelfand *et al.*, 1970; Patil and Elem, 1988; Mikhail *et al.*, 1988) where they may or may not provoke tissue inflammatory reaction. Gelfand *et al.* (1970) reported that granulomatous masses caused by schistosome egg deposition could lead to sterility if the ejacultory ducts of the vas deferens become blocked. In another study haematospermia was observed to be a feature of mixed *S. mansoni*, *S. haematobium* and *S. intercalatum* infections (Corachan *et al.*, 1994). In that study schistosome eggs were isolated from semen of schistosomiasis patients with hemospermia. Schistosomiasis can also cause calcification in some parts of male genital organs in man including the ureters, bladder and seminal vesicles (Fataar *et al.*, 1990). Furthermore Fataar *et al.* (1990) observed vesical carcinoma and seminal vesicle granuloma in one patient. Malik and Ibrahim (1982) pointed out that scrotal swelling should be considered as an important clinical feature in diagnosis of male genital schistosomiasis especially for clinicians practicing in tropical area.

1.2.3. Gender-Related Differences in Schistosome Infection

Differences in susceptibility to parasitic infection based on host gender have been described in a variety of experimental models including humans. Many parasitic diseases have been observed to affect males and females differently (Benten *et al.*, 1992; Greenblatt and Rosenstreach 1984; Nakazawa *et al.*, 1997). It is now accepted that social, epidemiological and immunological factors may contribute to these differences. Visceral leishmaniasis has been found to predominate in males (Hoogstraal and Heyneman, 1969). In Kenya a 60% preponderance of male cases was observed by Southgate and Oriendo (1962) in which house-to-house investigations on prevalence of visceral leishmaniasis were conducted. It has also been reported that male mice are more susceptible to infection with *Trypanosoma rhodesiense* (Greenblatt and Rosenstreich, 1984) and female mice are capable of self-cure following *Plasmodium chabaudi* infection, whereas males are not (Benten *et al.*, 1992).

In a variety of experimental models, females display greater resistance to infection than males. However in experimental *S. mansoni* infections, the opposite phenomenon occurs (Eloi-Santos *et al.*, 1992; Nakazawa *et al.*, 1997). Upon equal subcutaneous exposures to 45 cercariae, female mice present a more severe clinical course with consequent higher mortality than male mice (Eloi-Santos *et al.*, 1992). Further studies

have shown that fewer adult worms develop in intact male than in female mice infected with the same number of *S. mansoni* cercariae, whereas castrated male mice exhibit infection level and survival characteristics of female mice (Nakazawa *et al*., 1997).

Increased susceptibility of young girls than boys was implied in a study of *S. mansoni* in Machakos District (Kenya). In this study girls less than 9 years of age were observed to have higher mean egg counts of *S. mansoni* than boys of similar age although the latter had less water contact than boys (Arap Siongok *et al.*, 1976). In Gambia, girls have been observed to have higher reinfection rates of *S. haematobium* following treatment with praziquantel than boys of the same age, and these differences remain after controlling for levels of exposure and eosinophil counts (Hagan *et al.*, 1985). Although studies on gender-related differences in schistosome infection are scarce, it is likely that susceptibility and mortality differences between male and female in schistosomal infections do occur. It is possible that these observed differences in susceptibility are due to hormonal interraction between parasite and the host. Since the levels of reproductive hormones differ in male and females, it is possible that sex hormones play a crucial role in gender related differences frequently observed during schistosome infection.

The importance of hormonal factors in modulating helminth infection has been demonstrated by Knopf (1982). Furthermore, differences in the relative susceptibilities of male and females to parasitic infection has been observed to be modified by gonadectomy

or the administration of exogenous sex hormones (Brabin and Brabin, 1992; Nakazawa, *et al* 1997). This showing that sex hormones may play an important role in immune modulation (Grossman, 1985).

Several authors have suggested that sex steroid hormones may play a vital role on host immunity against schistosome parasite. It is now known that sex hormones can interact specifically with, and profoundly modify many facets of the immune response. Androgen receptors have been found on peripheral blood mononuclear cells (Kuhale, 1994) and treatment of animal with androgen has been reported to increase the number of $CD8^+$ T cells in various tissues (Ahmed *et. al.*, 1985). It has been reported that male mice develop lower worm burdens and survive longer than female mice exposed to the same number of *S. mansoni* cercariae (Eloi-Santos *et. al.*, 1992). More recently Nakazawa *et. al.* (1997) demonstrated that female mice treated with testosterone 10 days prior to infection had reduced worm burdens but not if the testosterone was given 10 days or 5 weeks after infection. The sex bias observed in parallel-infected male and female mice appears to be related to the presence of male gonadal tissue or testosterone early in infection, during the development of immature schistosomules (Nakazawa *et al.*, 1997) Also it is of interest to note that Schistosomula express androgen and oestrogen receptors (Nakazawa *et al.*, 1997; Barrebes *et al.*, 1986). Therefore, the possible explanation of the the above observation is that, schistosomula interact with host immune system which may respond by increased levels of steroid hormones. This response may contribute to the

partial prevention of primary infection by interrupting schistosomula development to adult worm. However, the functional role of steroid receptors on schistosomula remain undefined (Nakazawa *et. al.*, 1997).

1.2.4. Some Biochemical Changes in *Schistosoma mansoni* Infection

Several biochemical changes have been associated with schistosome infection in both humans and animal models. Dalmo *et al.* (1977) reported the decreased levels in the esterified cholesterol concentration in the liver of the infected mice but not of the free cholesterol. They concluded that an impairment of plasma cholesterol esterifying enzyme caused the low esterified cholesterol concentration in the plasma of the infected animals. In another study, the changes in plasma cholesterol and lipids was observed in human patients and mice infected with. *S. mansoni* (Michael *et al.*, 1978). Changes in enzyme activities in blood serum have been frequently reported in bilhaziasis. Elevation of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) in infected mice was reported by Elvio *et al.* (1966). Schistosomiasis has also been shown to alter the blood concentration of serum proteins. A decrease in albumin and increase in globulins, particularly the α and γ-fraction have been reported (Bruce *et al.*, 1963; Elvio *et al.*, 1966). The latter is said to be associated with an increased production of immunoglobulins (Bassily *et al.*, 1972). Schistosomiasis is also associated with changes in

serum glycoprotein and Wagdy *et al.* (1976) observed that α-1 and γ-glycoproteins were significantly higher while β-glycoprotein was lower in bilharzial patients.

The effects of *S. mansoni* infection on hepatic drug metabolizing enzymes function has been also studied. The hepatic drug metabolizing capacity is severely reduced in mice infected with *S. mansoni* (Cha and Edware, 1976; Cha, 1978). Furthermore it has been observed that hepatic drug metabolism is not impaired in unisexual infection (Cha *et al.*, 1980a) and that the severe reductions of the hepatic drug-metabolizing functions occur only in mice that are immunologically competent, therefore are dependent on the host's response to the parasite eggs (Cha *et al.*, 1980b).

1.2.5. Influence of Schistosome Infection on the Host's Endocrine System

1.2.5.1. Effects on the Invertebrate Host (Fresh Water Snails)

Schistosomes reduce or completely stop reproduction in their snail host, a phenomenon referred to as "parasitic castration". They also inhibit or unexpectedly enhance body growth "giant growth" (De Jong-Brink and Bergamin, 1989 Hurd, 1990). Several species of snails have been shown to produce fewer egg when infected with schistosomes (McCelland and Bourns, 1969; Sluiters, 1981; De Jong-Brink, 1995).

Host starvation, has been suggested to be responsible either directly or indirectly for reproductive dysfunction in infected hosts. This is possibly as a result of parasite induced blockage of nutrient supply to the reproductive tissue (Hurd, 1990). However, parasite-mediated effects on the reproductive system of snails are still observable earlier during infection, when nutritional needs of the parasite are limited (Sluiters, 1981), suggesting that those effects are due to interference with the snail neuroendocrine system which regulates reproduction and growth.

The reduction or elimination of host reproductive potential is manifested in a variety of ways including suppression of development and maturation of gonads, suppression of sex cell formation and physical destruction of the reproductive tissues (Thompson, 1993). McClelland and Bourns (1969) observed that *L. stagnalis* infected with the avian schistosome trichobilharzia, grew rapidly, but showed a lower reproductive capacity than uninfected snails. These changes may be related to alterations in reproductive hormone levels. Hordijk *et al.* (1991) reported reduced level of female gonadotrophic hormones in schistosome infected snails. A peptide factor called schistosomin which inhibits the production of female gonadotrophic hormones has been isolated in infected snails (De jong-Brink *et al.*, 1988a; De jong-Brink *et al* 1988b; Hordijk *et al.*, 1991 Joose *et al.*, 1988;).

1.2.5.2. Effects on Vertebrate Host (Mammals).

Alteration in mammalian host endocrine function following various parasitic infection has been reported by several authors (Mutayoba and Gombe 1989; Swanson *et al.*, 1984; Sharp *et al.*, 1982). One possible strategy for a successful adaptation of a parasite to its host, is the induction of conditions favourable to its own development and metabolism by manipulating the endocrine system of the host. Such favourable conditions could involve maintaining an adequate body size and food supply, inducing immunosuppression and analgesia in the host and controlling the speed of development of the host (Fiore *et al.*, 1996; Kavaliers *et al.*, 1984; Spindler, 1988). These modification of the reproductive endocrine system of the host by a parasite may lead to a reduced reproductive efficiency.

Although a considerable amount of research has been conducted on schistosomiasis, less attention has been paid to the pathophysiology of the infection. Few studies have been conducted on parasite induced changes on mammalian reproductive endocrine system. Furthermore, available literatures on that subject are contradictory, some authors found an impairment in cortisol secretion (Abdel *et al.*, 1969; Aboul-Dahab *et al.*, 1973) while others did not observe any overt signs of adrenal failure and the cortisol secretion remained normal in bilharzial patients (Zaki *et al.*, 1971; Zaki *et al.*, 1972). Significant decreases in the levels of the pituitary gonadotropin (i.e. FSH, LH) and

testosterone has been observed in rats and hamsters experimentally infected with *S. mansoni* (Lansoud-Soukate *et al*, 1991). The mRNAs for collagen have been shown to increase in schistosome infected animals whereas those for albumin and the major urinary proteins are decreased. The latter proteins are under the control of testosterone, the concentration of which is drastically lowered in schistosomiasis infected mice (Isserrof *et al.*, 1986). The seminal vesicle weight is also decreased significantly in such mice. Tibold *et al.* (1979) reported that the mean serum levels of progesterone is significantly lowered in mice with acute *Schistosoma mansoni* infection. However, more recent studies have reported increased levels of testosterone, progesterone and 17β -estradiol in schistosome infected mice (Abdalah *et al.*, 1994). Further studies are therefore needed to clarify the effects of schistosomes on mammalian sex hormones.

1.3. Justification of the Study

Schistosomiasis is now prevalent in 79 countries causing about 500,000 deaths annually (Capron *et al.*, 1995). The development of irrigation schemes and the construction of dams to conserve water, together with the inevitable concentration of human population in these areas have enhanced the transmission of the disease. In East Africa the current spread of water hyancith in Lake Victoria provides a breeding habitat for intermediate host (fresh water snails) thereby increasing the risk of contracting the infection by people living along that lake.

Although schistosomiasis is recognized as one of the most widespread parasitic disease of man and an important public health problem (WHO 1987), less attention has been paid in studying the pathophysiology of the infection. Human studies have shown that schistosomiasis may cause anatomical anomalies of the genital organs and other studies suggested that this parasitic infection may have adverse effects on the endocrine system (Zaki *et al.*, 1971; Prasad, 1984). Furthermore many complications associated with male and female genital schistosomiasis remain largely undocumented. Only limited data are available in literature. Those data mostly describe clinical case studies which are limited by ethical constraints because controlled and invasive studies in humans are not possible. This study therefore was aimed at investigating the pathophysiology of *S.*

mansoni in a rabbit model by assessing the reproductive endocrine system and histology of gonads during schistome infection.

1 .4. Research Objectives

The objectives of this study were as follows:

i) To determine the effects of *S. mansoni* infection on the levels of the bioactive LH and plasma testosterone in male rabbit.

ii) To examine the effect of *S. mansoni* infection on the histology of the testes, epididymides and livers in male rabbits.

It was hypothesized during this study that:-

(1) *S. mansoni* infection induces changes in pituitary and testicular endocrine functions which might impair reproductive function of male rabbit.

(2) *S. mansoni* infection induces pathologies in the testes, epididymis and liver of male rabbit which may alter their function.

CHAPTER 2

2.0. MATERIALS AND METHODS

2.1. Study Area, Parasites and Experimental Animal

This study was conducted at the Chiromo Campus of the University of Nairobi, within the Reproductive Biology Unit of the Department of Animal Physiology. Twenty two sexually mature Newzealand male rabbits aged between 9 and 12 months were used in the study. Four weeks prior to the commencement of the study, the animals were transferred to the experimental animal house where they were caged singly. Animals were monitored for changes in body weight and demeanor. Rectal temperature was measured at four day intervals before and after infection. Standard rabbit pellets, (From TAMFEED LTD., Nairobi), vegetables and fresh tap water were supplied *ad-libitum*. The animals were subjected to natural lighting condition 12:12 hours light/darkness and at average room temperature of 23^0C.

The cercariae which were used in this experiment were shed from the infected snails (*Biomphalaria pfeifferi)* which were infected with miracidia of *S. mansoni* obtained from baboons chronically infected with a strain of *S. mansoni* at the Institute of Primate Research (IPR), Nairobi.

2.2. Experimental Design and Infection:

The animals were divided into four groups (I-IV). The number of animals in each group were five, six, five and six for group I, II, III and IV respectively. Group I and II were exposed to an average of 1000 and 100 cercariae of *S. mansoni* respectively. Group III was exposed to an average of 1000 cercariae followed by treatment with praziquantel five weeks after infection, This was administered orally at dose rate of 1000 mg/Kg body weight. Group IV was not exposed to cercariae and served as uninfected control. The animals were exposed to cercariae percutaneously according to the procedures described by Miriam *et al* , (1991). Ten weeks after infection all experimental animals were sacrificed.

2.3. Blood Sampling:

Blood samples were collected into heparinized LP3 tubes through lateral ear vein puncture. For haematological studies (Packed Cell Volume, Haemoglobin concentration and Red Blood Cell count) 0.5 ml samples were collected two weeks before infection and at two week intervals for 10 weeks. Blood for hormonal studies was collected at 20 min. interval of 4 hr 14 days prior infection, and at days 14, 42 and 70 post-infection. The collected blood was centrifuged at 1500g for 10 min and the plasma was frozen at -20^{0}C until assayed for LH and testosterone.

