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Experimental *Trypanosoma brucei* infection in rats (*Rattus norvegicus*): effects on different stages of gestation and the neonatal period

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Abstract

The purpose of the research was to examine the effects of *Trypanosoma brucei* infection on different stages of gestation in female albino rats (*Rattus norvegicus*). The animals were divided into ten groups as follows: 15 animals infected on each of the following periods of pregnancy (days) 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, and 19-21; 35 uninfected animals, out of which 10 were sacrificed immediately after littering; 10 infected non-pregnant rats, and 45 uninfected females used as foster mothers. Infection with *T. brucei* between days 1-9 of pregnancy increased the length of gestation ($P < 0.05$) as well as the survival time of infected animals. The live birth index was significantly ($P < 0.05$) reduced in the groups infected between days 1-9 of gestation. Only the group infected on days 1-3 showed evidence of reduced post-implantation survival index. At birth and up to day 7 postpartum, litter weight was comparatively lower ($P < 0.05$) in the groups infected on day 1 through day 12 of gestation. However, by days 14 and 21 postpartum, litter weight in all infected groups were significantly reduced ($P < 0.05$) compared with the uninfected control. Litter size at birth was not significantly ($P > 0.05$) different in all the groups, but was lower on day 7 through day 21 postpartum. Partial fetal resorption was a prominent feature in the groups infected between days 1 and 6 of pregnancy. Placental histology revealed a placentitis characterized by diffuse perivillous fibrin deposition and lymphoplasmacytic infiltrate. It was concluded that *T. brucei* infection, especially during the first week of pregnancy, caused partial fetal resorption, increased the length of gestation and reduced the litter size as well as the litter weights of infected rats.

Keywords: fetal resorption, infection, placental pathology, prolonged gestation, rat, *Trypanosoma brucei.*

Introduction

Trypanosomosis has been reported to affect humans and domestic animals (Fraser and Mays, 1986). It is described as a complex debilitating and often-fatal condition caused by infection with one or more of the pathogenic tsetse-transmitted protozoan hemoflagellate parasites of the genus Trypanosoma (Anene *et al*., 2001). From all indications, the disease has been a great challenge to the livestock industry where the barrier imposed has been difficult to surmount by any form of chemotherapy, prophylaxis or control (Holmes *et al*., 2004; Van den Bossche and Doran, 2004). Over four decades ago, the disease, along with malaria, cancer and heart diseases, was considered by the World Health Organization (WHO) as being among the ten major health problems facing mankind (Kershaw, 1970).

According to Sekoni (1993), trypanosomosis is among the important diseases which cause various reproductive disorders in both male and female animals. Although the protozoan parasites localize in internal organs of the infected host, the gonads are obviously their preferred site (Ashman and Seed, 1974). Hence, severe degenerative changes have been observed in the reproductive organs of animals, especially with *T. brucei* infection (Ikede, 1979; Ikede *et al*., 1988; Edeghere *et al*., 1992), resulting in abortion, stillbirth, neonatal death, irregular estrous cycle, infertility, anestrus, cystic degeneration of the ovaries and irregularities in reproductive hormone production in females. In males, the infection is characterized by elevated sperm morphological abnormalities (Akpavie *et al*., 1987; Sekoni, 1994). Sporadic cases of congenital transmission of trypanosomes have also been reported in humans (Rocha *et al*., 2004), sheep (Ikede and Losos, 1972) and mice (Ijagbone and Agbede, 2000). Among the three subspecies of *T. brucei*, *T. brucei brucei* is considered the most virulent in domestic animals (Ikede, 1983). Human trypanosomosis or sleeping sickness is caused by the other two subspecies namely *T. b. rhodesiense* and *T. b. gambiense*. While sleeping sickness associated with *T. b. rhodesiense* manifests in the form of acute inflammatory disease, the *T. b. gambiense* variant develops as a chronic autoimmune disease (Van Meirvenne, 1999).