2.4. Perfusion Technique and Schistosomes Recovery

After 10 weeks post-infection, all animals were killed by intravenous administration of heparinized sodium pentobarbitone (Euthatal $^{(R)}$) from May and Baker Ltd, UK, administered at a dosage of 100 mg/Kg body weight. The perfusion technique was done according to method of Muchemi, (1992). After the animal were killed, a mid-line incision was made from the neck to the groin, and the visceral organs were exposed. The intercostal muscles were severed to allow clamping of the aorta and the posterior vena cava. The abdominal aorta was clamped immediately before the bifurcation into iliac arteries. The posterior vena cava was clamped to exclude the heart. A small incision was made into the abdominal aorta just after it leaves the heart and a small cannula was inserted in the incision and connected to the perfusion pumping equipment (Masterflex$^{(R)}$, Cole Palmer Instrument Company, USA). Another incision was made into the hepatic portal vein, then the perfusion fluid (0.85% sodium chloride and 1.5% sodium citrate) was introduced into the abdominal aorta via the perfusion pump. The mesenteric veins, the liver and other abdominal organs were perfused excluding the heart and lower limbs. The perfusate was collected at the incised hepatic portal vein by creating a depression between the liver and abdominal muscles and by using a plastic tube connected to a standard vacuum pump (Milliporer, Bedford, Massachussets, USA). The perfusate was aspirated into a 10 litre bell jar and was thereafter poured through a 105-mesh sieve

(Arthur Thomas Company, Philadelphia, USA). Collected worms were transferred to petri dishes containing normal saline and counted under the low power dissecting microscope (x 40 magnification). After perfusion, the livers, testes and epididymides from all 22 animals were collected, livers were fixed in 10% neutral formalin while testes were fixed in Bouin's solution.

2.5. Sample Processing

2.5.1. Haematological Parameters

The Packed Cell Volume (PCV) was measured using the standard haematocrit method. Haemoglobin concentration and Red Blood Cell count (RBC) was done using Acid Hematin and Haemocytometer methods, respectively as described by Benjamin, (1985)

2.5.2. Hormonal Analysis

2.5.2.1. Radioimmunoassay for Testosterone:

Plasma testosterone levels were determined using a competitive Radioimmunoassay method which utilizes dextran-charcoal for separation of free from antibody bound hormone (WHO, 1996). Reagents used for this assay were provided by

WHO Matched Reagent Programme. The plasma samples were thawed and whirl mixed, 200µl from each samples were transferred to clearly labelled extraction glass tubes and 200µl of Phosphate Buffer Saline (PBSG) (2.35g NaH_2PO_4, 11.6 g Na_2HPO_4, 8.8g NaCl, 1.0g gelatin and 0.1g NaN_3 in 1 litre of double distilled water, pH 7.4) was added to each tube. Then 2 ml of diethyl ether was added to each tube. The tubes were vortexed and thereafter shaken vigorously for 3 min. using Hover motor shaker.

The aqueous phase was frozen using dry ice and the supernate decanted into 7.5 ml grass tubes. The solvent was evaporated in vacuo and 2 ml of assay buffer added to each tube. The tubes were vortex mixed and left to stand for 20 min., then vortexed again. From each of the redissolved sample 500µl aliquots were taken in triplicate and each put into labelled LP3 assay tubes. In each tube 100µl of tritiated testosterone and 100µl of testosterone antiserum were added, vortexed and incubated overnight at 4^0C. Total counts tube (Tc approximate 10000 cpm), standard tubes(S), non-specific binding (NSB), zero antigen tubes (Bo) high and low quality controls (Qc) samples were included to each assay. After overnight incubation period, the samples were transferred to ice baths, and 200µl of ice cold dextran coated charcoal (0.625g dextran, 0.0625g dextran in 100ml assay buffer) was added to each tube except the Tc tubes. The assay tubes were vortexed then incubated for 15 min. at 4^0C. Centrifugation was done at 1500 g for 10 min. at 4^0C using Beckman Model J1-6 centrifuge connected to TJ-R Refrigeration unit. The supernate from each tube was decanted into labelled scintillation vials then 4 ml of

toluene scintillation fluid was added and counted in a packard prias counter. The intra-assay Coefficient of Variation (CV) for the assay was 5.9% (n=32) at 5.62 nmol/l and 6.9% (n=32) at 1.71 nmol/l. The Inter assay CV was 5.5% at 5.62nmol/l (n=32) and 4% at 1.71 nmol/l. The detection limit of the assay, defined at 90.9% confidence limit was 0.3 nmol/l.

2.5.2.2. Luteinizing Hormone Assay (BioAssay)

Plasma LH was determined by bioassay as described below, using materials provided by WHO Matched Reagent Programme (WHO, 1996).

Interstitial Cell Preparation:

Testes removed from adult mice of 5-6 weeks were decapsulated and fat was trimmed off then rinsed in ice-cold 10 ml Basal Medium Eagle (BME) on plastic petri dish. The testes were minced with scissors, cells were dispersed mechanically using a pipette by sucking up and down several times. Minced tissues were transferred to a 50 ml polythene conical flask containing 100 ml medium containing 2% Bovine Serum Albumin (BSA), then mixed gently on magnetic stirrer for 15 min. at 4^0C. The Leyding cells separated from other testicular cells by filtering through fine nylon gauze and transferred into 100 ml polythene conical flask. The collected cells were then incubated for 15 min. in 5% CO_2 water bath at 4^0C. The collected cells in the medium were divided between six LP3 tubes and spun at 1500g at 4^0C for 10 min. and the supernatant was discarded. The

cell pellets were washed once in 20 ml BME medium, centrifuged and thereafter resuspended in 10 ml medium. The cells in all tubes were pooled and counted using haemocytometer. In the assay, 150,000cell/ 200µl, that is 7.5 x 10^5 cell/ml were required. Viability of the cells was determined by the dye exclusion method using Trypan Blue.

Bioassay of LH

Incubations were perfomed in LP3 tubes at 37^0C under 5% CO_2. Each tube contained 200µl of the Leyding cell suspsension and 100µl of standard hormone solution or plasma samples. The standard tube was incubated in triplicate, plasma samples were assayed in dublicate and after incubation for 3 hr with gentle shaking at 150 cycles/min. After incubation, the cell suspensions were centrifuged 1500g for 15 min. The supernatant solutions were immediately diluted 1:25 with PBSG for testoterone assay (RIA) as described previously. Bioactive LH levels was estimated as testosterone in the sample vials and was compared with that of standard preparations. The Intra-assay CV for the assay was 6.5% (n=16) at 3.29 IU/L. The Inter assay CV was 8.4% at 3.29 IU/L (n=16). The assay limit of detection at 90% confidence limit was 0.41 IU/L.

2.5.3. Histology Samples Processing

The fixed tissue samples were dehydrated by passage through progressively higher concentration of ethyl alcohol (50%, 70%, 80%, 90% and absolute alcohol). Then they were processed through benzene, benzene-paraffin wax mixture in an oven, and thereafter embedded in paraffin wax. Seven micron-thick sections were cut using a rotary microtome and stained with hematoxylin and eosin (H&E). Some liver sections were stained with Masson's Trichrome stain which is used for demonstrating liver fibrosis. Slides prepared for each tissue were observed under a light microscope and compared to those of uninfected controls to detect histological changes associated with the infection.

2.6. Statistical Analysis

All the values are expressed as the mean ± SEM standard error. Hormonal data were tested by One way Analysis of Variance at 95% level of significance using SPSS/PC+, and Scheffe' Test was used to pin point the actual difference. The effect of the infection on clinical parameters were analysed by using Unpaired T-test, and Mann-Whitney Test was performed when the T-test indicated a significant difference. A $P<0.05$ was considered statistically significant. In samples collected over 4 h, the pulsatile variations in hormone concentration were analysed by using criteria of Mutayoba *et al.* (1994) that a pulse was present when (i) a value exceeded the previous value by at least

three SDS of the estimate of the previous value calculated from duplicate sample results, and (ii) when the peak value was followed by either a decline or no significant increment. The pulse amplitude was calculated by subtracting the concentration at the onset of the pulse from the peak concentration. The pulse frequence was calculated by counting the number of peaks, and mean hormone concentration by averaging the concentrations over the 4 hr sampling period. In addition, the area under the curve was calculated using the trapezoid rule (Mutayoba *et al.*, 1994).

CHAPTER 3

3.O RESULTS

3.1. Clinical Observation

All animals in all groups appeared to be in good health, rectal temperatures remained within normal range (38.2-39.5°C) in the course of the experiment and haematological parameters (i.e PCV, Hb and RBC) showed no significance difference ($P>0.05$) between the infected and control animals (Fig.1, Fig.2 and Fig.3). The animals in Group I and Group II showed a significant decrease ($P<0.05$) in body weight 10 weeks after infection (Fig.4)

3.2. Gross Pathology and Worm Recovery

The gross pathology was examined when rabbits were sacrificed 10 weeks post-infection. The livers were friable, enlarged with rounded edges, indicating inflammation in Group I and II animals which remained untreated throughout the experimental period, but the lesions were severe in Group I. There were also numerous creamy foci characteristic of *S. mansoni* granulomas distributed on the surface of the lobes. In contrast to the other two groups, Praziquantel treated (PZQ-treated) group (Group III) had tiny inconspicuous granulomas on the liver lobes and there were no signs of inflammed liver in all animals of

this group. No gross involvement of the intestine was observed in all infected animals. The percentage worm recovery was about 78.7%, 57.5% and 0.004% in animals exposed to 1000 (Group I), 100 (Group II) and 1000 cercariae+PZQ-treated (Group III), respectively (Fig.5). No worms were recovered from uninfected control animals.

3.3. Histopathology

Some regions in livers of infected animals showed periportal fibrosis characterized by marked increase of collagen matrix and fibroblasts. Other main histopathological findings were bile duct hyperplasia (Plate 2), adult worm granulomas, necrotic foci within the liver parenchyma, diffuse infiltration of monocytes around the portal tracts and sinusoidal dilatation. Portal triad areas revealed the marked increase in fibrous tissues accompanied by proliferation of bile ducts and blood vessels (Plate 2). The above mentioned lesions were found in all animals in Group I which were infected with 1000 cercariae, while four out of six expressed the same lesions in Group II animals, which were infected with 100 cercariae. The commonest pathological finding in livers of infected and praziquantel treated animals was periportal fibrosis and infiltration of round cells (Plate 3). Remnants of adult worms in the hepatic vein, were also observed in livers of GroupI and GroupII animals (Plate 3). However, the typical egg granulomas was not observed. The liver sections from uninfected control rabbits showed normal hepatic

tissues and the morphology of the portal triads was normal with only single structures for the portal vein, portal artery and bile duct (Plate 1).

Testicular morphorogy showed mild changes in the seminiferous tubule epithelia. In animals infected with either 100 or 1000 cercariae, the seminiferous tubules epithelia showed similar histological changes. There were very few mature spermatozoa or late-stage developing spermatids and most of seminiferous tubule lumina were without spermatozoa (Plate 6). Degenerating young round spermatids with shrunken nuclei appeared in the luminal areas of epithelium (Plate 6). Additionally, seminiferous epithelial thickness was reduced compared to those of uninfected controls. However, other epithelial cells appeared to be normal. The germinal epithelium of uninfected control seminiferous tubules consisted of normally arranged spermatogenic cells in different stages of development (Plate 5).

Epididymal tissues from infected animals showed normal morphology with similar features as in control animals (Plate 7), and the stereocilia characteristic of epididymides epithelium was evident. However, few mature spermatozoa were present in the epididymal lumina of infected animals, and the latter showed marked accumulation of inflammatory cells in the central mass of spermatozoa. (Plate 8).

3.4. Effect of *S. mansoni* on Bioactive Luitenizing Hormone.

Changes in plasma bioactive LH in representative control and *S. mansoni* infected rabbits in each group are presented in Figures 8 to Figure 11. Pulsatility in LH was observed in all experimental animals in samples collected at 20 min intervals for 4 h on day -14 (Fig.8). The pulse frequence in control animals ranged between 2.0±0.4 to 2.67±0.81 and did not differ significantly at all sampling periods. Similar observations were made in rabbits which were infected and treated with PZQ (Group III). Pulse frequence declined significantly on day 42 and 70 ($P<0.05$) in rabbits infected with 1000 cercariae (Group I) and Group II animals (100 cercariae) compared with Group III and Group IV values (Fig. 6c). Changes in Group I and Group II were not significant from each other (Fig 6c). Changes in pulse frequency were associated with changes in pulse amplitude in Group I and Group II animals (Fig. 6b). Similar decline in mean area under LH curve (Fig. 6a) was observed in infected groups (Group I and II) on days 42 and 70 compared to uninfected controls.

The mean plasma bioactive concentration over 4 hr on day 14 before infection were 10.62±1.51, 8.59±1.08, 7.72±1.13 and 9.63 ±1.66 IU/L for Groups I, II, III, and Group IV respectively. Comparison of mean plasma bioactive LH levels collected over 4hr in infected and uninfected control animals at different stage of infection are shown on Figure 7. In all infected untreated animals (Group I and Group II), a significant fall in bioactive LH levels was recorded on day 42 and day 70 post infection ($P<0.05$).

However no statistically significant difference was recorded in mean bioactive LH levels of infected PZQ-treated group (Group III), on day 14, 42, and 70 days post infection (7.31 ±1.55, 5.13 ±0.85, and 7.28 ±0.89 IU/L respectively), compared with day -14 values (7.72 ±1.13 IU/L). The greatest suppressive effect on plasma bioactive LH levels was observed 70 days post infection in two groups of infected untreated animals (Fig.7). These recorded minimum levels of LH were 0.89±0.1, and 1.47±0.25 IU/L for Group I and Group II respectively (Fig. 7). Plasma bioactive LH profiles in representative infected and control rabbits in samples collected every 20 min. for 4hr on day 14 before infection and on days 14, 42, and 70 post infection are shown on Figures 8, 9, 10 and 11 respectively. The pulsatile pattern of LH secretion were similar in all groups 14 days before infection (Fig.8) and on day 14 after infection (Fig.9), however those patterns were lowered in the infected groups on day 42 (Fig.10) and 70 days post infection (Fig.11). In infected PZQ-treated animals and uninfected controls, the LH pattern secretion did not change over the entire experimental period.

3.5. Effect of *S. mansoni* on Plasma Testosterone Concentration

Mean plasma testosterone concentration and the area under the response curve in infected rabbits and controls collected over 4 hours on day 14 before infection and days 14, 42 and 70 post-infection are shown in Figure 12 and Figure 13. The Mean testosterone concentration and the area under testosterone curve had decreased

significantly on day 42 and 70 ($P<0.05$) in Group I and Group II animals. In Group I animals which were exposed to 1000 cercariae then remained untreated, *S. mansoni* induced a mild decrease in plasma testosterone concentration from a mean of 3.69 ±0.89nmol/l over 4 h on day -14 to 2.95 ±0.54 and 1.54 ±0.35 nmol/l on days 42 and 70 respectively. In the group exposed to 100 cercariae (Group II) a similar trend was observed, plasma testosterone concentration decreased from a mean of 3.48 ±0.48 nmol/L on day -14 over 4h to 2.78 ±0.39 nmol/L on day 42 and 1.41 ±0.19 nmol/l on day 70 (Figure 12). In contrast, rabbits which were infected with 1000 cercariae then treated with PZQ after five weeks, and uninfected control rabbits showed no alteration in plasma testosterone over the entire experimental period (Fig. 12). The patterns of the pulsatile release of testosterone was altered in Group I and Group II 70 days post infection (Fig. 17) compared to 14 days before infection (Fig. 14). But plasma testosterone pattern for controls and infected PZQ-treated animals was not altered over the entire experimental period.