Food and Agriculture Organization (1983) reports showed that nearly two-thirds of the world's cattle and almost all the buffalo are in the developing countries where trypanosomosis is mostly endemic. Paradoxically, these areas put together produce slightly less than one-third of the world's beef and buffalo meat. Similarly, while 58% of the world's pigs are found in

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the developing countries, they contribute just about one-third of the world's pig meat. In developing countries, the problem with food animal production seems to lie, among others, with understanding of the requisite management procedures and the role some tropical diseases such as trypanosomosis play in the reproductive performance of the domestic farm animals.

Although the clinical and pathological manifestations of trypanosomosis have been widely reported, there is limited information on its effects on reproduction, especially at the different stages of gestation. Since trypanosomosis is endemic in most tropical countries, it is possible for the animals to become infected at any stage of their reproductive cycle. The purpose of the present study therefore, is to examine the effects of *T. brucei* infection on the reproductive performance of rats at different stages of gestation and during the neonatal period.

Materials and Methods

Animals

Two hundred and thirty five mature outbred Sprague-Dawley albino rats (*Rattus norvegicus*) comprising 195 females and 40 males were used for the study. Just before commencing the study, all the animals weighed between 130 and 170 g, and were aged between 12 and 15 weeks. Throughout the duration of the experiment, they were housed at room temperature (28-32^{o}C) in the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, using stainless steel cages. They were fed *ad libitum* with commercial feed (Top Feed Nigeria Ltd.) containing 16% crude protein and 4.5% fibre. Clean water was provided *ad libitum*.

Source of trypanosomes

The Faculty of Veterinary Medicine, University of Nigeria, Nsukka (FVM-UNN) *Trypanosoma brucei brucei* stock strain maintained on albino rats was used for infecting the rats. This strain was originally isolated in the year 2002 from a clinically infected dog and was identified at the Clinical Laboratory of the University of Nigeria Veterinary Teaching Hospital, Nsukka. Each infected animal received 2.50×10^5 trypanosomes administered intraperitoneally as 0.1 ml suspension in normal saline. At this dose, the strain usually causes progressively high parasitemia leading to death of infected rats in approximately 20 days (Ihedioha *et al*., 2003). Confirmation of infection was done starting from 4 days post-infection using hematocrit method as described by Murray *et al.* (1977).

Determination of successful mating

The vaginal plug method of Bennett and Vickery (1970), modified by Ochiogu *et al.* (2006) was used in determining successful mating in the animals. Briefly, three female rats were placed in a cage with a male of proven fertility. Vaginal smear of each female was made on a labeled clean glass slide with the aid of wet cotton swab dipped in fresh normal saline and inserted into the vagina to a depth of approximately 1.5 cm. The wet smear was examined grossly for the presence of protein coagulates (remnants of copulatory plug) as evidence of successful mating. This procedure was carried out at 12 hours intervals. The day remnants of the plug were found was regarded as day 1 of pregnancy. Thereafter, the females were separated from the males.

Increase in body weight was used to monitor the progress of pregnancy. The body weights although noted, are not shown in the results.

Determination of Packed Cell Volume (PCV) and level of parasitemia

Packed Cell Volume was determined by the microhematocrit method (Coles, 1968), while the level of parasitemia was assessed using rapid matching method as described by Herbert and Lumsden (1976) starting from day 4 post-infection and at 4-day intervals until day 24 post-infection. Body temperature was monitored with clinical thermometer.

Screening of neonates for trypanosomes

The offspring of infected rats were screened for trypanosomes at birth in order to verify the possibility or otherwise of transplacental infection. Blood samples were collected from tail tips of the animals and parasitemia determined by hematocrit method (Murray *et al*., 1977).

Foster mothers

A total of 45 uninfected nursing mothers that recently littered were used as foster mothers to nurse, up to the weaning age, the litters that lost their mothers to infection. This enabled conclusion of the studies on litter size and weight started at birth, and that ran through days 7 to 21 postpartum.

Experimental design

The 195 females used for the study were divided into ten groups, identified with letters A to H (in parentheses), as follows: 35 uninfected controls out of which 10 were sacrificed immediately after littering (A1); 10 infected non-pregnant control (A2); 15 animals infected at each of the different stages of gestation (in days) as follows: 1-3 (B), 4-6 (C), 7-9 (D), 10-12 (E), 13-15 (F), 16-18 (G) and 19-21 (H). The remaining 45 uninfected females served as foster mothers as indicated above. The 40 uninfected males of proven fertility were used solely for mating purposes.