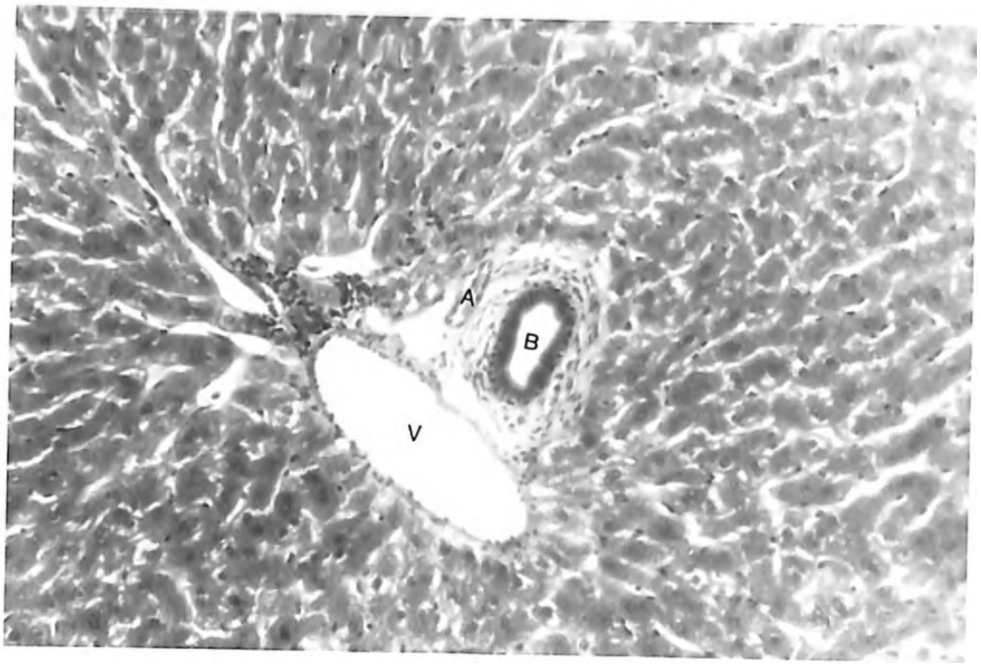

Plate 1: Liver section from a normal uninfected rabbit, showing the normal morphology of the liver around the portal triad with single structures for portal vein(V), portal artery(A), and bile duct(B). (Masson's Trichome stain. x40)

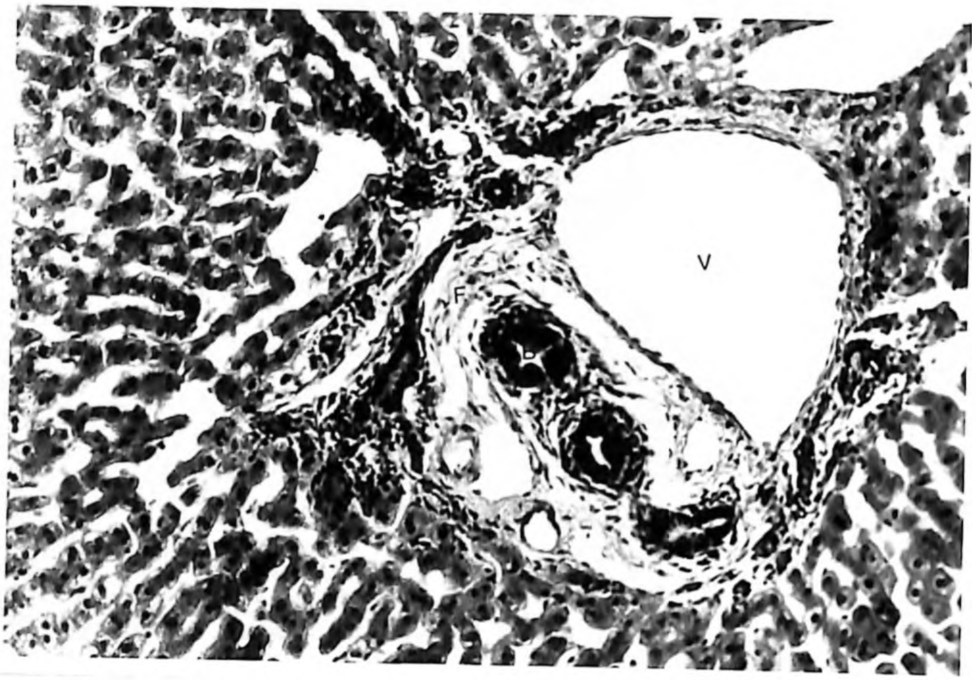

Plate 2: Liver section of a rabbit infected with 100 *S. mansoni* cercariae showing distorted morphology of portal triads as evidenced by the proliferation of bile duct(B), blood vessels(V), and increased amount of fibrous tissues(F). (Masson's Trichome stain x100)

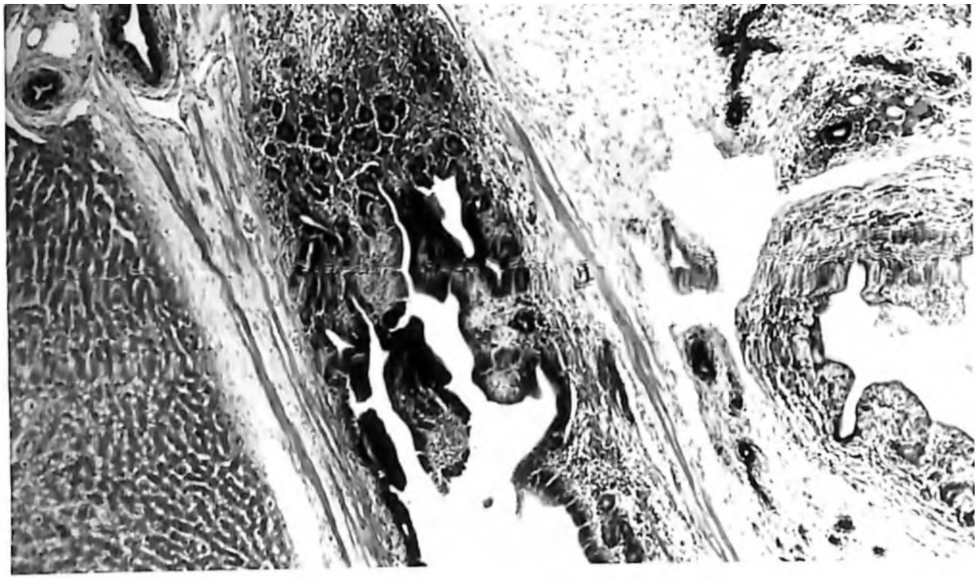

Plate 3: Liver section from infected PZQ-treated rabbit. Note the extensive fibrosis(F) around the portal triads, and the coalition of several portal triads to form pipestem fibrosis. (Masson's Trichome stain x40)

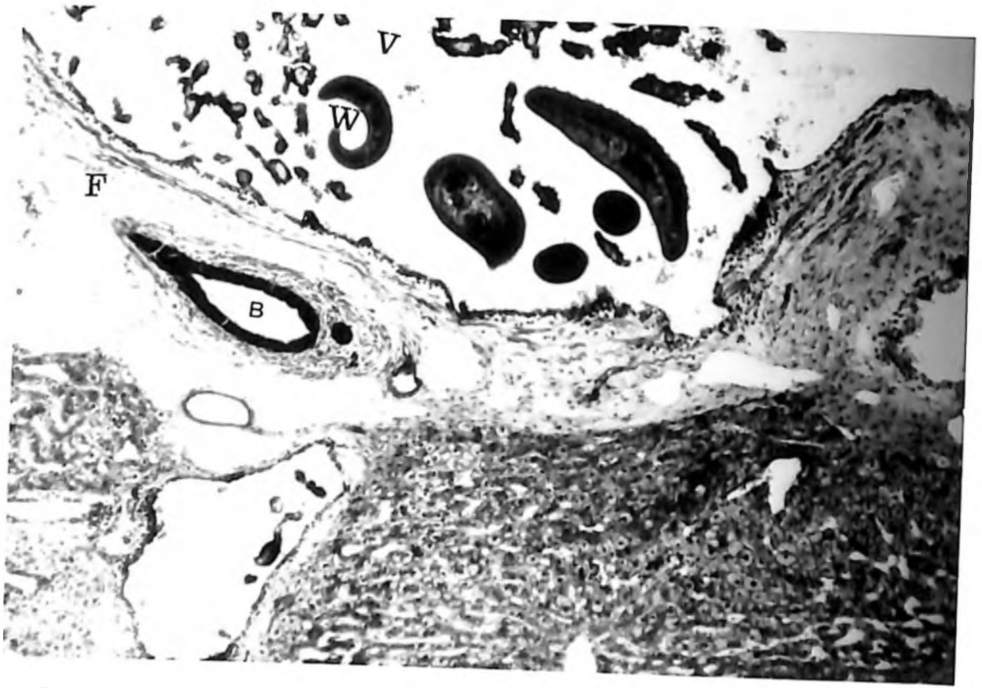

Plate 4: Liver section from rabbit infected with 1000 *S. mansoni* cercariae, showing the dilated portal vein(V) containing schistosome worms(w). Note also the extensive periportal fibrosis(F), proliferation of bile duct(B) and blood vessels. (Masson's Trichome stain x40)

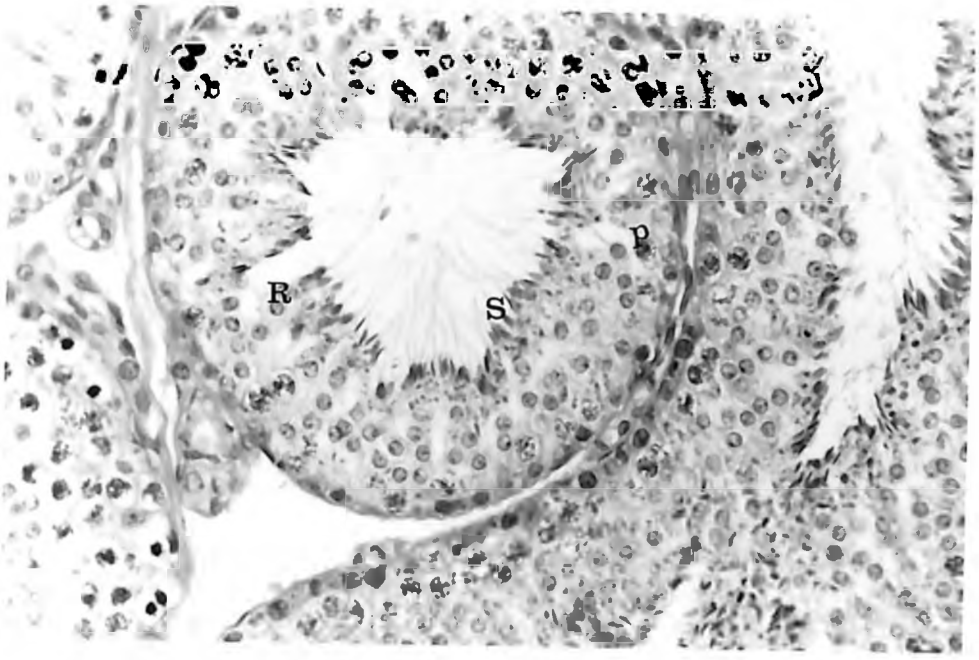

Plate 5: Seminiferous tubules of uninfected control rabbit showing normal testicular morphology, consisting of proliferating primary spermatocytcs(P), round spermatids(R) and mature spermatozoa(S). (H&E stain x 200)

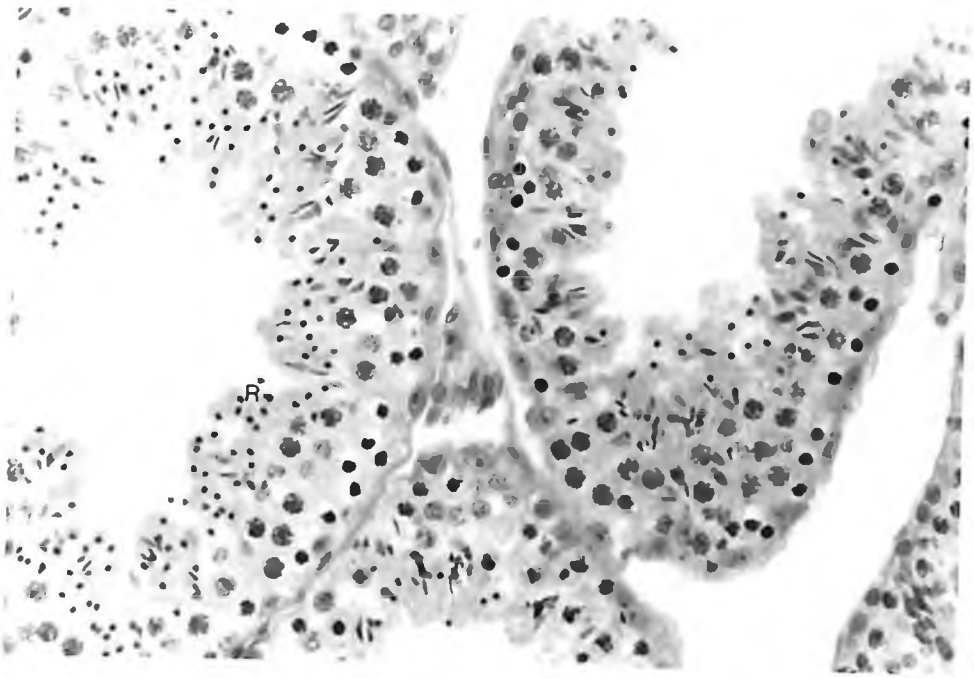

Plate 6: Seminiferous tubules of rabbit infected with *S. mansoni*. Note the reduction in the epithelial thickness and the scanty mature spermatozoa. The round spermatids (R) have clearly shrunken nuclei and located in the luminal areas of epithelium. (H&E stain x 200)

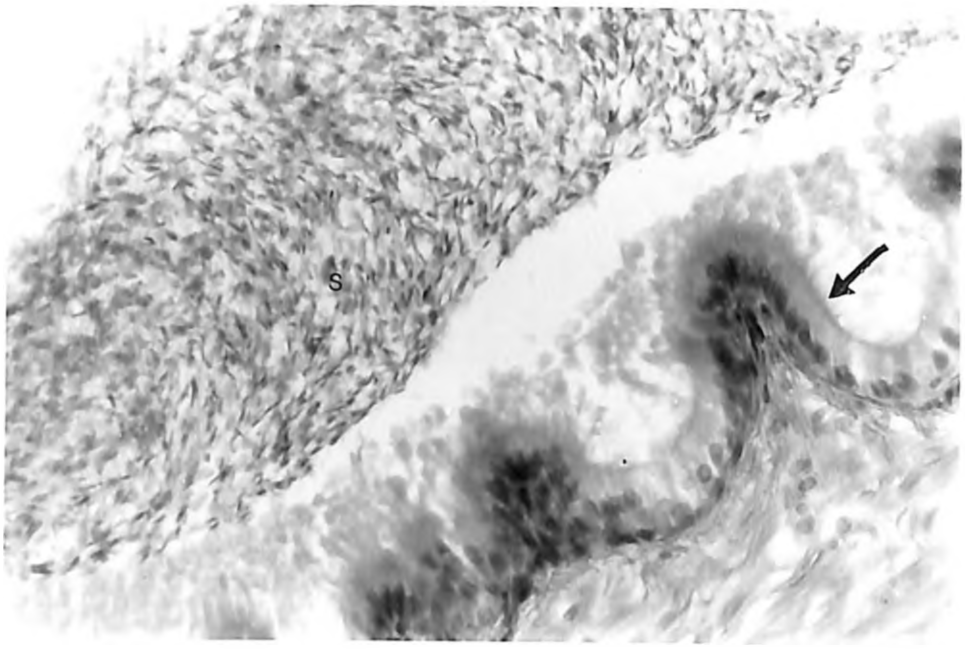

Plate 7: Cauda epididymis of uninfected control rabbit showing epithelial lining with steriocilia(arrow). Notice the absence of inflammatory cells in the central mass spermatozoa(s). (H&E stain x 200)