The following parameters were monitored: gestation length, PCV, level of parasitemia, litter size and weight at birth, and at days 7, 14 and 21

postpartum, transplacental infection, post-infection survival time, and areas of fetal attachment and hence fetal resorption (determined at death). The survival time denotes the length of time (days) the animal was able to live after infection.

The gestation index, post-implantation survival index, and live-birth index were calculated as follows:

$$\text{Live-birth index} = \frac{\text{Number of live offspring at PND1}}{\text{Total number of offspring born}} \times \frac{100}{1}$$

$$\text{Post-implantation survival index} = \frac{\text{Total number of offspring born}}{\text{Total number of implantation sites}} \times \frac{100}{1}$$

$$\text{Gestation index} = \frac{\text{Number of live fetuses born}}{\text{Number of animals pregnant}} \times \frac{100}{1}$$

Where PND1 represents Post-natal day 1. i.e., 6 hours after delivery.

Histopathology

For the histopathology, sections of the placentae collected from infected and control rats were fixed in 10% buffered formal saline. Tissues were processed as routinely done to paraffin wax (Drury and Wallington, 1979) and cut at 5 micron thickness. All sections were stained with hematoxylin and eosin (H & E) stain; while selected sections were stained by Price's Giemsa method (Luna, 1968).

Statistical analyses

Data were subjected to Analysis of Variance (ANOVA) and variant means separated using least significance difference (LSD). The results are presented as the mean ± standard error of the mean (SEM).

Results

Effect of T. brucei on length of gestation

The effect of *T. brucei* on the length of gestation in rats is presented in Fig. 1. For uninfected pregnant animals (group A1), the mean gestation length was 21.16 ± 0.07. However, for animals infected during 1-3 days of pregnancy (group B), the length of gestation was extended, with a mean value of 23.3 ± 0.71. The increase was significant ($P < 0.05$). In addition, 5 out of 15 animals infected during the same period (1-3 days of pregnancy; group B^+) died in the course of pregnancy and similarly had an extended gestation period, with a mean value of 24.70 ± 0.71. One animal aborted in the group infected at 4-6 days of pregnancy. With the exception of these six animals, all other infected but pregnant rats were able to litter before death.