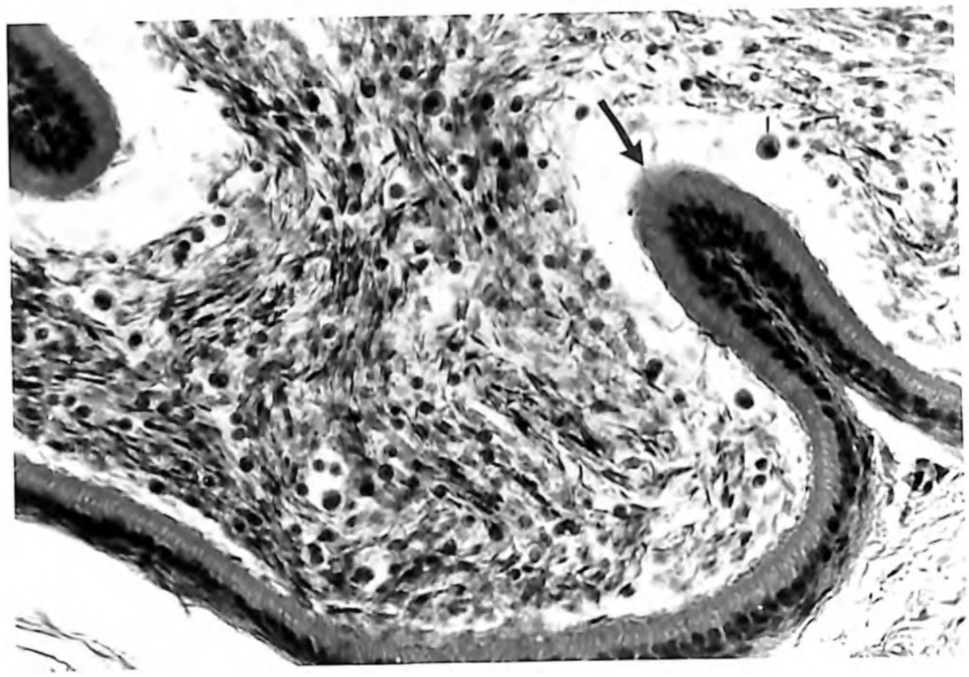

Plate 8: Cauda epididymis of rabbit infected with *S. mansoni* showing the accumulation of inflammatory cells (I) in the central mass of spermatozoa and presence of steriocilia (arrow) on the epithelial lining. (H&E stain x 200)

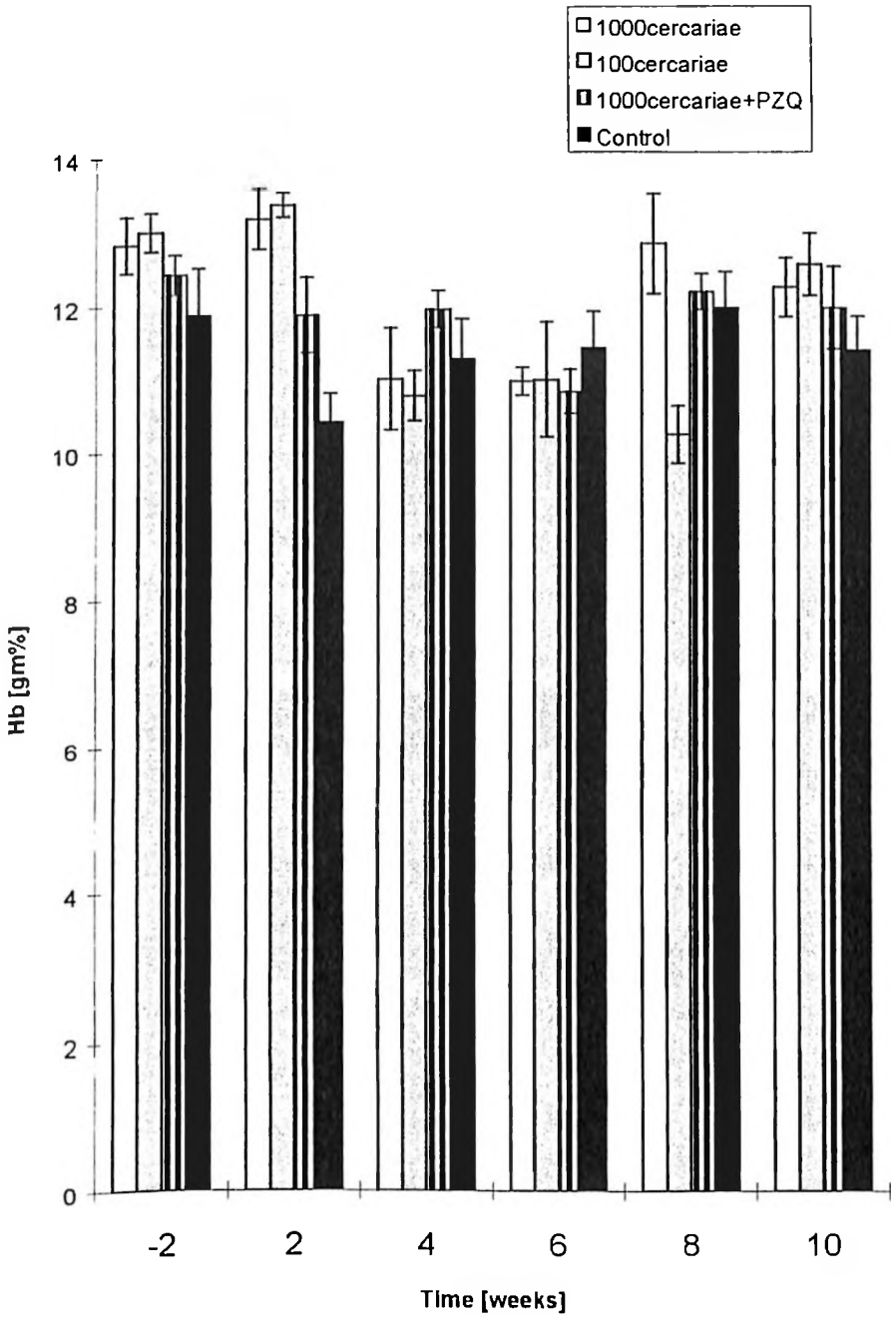

Figure 1: Mean haemogolobin concentration of infected and control animals. There was no significant differences [P>0.05] between *S. mansoni* infected and controls animals throughout the experimental period.

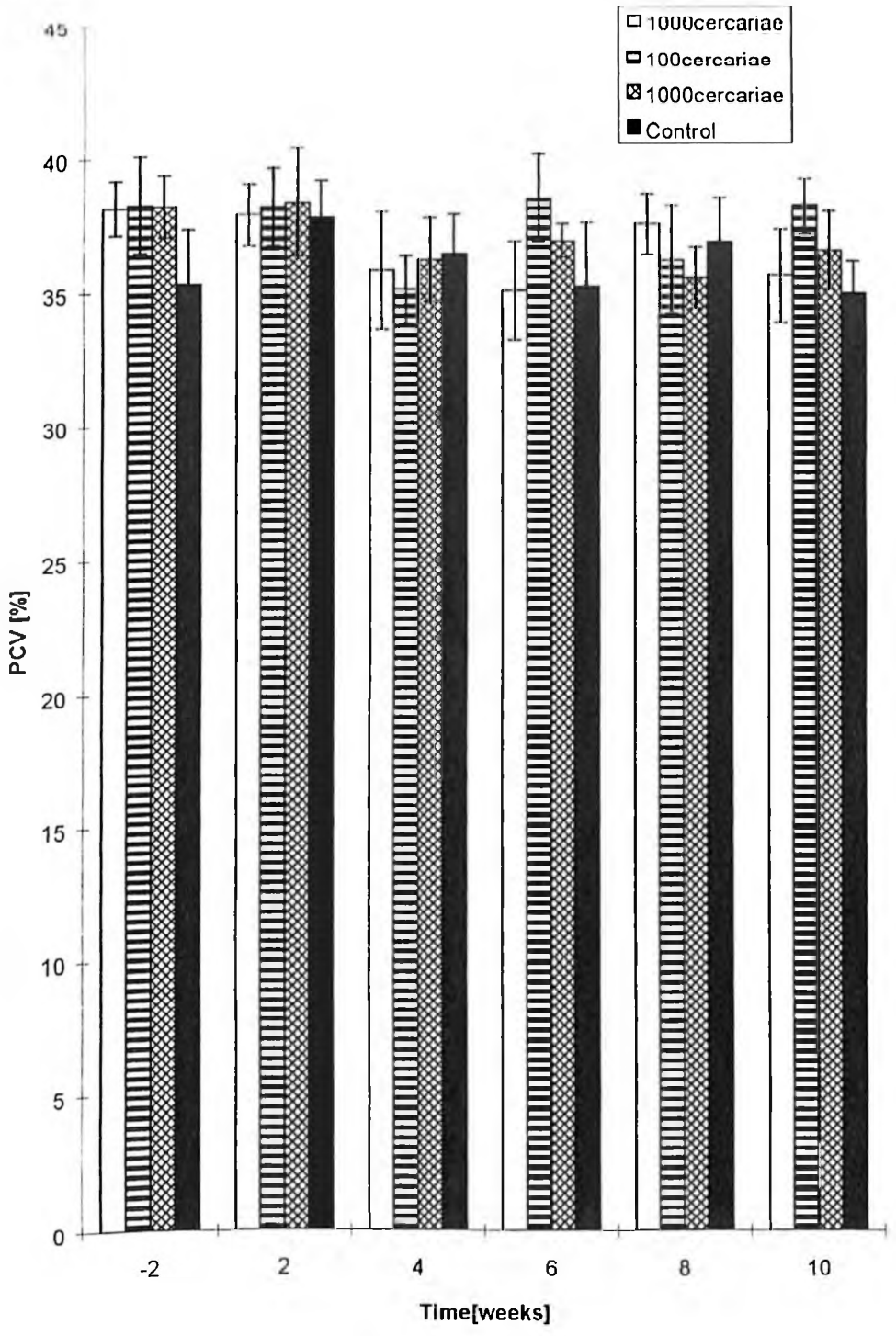

Figure 2: Mean PCV of infected and control animals. There was no significant differences between *S. mansoni* infected and control animals throughout the experimental period.

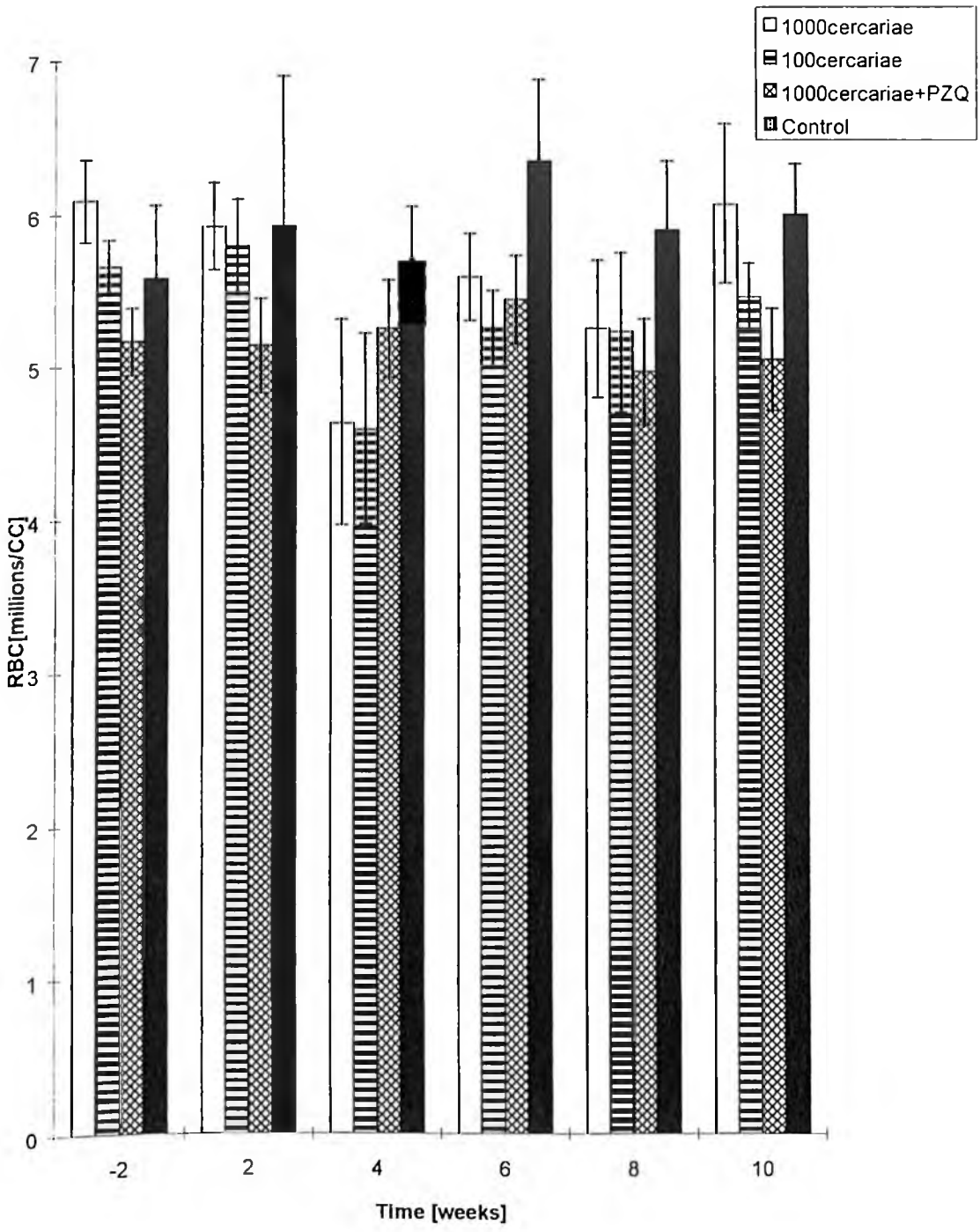

Figure 3: Mean RBC count of infected and control animals. There was no significant difference between *S. mansoni* infected and control animals throughout the experimental period.

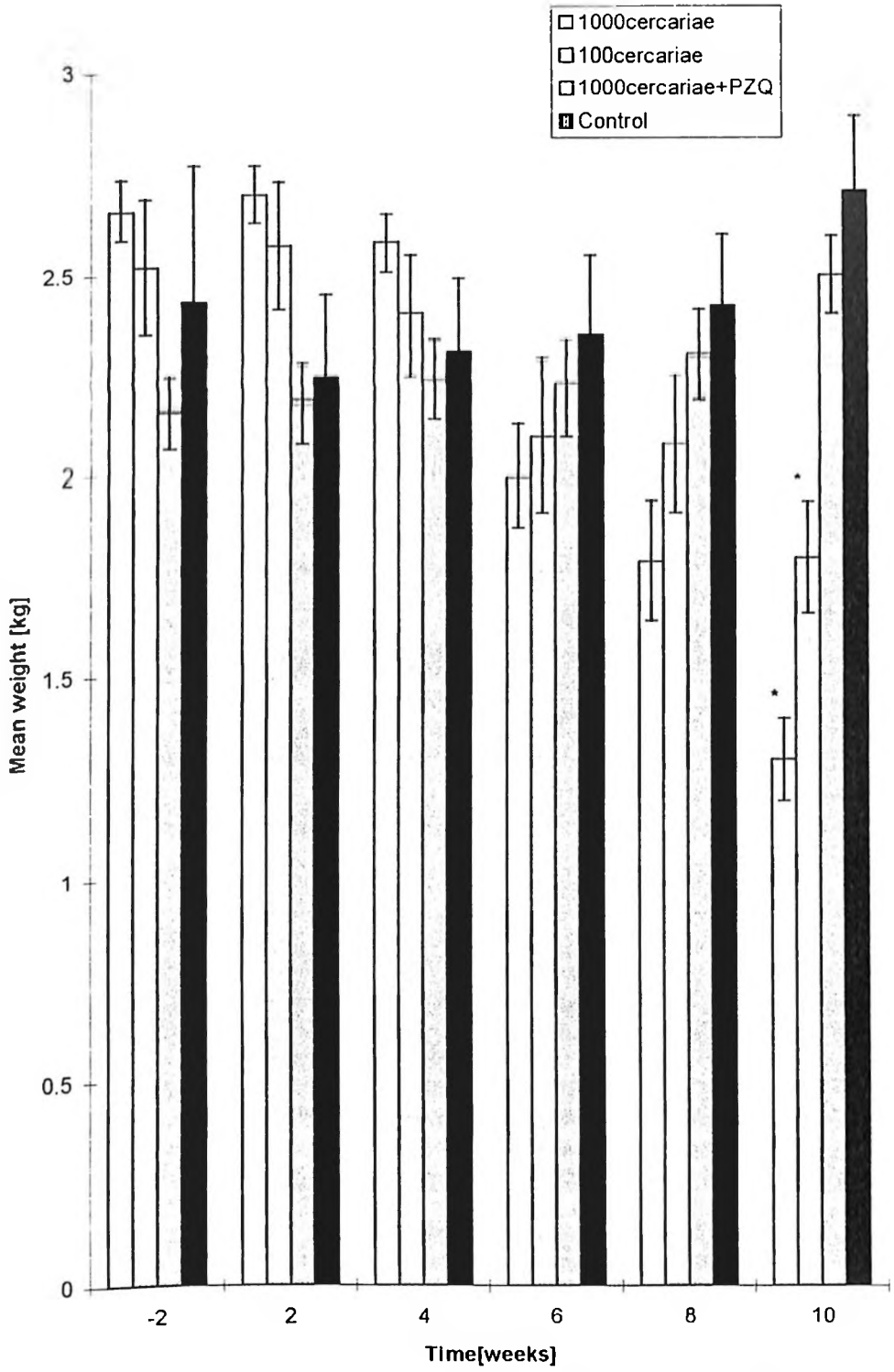

Figure 4: Mean weight of infected and control animals. Significant weight loss were seen 10 weeks post infection in animals infected with 100 and 1000 *S. mansoni* cercariae as compared to controls (* =P<0.05).

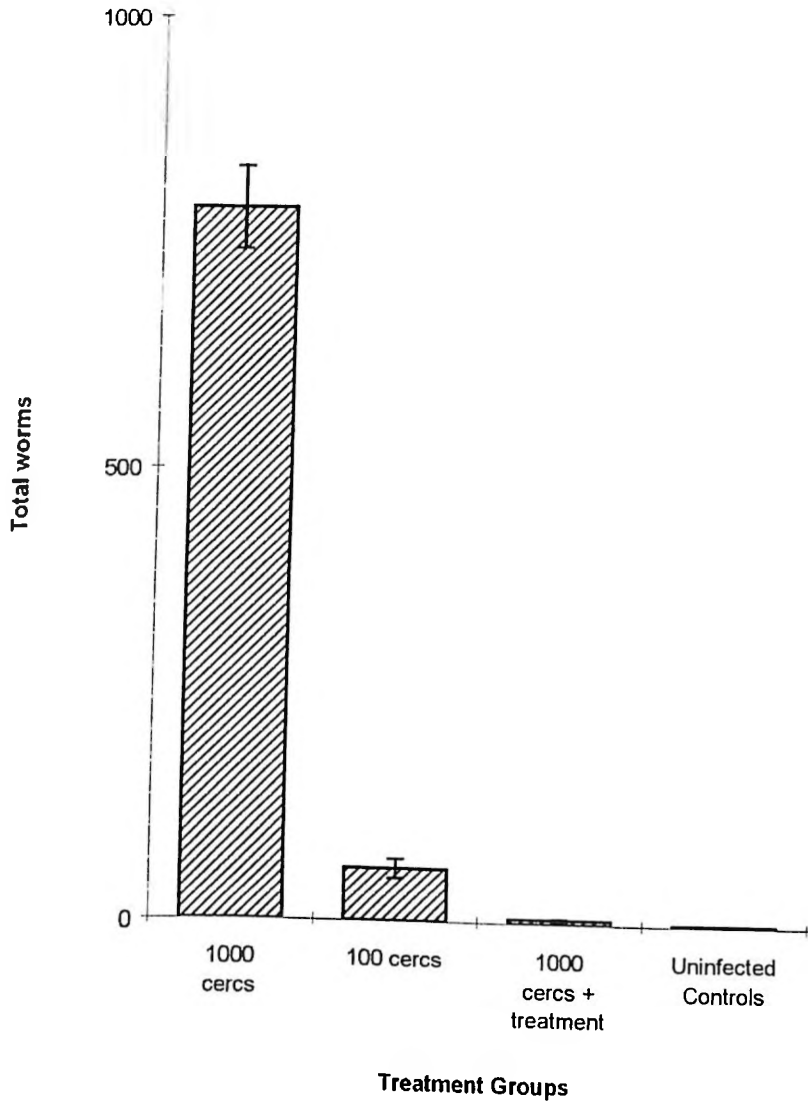

Figure 5: Worm recovered at perfusion 10 weeks after infection.

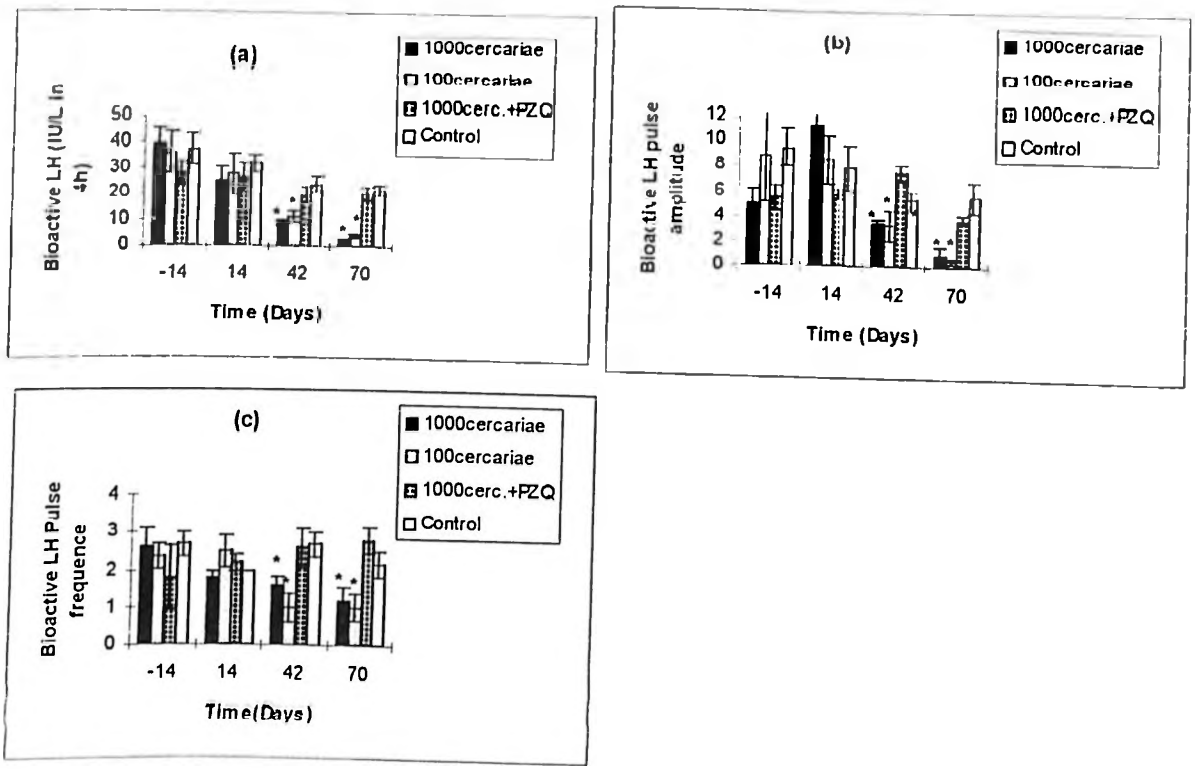

Figure 6: Mean +SEM bioactive LH area under the response curve (a), LH pulse amplitude (b), and LH pulse frequence (c) in controls and *S. mansoni* infected rabbits in samples collected at 20 min. intervals for 4h, 14 days before infection (day -14) and on days 14, 42 and 70 after infection (* = P<0.05).

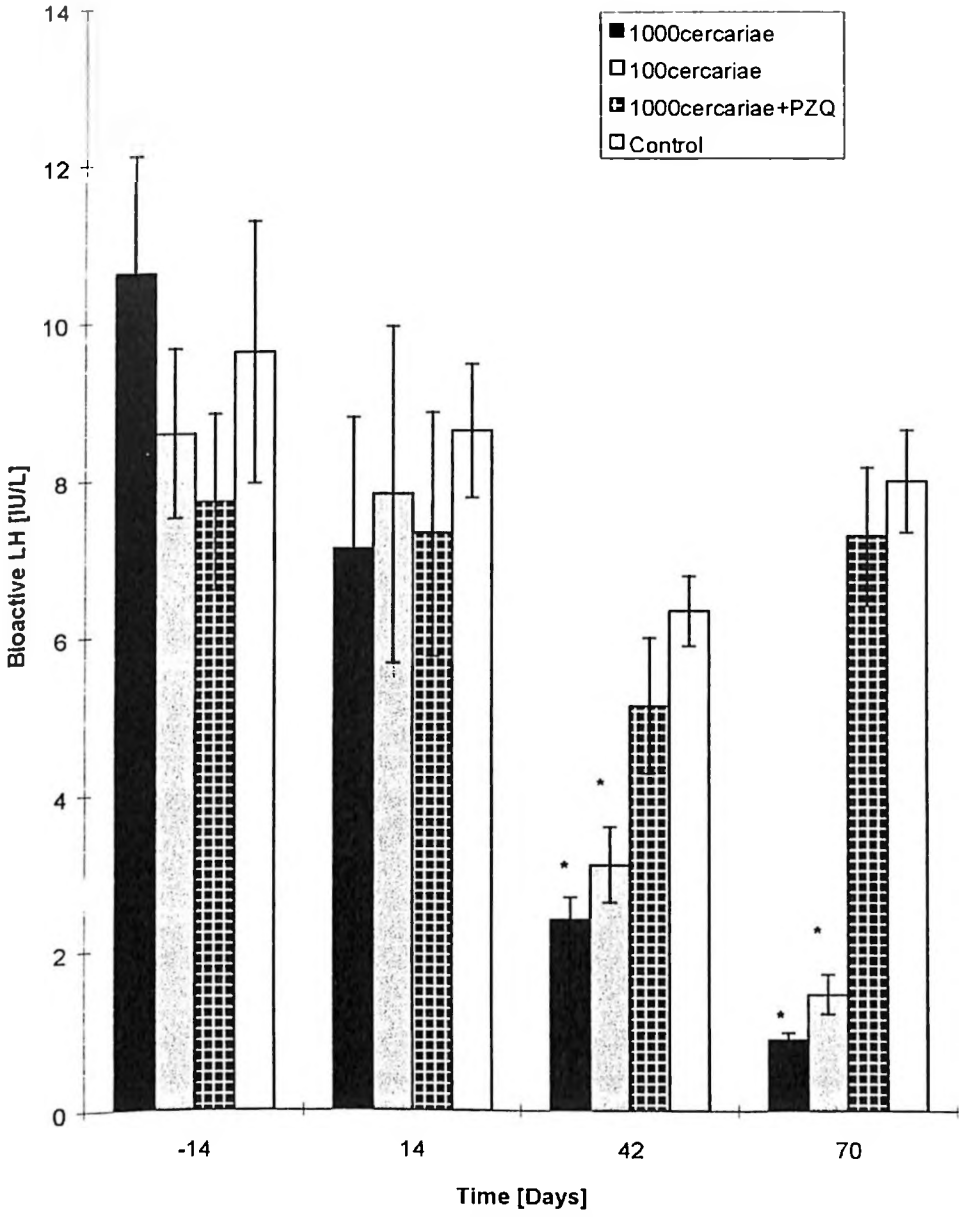

Figure 7: Mean plasma bioactive LH in controls and *S. mansoni* infected rabbits in samples collected at 20 min intervals for 4h on day 14 pre infection and days 14, 42 and 70 after infection. *Significant difference (P<0.05) in control Vs infected groups