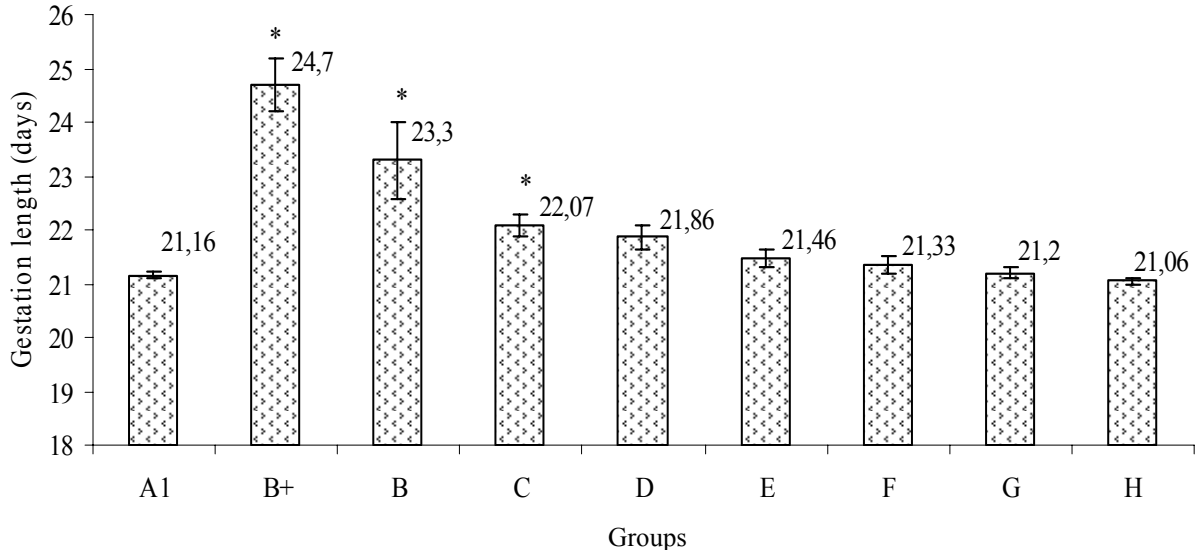

Figure 1. Effect of *Trypanosoma brucei* on the length of gestation (days). *$P < 0.05$ compared with uninfected control. Group A1 represents uninfected control rats; B^+ represents rats infected 1-3 days of pregnancy but died in the course of pregnancy without littering; Groups B - H were infected on days 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, and 19-21 of pregnancy, respectively.

Effect of T. brucei on survival time

The survival time of the groups infected with *T brucei* at 1-3, 4-6 and 7-9 days of pregnancy (groups B to D) was comparatively longer ($P < 0.05$) than that of their infected non-pregnant control counterpart (Fig. 2).

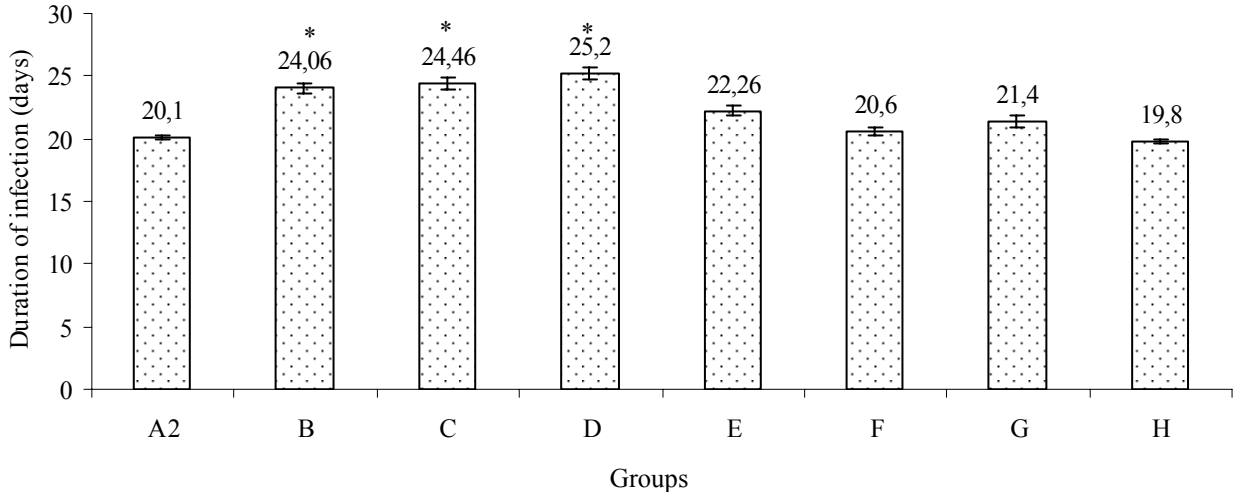

Figure 2. Effect of *Trypanosoma brucei* on the survival time (days) of rats. *P < 0.01 compared with control. Group A2 represents infected non-pregnant rats. Groups B – H were infected on days 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, and 19-21 of pregnancy, respectively.

Effect of T. brucei infection on fetal resorption

Only 1 out of 15 animals infected on days 1-3 of pregnancy had total fetal resorption. Even though all the groups had partial fetal resorption, only those animals inoculated with the parasite on days 1-3 and 4-6 of pregnancy (groups B and C) had significantly ($P < 0.05$) higher values than the control (Fig. 3).