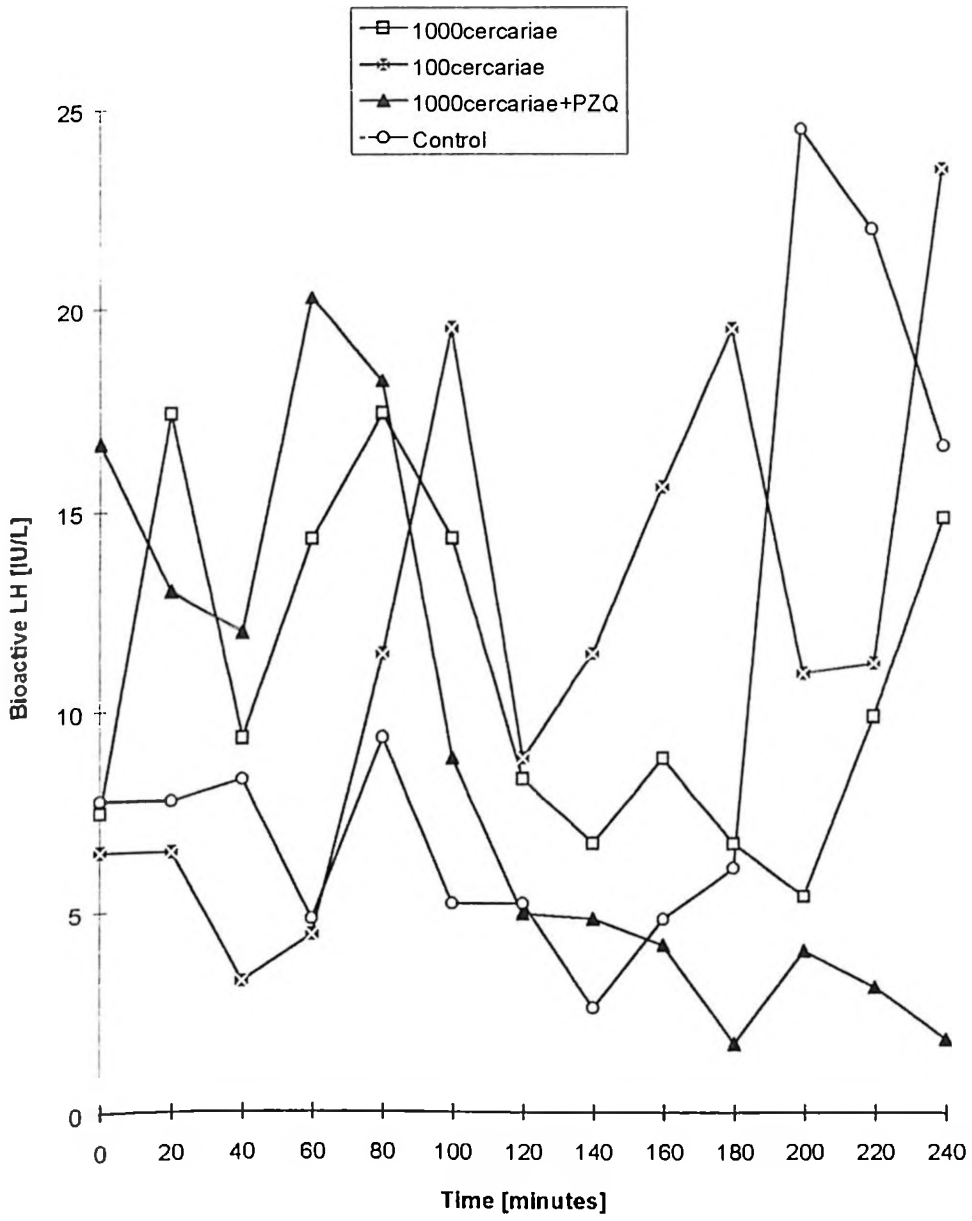

Figure 8: Pattern of plasma bioactive LH concentration in a representative uninfected control and *S. mansoni* infected rabbit in samples collected at 20 min intervals for 4h, on day 14 before infection

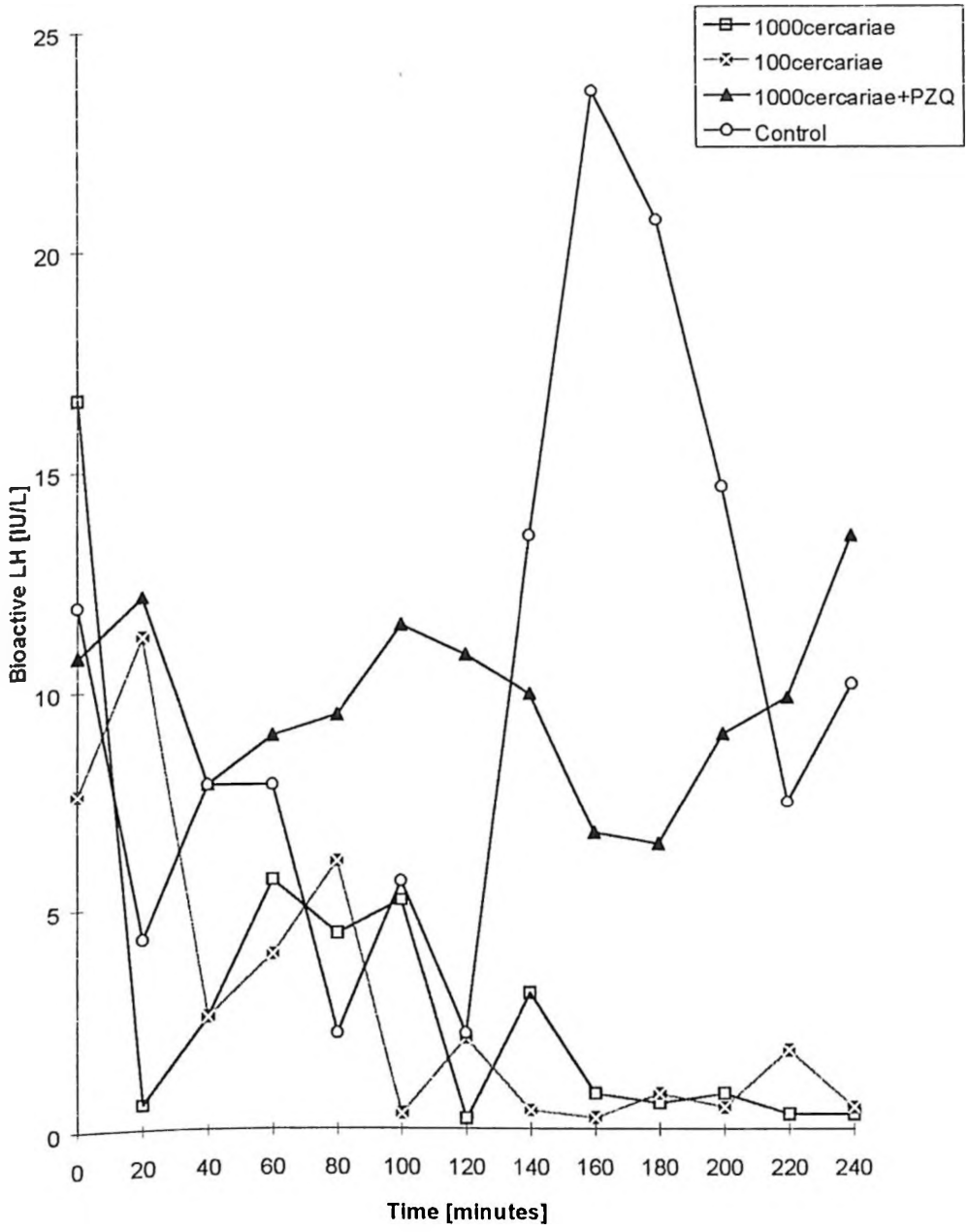

Figure 9: Pattern of plasma bioactive LH concentrations in a representative uninfected control and *S. mansoni* infected rabbit in samples collected at 20 min interval for 4 h, on day 14 after infection.

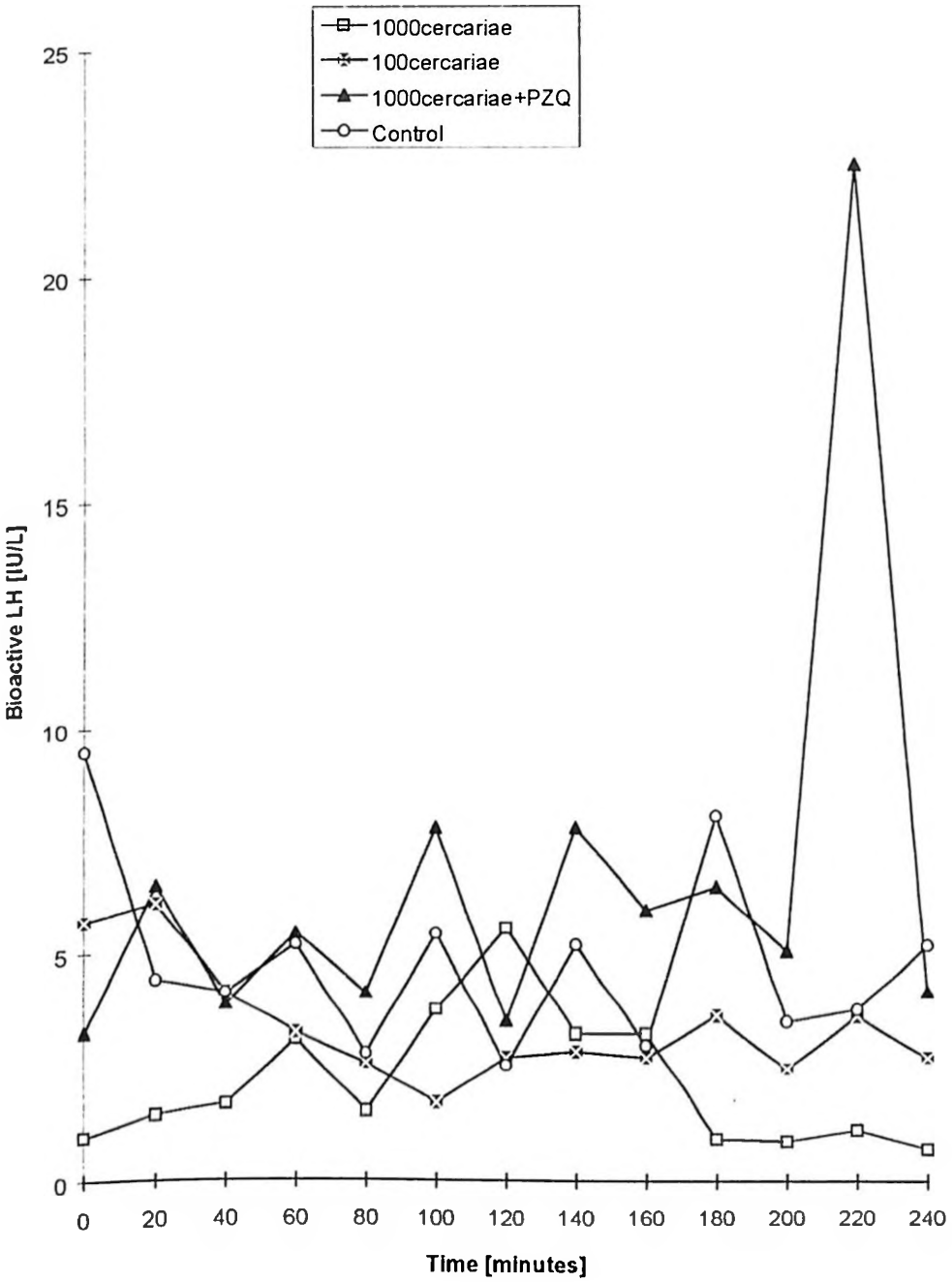

Figure 10: Pattern of LH concentration in a representative uninfected control and *S. mansoni* infected rabbit in samples collected at 20 min intervals for 4h, 42 days after infection

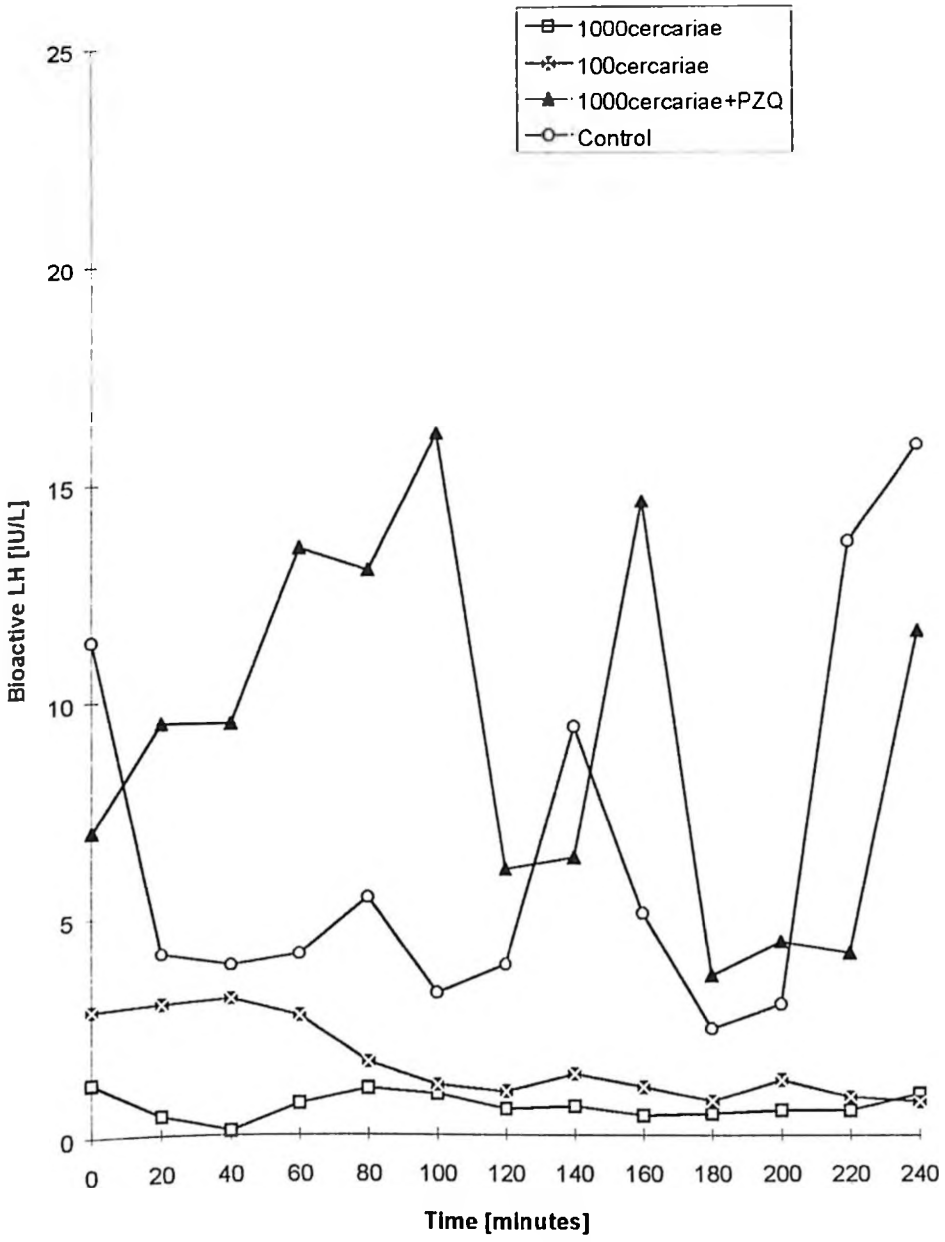

Figure 11: Pattern of plasma LH concentration in a representative uninfected control and *S. mansoni* infected rabbit in samples collected at 20 min intervals for 4 h, 70 days after infection

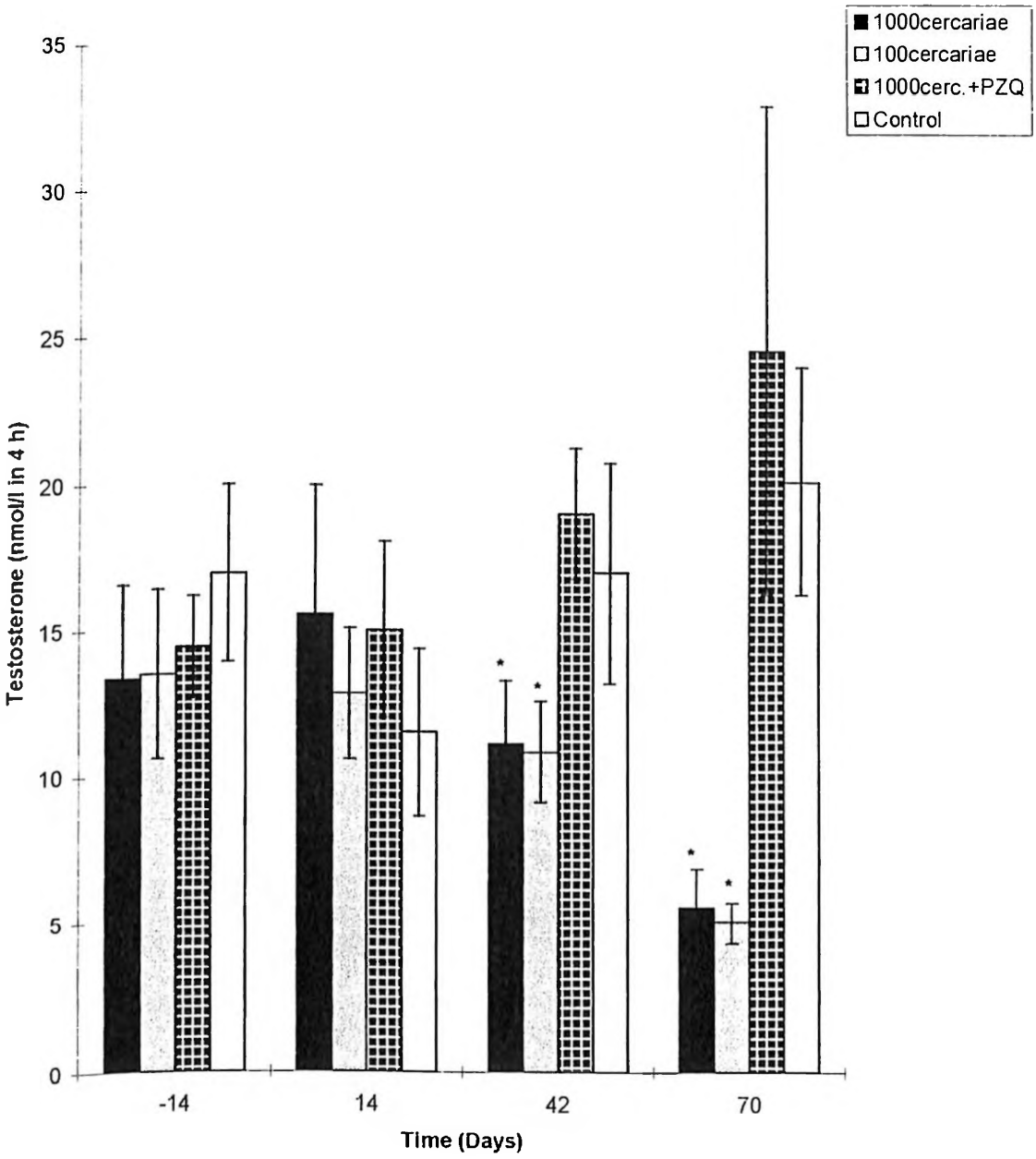

Figure 12: Area under response curve for testosterone in samples collected at intervals of 20 min for 4 h in S. mansoni infected and control rabbits on day 14 before infection and days 14, 42 and 70 post infection

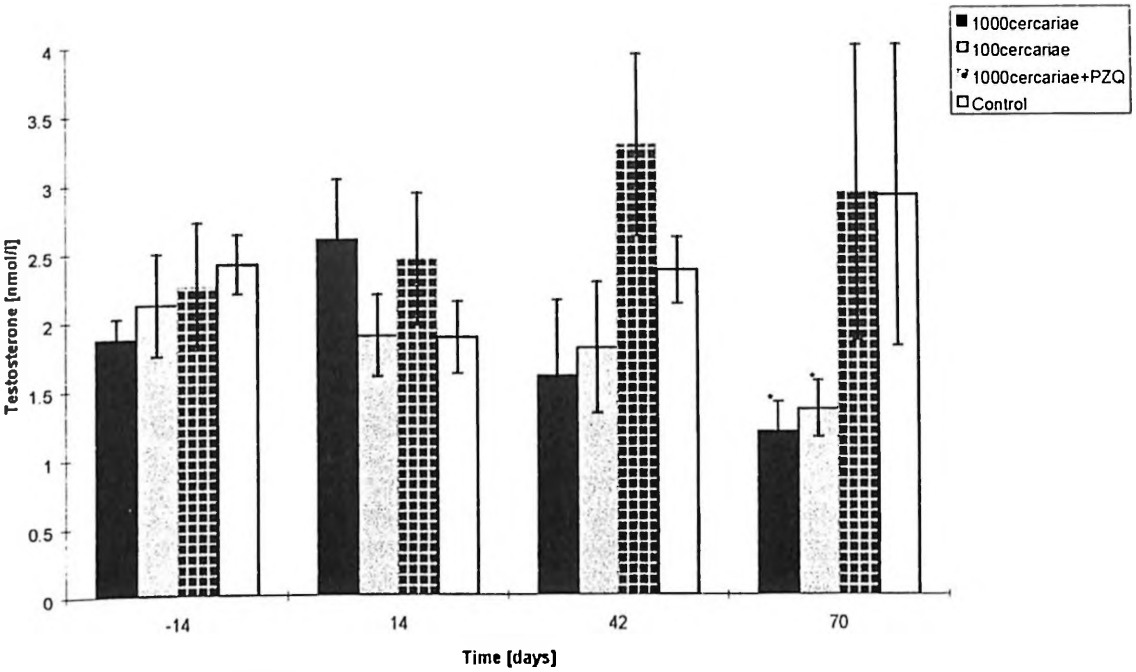

Figure 13: plasma testosterone levels in control and *S. mansoni* infected rabbits in samples collected at 20 min intervals for 4 h, 14 days before infection and on days 14, 42 and 70 post infection [*P<0.05 Vs uninfected control].

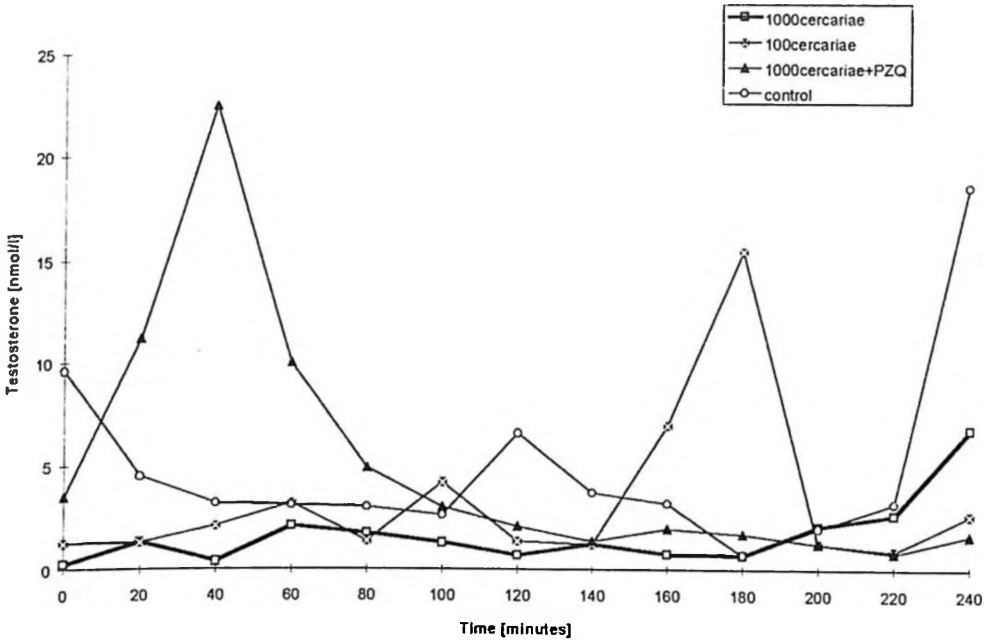

Figure 14: Pattern of Testosterone concentrations in a representative uninfected control and *S. mansoni* infected rabbit in samples collected at 20 min intervals for 4 h, 14 days before infection

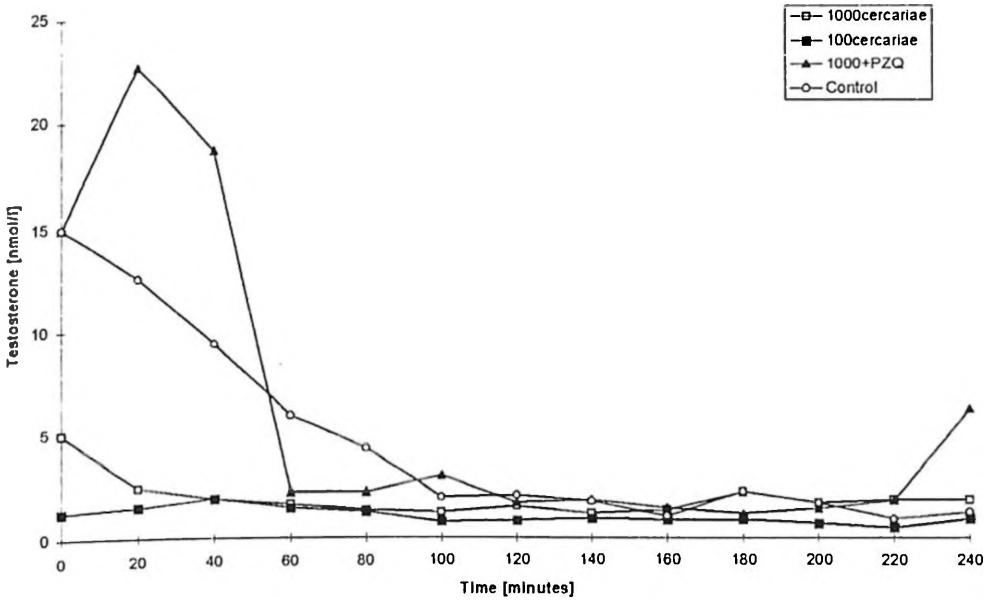

Figure15: Pattern of plasma Testosterone concentrations in a representative uninfected control and S. mansoni infected rabbits in samples collected at 20 min intervals for 4h on day 14 after infection

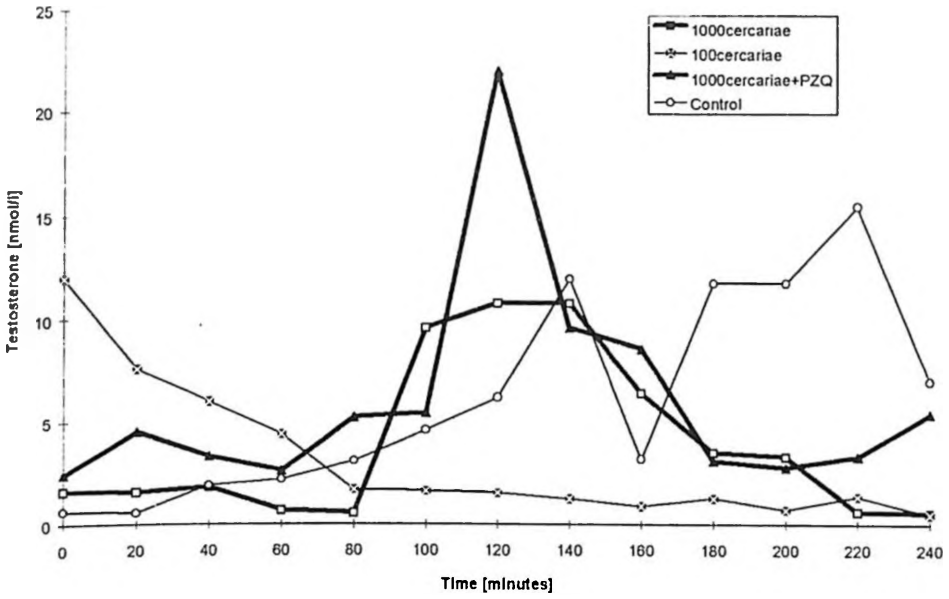

Figure 16: Pattern of testosterone concentrations in a representative uninfected control and *S. mansoni* infected rabbit in samples collected at 20 minutes intervals for 4h, 42 days after infection

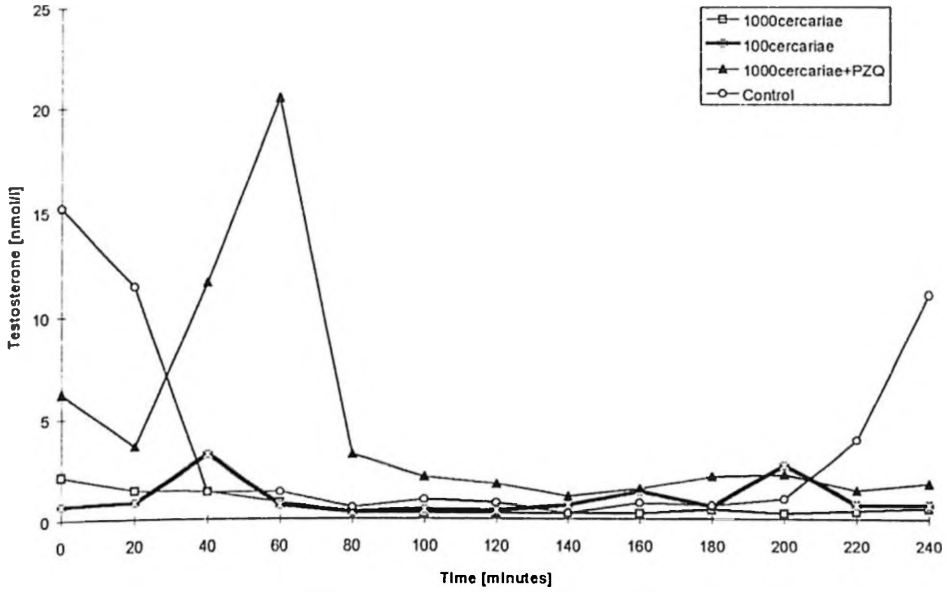

Figure 17: Pattern of Testosterone concentrations in a representative uninfected control and *S. mansoni* infected rabbit in samples collected at 20 min intervals for 4h, 70 days after infection

CHAPTER 4

4.0 DISCUSSION

In the present study, *S. mansoni* infection caused changes in both plasma testosterone and bioactive LH concentrations. Plasma testosterone declined progressively in both groups of rabbits infected with 1000 cercariae (Group I) and 100 (Group II) and reached minimum level by day 70 of infection ($P<0.05$). Early treatment of infected rabbits with PZQ inhibited these changes. Control rabbits maintain normal testosterone pulsatility over the entire experimental period.