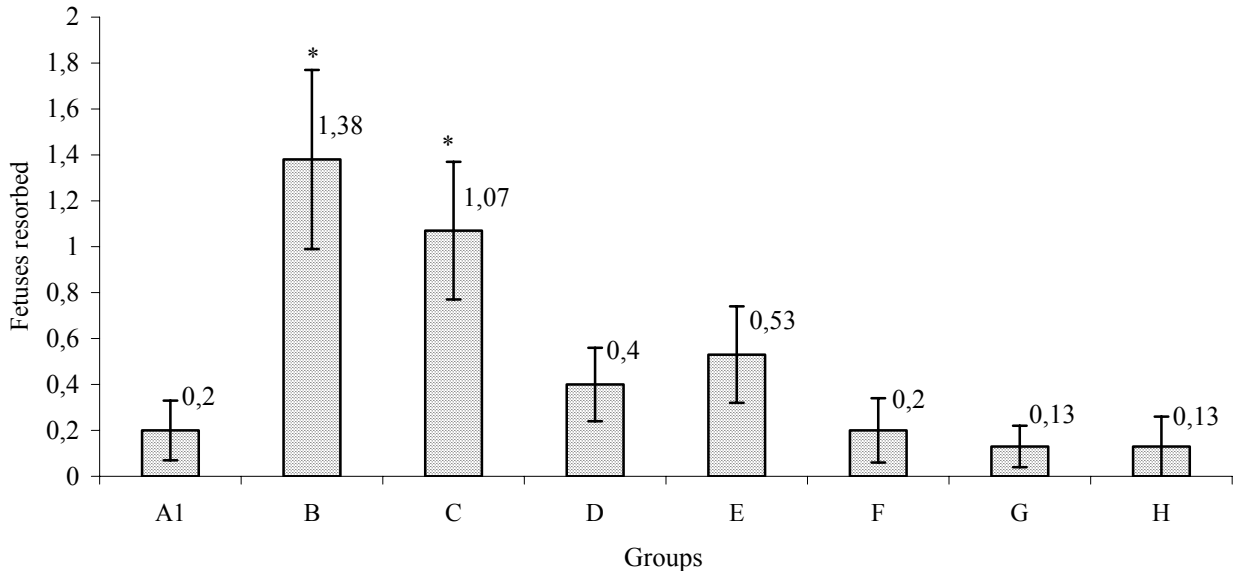

Figure 3. Effect of *Trypanosoma brucei* on partial foetal resorption. *P < 0.05 compared with control. Group A1 represents uninfected pregnant control rats. Groups B - H were infected on days 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, 19-21 of pregnancy, respectively.

Effect of T. brucei on litter size and litter weight

As shown in Table 1, litter size at birth did not differ significantly ($P > 0.05$) between the groups but was, however, lower ($P < 0.05$) in all the infected groups (groups B to H) at days 7, 14 and 21 postpartum when compared with the uninfected control group.

Table 1. Mean (± SEM) effect of *Trypanosoma brucei* infection on litter size of rats.

Time period	Uninfected control (A1)	Infected during the following days of pregnancy						
		1-3 (B)	4-6 (C)	7-9 (D)	10-12 (E)	13-15 (F)	16-18 (G)	19-21 (H)
At birth	7.84[a] (0.32)	6.86[a] (0.62)	6.92[a] (0.28)	7.10[a] (1.66)	6.86[a] (0.29)	6.86[a] (0.30)	7.73[a] (0.25)	7.80[a] (0.31)
Day 7 after birth	6.92[a] (0.40)	4.60[b] (0.71)	4.56[b] (1.52)	5.55[b] (0.45)	5.00[b] (0.58)	4.46[b] (0.64)	5.50[b] (0.56)	5.46[b] (0.60)
Day 14 after birth	6.68[a] (0.41)	3.60[b] (0.68)	3.12[b] (0.58)	3.78[b] (0.46)	3.10[b] (0.48)	3.20[b] (0.53)	4.40[b] (0.60)	4.82[b] (0.52)
Day 21 after birth	6.64[a] (0.40)	3.40[b] (0.60)	2.86[b] (0.62)	3.28[b] (0.61)	3.33[b] (0.66)	3.00[b] (0.47)	4.12[b] (0.61)	4.40[b] (0.56)

[a,b]Different superscripts in a row indicate significant difference among means at $P < 0.05$.

The litter weight of the groups infected between days 1-15 of pregnancy (groups B to F) was significantly ($P < 0.05$) reduced at birth and at day 7 postpartum. However, by days 14 and 21 postpartum, all the groups infected during pregnancy (groups B to H) had a significant ($P < 0.05$) reduced litter weight (Table 2).