These studies also revealed that plasma bioative LH was significantly suppressed on day 42 and day 70 post infection in both Group I and II of rabbits which were infected with 1000 and 100 cercariae respectively. However treatment of infected animal (Group III) with praziquantel five weeks after infection inhibited the LH changes caused by *S. mansoni* infection. Changes in reproductive hormones following *S. mansoni* infection have been described by previous workers, but the results are contradictory. According to Lansoude-Soukate *et al* (1991), *S. mansoni* caused a significant decreases of FSH, LH and testosterone in infected mice. In another study Saleh and Hamdy (1979) observed a marked and persistent decrease in serum FSH in mice experimentaly infected with *S.*

mansoni, while Marzouki and Amin (1997) reported a decreased serum level of testosterone but the serum levels of pituitary gonadotropins (i.e FSH and LH) showed no significant changes in infected as compared to control mice. Abdalah *et al.* (1994) observed elevated levels of sex hormones in murine *S. mansoni* infection at 60 and 70 days post-infection, but also recorded a significant fall in testosterone and 17β-estradiol in female and male mice respectively 80 days post infection.

Several hypotheses have been made to explain the changes recorded in plasma LH and testosterone concentration in schistosomiasis. Ramirez *et al.* (1961) and Sadum and Williams (1966) reported that there is an increase in globulins in *S. mansoni* infection which may results in an increase in the protein-hormone bound fraction with a decrease in free hormone concentration assayed in infected cases. Some authors attribute it to hepatic dysfunction. Ghareeb (1973) suggested that, liver derangement may reflect itself by diminished hormone inactivation, resulting in an increase of these inactivated hormones.

In this study, the reason for decreased levels of bioactive LH remains unclear, but it is possible that the disease immunopathology may play a role. The decrease in bioactive LH and testosterone concentrations has been observed to develop with the peak granuloma size and inflammatory response occurring 6-10 weeks after *S. mansoni* infection (Farah *et al* 1997), and this coincided with elevated cytokine production (Boros, 1994). Chronic schistosomiasis is associated with elevated Th2 cytokines expression

(Araujo *et. al.*, 1996). It is now well established that Th2 stimulation has a strong relation with the disease immunopathology (Scott *et. al.*, 1989). Boros, (1994) cited 12 cytokines that are produced by granuloma cells, some of these cytokine are known to inhibit release of gonadotropin hormones (Kaira *et. al.*, 1990). Cytokines have been observed to exert a direct or indirect effect via hypothalamus, in modulating the secretion of LH from the pituitary. Kaira *et al.* 1990 reported that interleukin-1 (IL-1) inhibits the release of hypothalamic luteinizing hormone-releasing hormone (LH-RH) in rat. It is now known that, the secretion of pituitary hormones such as growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH), LH and FSH are usually influenced by cytokines such as IL-1α and β, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (INF-γ) (Scarborough, 1990). It has been reported that pituitary cells express cytokine receptors for IL-1, IL-2, and IL-6 (Marquette *et al.*, 1990). Therefore it is proposed that cytokines may play the essential role in the hormonal changes observed during this study.

An alternative explanation for the observed changes in bioactive LH concentration is that schistosomes probably induced the activation of the host's endogenous opiate system which has been observed to decrease release of the hypophyseal LH (Kavaliers *et al.*, 1984). The result is that the rate of testosterone synthesis is compromised. Kavaliers *et al.* (1984), suggested that infection with *S. mansoni* results in a chronic activation of the endogenous opiates system. Indeed, mice infected chronically for 15 weeks showed

an increase in pain sensitivity in the hot-plate test (Fiore *et al.* 1996) while hamsters infected with *S. mansoni* display a gradual analgesic response immediately after infection which was suppressed by the opioid antagonist, Naloxone(Kavaliers and Podesta, 1988). In another study, Isseroff *et al* (1989) showed that β-endorphin increases while estrogen and androgen levels decrease in mice with chronic *S. mansoni* infection. This may imply that the inhibition of hypothalamic-pituitary gonadal function that occurs in chronically infected male and female mice might result from excessive β-endorphin. It has also been reported that cytokines also influence animal endogenous opiate system (Bianchi *et. al.*, 1992). Thus, evidence from the above studies, suggest that cytokines are produced during schistosome infection and these might be involved in the pathogenesis of the disease. These cytokines may play an important role in the regulation of hormonal effects acting as the cellular mediators as well as the cellular link in the interaction between the endocrine and immune system.

However, the direct effect of schistosome derived antigen on Leydig cells steroidogenesis cannot be ruled out. The immunoevasion strategies employed by schistosomes in their hosts comprise among other changes; antigen shedding, secretion of detoxifying anti-oxidant enzymes and producing signaling molecules such as cytokine or cytokine-like molecule and β-endorphin (Abdul-Aal and Attallah, 1987; DeJong-Brink, 1995; Duvaux *et al.*, 1992; and Pearce *et. al.*, 1986). Duvaux *et al.* (1992) observed that,

co-incubation of adult worm of *S. mansoni* with human polymorphonuclear leucocytes led to the release by the parasite of β-endorphin and other proopiomelanocortin derived peptides. It is known that β-endorphin inhibit testosterone secretion and intratesticular injection of β-endorphin significantly decreased the testosterone response to LH treatment (Chandrashekar and Bartke, 1992). In addition, an in vitro study has shown that the exposure of Leyding cells of mice to β-endorphin reduces testosterone response to gonadotropin stimulation (Knotts and Glass, 1988). These excretory and secretory products released by all stages of schistosome parasites in the host body may have some direct effect on Leydig cell function, but this hypothesis remains speculative. It is suggested that in vitro study of isolated Leydig cells from schistosome infected animal should be conducted to ascertain the Leydig cells functioning by comparing testosterone levels produced by infected and non-infected animals.

DeJong-Brink (1995) suggested that schistosomes affect reproductive function in their definitive host by eliciting stress response. The stress response might be triggered by inflammatory reaction to schistosomula and eggs, comprising activation of macrophages. Stress affects the hypothalamic-pituitary-gonadal (HPG) axis, thus the secretion of gonadotropin releasing hormone (GnRH) in the hypothalamus is inhibited, which results in a decreased release of LH and FSH from the pituitary and subsequently the secretion of sex steroids from the gonads.

It was also observed that treatment of infected rabbits with praziquantel cleared almost all parasite at 10 weeks post infection. The percentage reduction in worm burden in this treated group was found to be 99.5 % five weeks after treatment. Interestingly, testosterone and LH levels in this group (PZQ-treated rabbit) showed no significant difference with those of uninfected controls at this time, indicating that early treatment with praziquantel is able to keep the hormonal levels to normal values. Mehlhorn *et. al.* (1982) pointed out that egg and worm granulomas regress rapidly after praziquantel treatment in mice. Results of the current study confirm this observation and is suggestive of a strong association between the changes in hormone levels and the immunopathology modulation caused by PZQ treatment. Plasma testosterone and LH profiles were lowered on day 70 post infection in infected untreated rabbits, and the pulsatile secretion of LH was completely inhibited in *S. mansoni* infected animals 70 days after infection. These observations suggest that the effect on reproductive gonadal hormones induced by *S. mansoni* in rabbit model is partly induced by alteration in pituitary LH secretion/ or release. Whether this is associated with abberation in LH synthesis/ or release or both , or due to reduced hypothalamic trophic effect influence on the anterior pituitary , was not investigated in this study. This is one area which needs to be investigated in future studies.

Histopathologically, this study demonstrated that, *S. mansoni* caused mild changes in testicular morphology. Most of seminiferous tubules in rabbits which were

infected with *S. mansoni* regardless of the cercarial dose used, were without spermatozoa in their lumen. The tubules contained very few mature or late stage developing spermatid, indicating that *S. mansoni* may cause reduction in testicular activity. Spermatogenesis was disturbed, leading to the appearence of young round spermatids with pathological condensed nuclei. These change may be an indication of on going degenerative processes induced by the disease. These results concur with other studies in human patients with bilharzial hepatic fibrosis, in which sperm count was decreased and alteration in endocrine profile was observed (Momen *et al.*, 1987). It has also been reported that *S. mansoni* significantly decreased serum levels of testosterone in mice but testicular tissue remained unchanged (Marzouki and Amin, 1997)). Although the present study did not show marked structural changes in testicular tissue, chronic endocrine disturbances may induce changes in testicular architecture. It is therefore reasonable to assume that severe testicular structural changes may occur in *S. mansoni* infection but might require a longer period of infection than that used in this study.

Changes in the biochemical compositon of the testicular cells have been reported during *S. mansoni* infection. Saleh and Hamdy (1979) observed marked alteration in the lipid composition of the testis associated with an elevation in the levels of triglycerides, cholesterol esters and decreased levels of phospholipids. Similarly Marzouki and Amin (1997) showed a significant increase in the concentration of total testicular cholestrol and a significant decrease in the concentration of total triglycerols in *S. mansoni* infected mice

as compared to controls. Therefore it is possible that, endocrine disturbances associated with *S. mansoni* infection results in changes in the biochemical composition of testicular cells which in turn respond by a marked reduction in sperm production.

In the present study, liver pathology was observed in all infected animals regardless of the PZQ treatment or the cercarial dose used to infect animals. The common pathological findings in all infected groups were periportal and presinusoidal fibrosis, which were more marked in animals infected with 1000 cercariae. Liver fibrosis has been studied extensively in murine schistosomiasis (Coutinho *et al.*, 1997; Marcos *et al.*, 1992; Cheever, 1986). Usually mice with relatively mild prolonged *S. mansoni* infection develop two different pathological features; one consisting of disseminated portal fibrosis caused by periovular granulomas concentrated at the portal spaces (pipestem or symmers fibrosis), and the other represented by scattered hepatic granulomas (Coutinho *et a.,l* 1997)

Human schistosomal hepatic fibrosis is characterized by periportal fibrosis, presinusoidal portal block and well preserved liver parenchyma (Da Silva and Carrilho 1992; Abdel-Wahab *et al.*, 1989; Fataar *et al.*, 1985). It has been reported that, typically fewer than 10% of infected patients develop potentially fatal clay pipestem fibrosis i.e "periportal fibrosis" (Mohmoud, 1977; Nash *et al.*, 1982), which results in portal

hypertension. The pathogenesis of periportal fibrosis is not well established, although experimental and clinical studies point to egg granulomata as the main pathogenic factor.

To date, animal models that have been used to study human *S. mansoni* infection do not develop portal fibrosis with a pattern resembling human symmers' fibrosis, thus rendering it difficult to study the pathogenesis and treatment of symmers' fibrosis (peripotal fibrosis). The chimpanzee is the only primate that has been observed to develop the typical clay pipestem fibrosis found in man (Lictenberg and Sadun, 1968), but being an endagered species it is unethical to be used as the model for studying the pathogenesis and treatment of human schistosomal liver fibrosis. This study has shown for the first time that the Newzealand rabbits can develop periportal fibrosis during *S. mansoni* infection and therefore can be used as an alternative model for studying human schistosomal liver fibrosis. Thus, further research using rabbit model can influence the development of new strategies to reduce the risk of serious morbidity in humans. The decreased body weight which were recorded 10 weeks post infection in infected untreated rabbits, are probably due to the schistosome-induced hepatic and intestinal pathology, a clinical feature also observed in baboons (Farah and Mramba, 1996).

Accumulation of inflammatory cells, especially eosinophils was evident in the liver parenchyma, hepatic veins and in the lumen of epididymides of infected rabbits in the study. It is of interest that eosinophils were found to be principal cells in granulomas

surrounding eggs in the liver and gall bladder of a human case (Hsu *et al.*, 1980). Ramalho-Pinto *et al.* (1979) pointed out that, eosinophils are responsible for inducing damage of schistosome worms. In normal mice, eosinophilia can be induced artificially by intravenous injection of soluble egg antigen (SEA), or schistosomula (Colley, 1972). However, it is not clear in the present study why the accumulation of inflammatory cells was marked and very prominent in hepatic tissues of PZQ-treated animals compared to infected untreated counterparts. It is possible that this data suggest an association between the host immune system and the schistosomicidal efficacy of PZQ.

Although PZQ has proved useful in the treatment of schistosomiasis, the precise mechanism by which PZQ kills the parasite has yet to be elucidated (Redman *et al.*, 1996). In animal models, curative doses of PZQ results in the exposure of schistosome worm antigens (Harnett and Kusel, 1986), and penetration of host defence cells into the worm after 17h (Brindley and Sher, 1987; Mehlhorn *et al.*, 1982). It has been shown that, the host immune system plays an important role in PZQ-induced parasite death (Fallon *et al.*, 1992), and the schistosomicidal efficacy is reduced in immunosuppressed mice (Fallon *et al.*, 1992). As yet there have been limited studies directly examining the relationship between PZQ efficacy and the host immune status. Until more comprehensive studies are performed, the involvement of host immunity in PZQ treatment of schistosome infected humans or animals can only be surmised.

Treatment with PZQ resulted in near total elimination of *S. mansoni* worms five weeks after treatment but liver fibrosis remained. These findings are in harmony with those of Chapadeiro and Pitanga (1996), who observed that parasites and granulomas were absent in all bilharzial patients with hepatosplenic form of *S. mansoni*, previously treated with oxaminiquine but the portal fibrosis was present. The findings in the present study indicate that, as much as schistosomicidal drugs are effective in eradication of parasites, another strategy is required for management of hepatic fibrosis.

This study also showed that, despite the recent reports concerning reduced praziquantel efficacy against *S. mansoni* (Fallon *et al.*, 1997, Fallon and Doenhoff, 1994), the strain of *S. mansoni* used in this study is still highly susceptible to PZQ. This data is important in that PZQ is the current drug of choice for human schistosomiasis. It is also noted that the occurrence of resistance against the oxaminiquine/ hycanthone family, the alternative schistosomicides is well documented (Cioli *et al.*, 1993). To date no vaccine exists for humans against schistosomiasis, hence the treatment and control of the disease relies mainly on PZQ chemotherapy. This finding is therefore significant in that it points to the fact that PZQ is still a effective against the strain of *S. mansoni* used in this study .

4.1. CONCLUSION AND RECOMMENDATIONS

4.1.1 Conclusion

In conclusion, the results of the present study has demonstrated that:

1) Schistosomes impair reproductive function in male rabbit by inducing changes in the reproductive hormones.

2) Early treatment with Praziquantel inhibit the endocrine changes induced by schistosome parasites.

3) The major pathological changes in livers of rabbit infected with *S. mansoni* includes periportal fibrosis, blood vessels and bile duct proliferation and lymphocytic infltration.

4) Hepatic fibrosis in infected rabbit remain even after treatment with praziquantel.

5) Testicular morphological changes in seminiferous epithelia induced by *S. mansoni* were found to be, round spermatid with pathological condensed nuclei, decreased mature spermatozoa, and reduced epithelia thickness.

4.1.2. Recommendations

The failure of schistosomes to induce endocrine changes in animals treated with PZQ at the early stage of infection suggests a strong association between the endocrine changes observed in this study and immunopathology caused by *S. mansoni*. Therefore it is recommended that in future, studies regarding the effect of schistosomes on

eproductive hormone, the reproductive system and immune system should be studied ogether

This study, also has shown that Newzealand rabbit model can potentially be used or studying human periportal fibrosis caused by *S. mansoni* infection. It is suggested that his simple, inexpensive and reproducible model should be utilized in studying the thogenesis and treatment of human hepatic fibrosis.

CHAPTER 5

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