Table 2. Mean (± SEM) effect of *Trypanosoma brucei* infection on litter weight (g).

Time period	Uninfected control (A1)	Infected during the following days of pregnancy						
		1-3 (B)	4-6 (C)	7-9 (D)	10-12 (E)	13-15 (F)	16-18 (G)	19-21 (H)
At birth	44.12[a] (1.62)	37.30[b] (1.6)	37.36[b] (1.46)	36.60[b] (1.46)	36.60[b] (1.44)	37.26[b] (1.46)	40.73[a,b] (1.26)	41.53[a] (1.40)
Day 7 after birth	76.52[a] (4.21)	34.23[b] (4.91)	37.86[b] (5.54)	41.17[b] (3.62)	40.16[b] (5.04)	33.24[b] (4.60)	48.23[a,b] (5.47)	47.43[a,b] (5.05)
Day 14 after birth	129.10[a] (7.13)	42.16[b] (7.50)	42.10[b] (7.46)	45.98[b] (6.75)	41.13[b] (7.10)	47.47[b] (7.57)	57.03[b] (7.98)	63.81[b] (6.88)
Day 21 after birth	186.90[a] (10.28)	56.56[b] (10.04)	46.40[b] (11.92)	9.36[b,c] (13.08)	60.06[b,c] (13.84)	57.63[b,c] (8.83)	80.33[b,c] (12.84)	89.67[c] (10.46)

[a,b,c]Different superscripts in a row indicate significant difference among means at $P < 0.05$.

Trypanosoma brucei infection and reproductive indices

From the results presented in Table 3, there was a significant ($P < 0.05$) reduction in the live-birth index of the groups infected between days 1-9 of pregnancy (groups B, C, D) while only the group infected on days 1-3 of pregnancy (group B) had a significantly ($P < 0.05$) reduced post-implantation survival index. Gestation index showed a graded increase from the group infected on days 1-3 of pregnancy up to the group infected on days 19-21 of pregnancy (group H) when compared with the uninfected control.

Level of parasitemia

Results in Table 4 show that beginning from day 4 post-infection, there was a persistent increase in the mean level of parasitemia in the infected but non-pregnant animals (group A2) until death. The level of parasitemia was positively correlated with pyrexia (unpublished data; Ochiogu et al., 2006). However, the level of parasitemia was comparatively ($P < 0.05$) reduced in the groups inoculated on days 1-3 (group B), 4-6 (group C), 10-12 (group E), 13-15 (group F) and 16-18 of pregnancy (group G) at day 8 post-infection and for groups 1-3 and 4-6 at the day 16 post-infection. There were no trypanosomes in the blood of any of the surviving offspring.

Table 3. Means (± SEM) effect of *Trypasoma brucei* infection on some reproductive indices of female rats.

	Uninfected pregnant control (A1)	Infected during the following days of pregnancy						
		1-3 (B)	4-6 (C)	7-9 (D)	10-12 (E)	13-15 (F)	16-18 (G)	19-21 (H)
Live-birth index	97.49[a] (1.05)	28.00[d] (8.39)	77.71[c] (6.46)	91.64[b] (2.88)	94.52[a,b] (1.80)	98.33[a] (1.14)	97.09[a,b] (1.62)	95.56[a,b] (1.77)
Post-implantation survival index	97.75[a] (1.51)	60.89[b] (11.65)	81.40[a,b] (6.63)	94.05[a] (2.56)	92.48[a] (2.40)	97.83[a] (1.52)	98.67[a] (0.91)	98.67[a] (1.33)
Gestation index	760.00	200.00	533.33	640.00	646.66	673.33	746.66	793.33

[a,b,c,d]Different superscripts in a row indicate significant difference among means at $P < 0.05$.

Table 4. Mean (± SEM) levels of parasitemia (10^6/ml) at specified periods of gestation.

Post-infection period (days)	Infected non-pregnant control (A2)	Infected during the following days of pregnancy						
		1-3 (B)	4-6 (C)	7-9 (D)	10-12 (E)	13-15 (F)	16-18 (G)	19-21 (H)
4	0.60 (0.38)	0.38 (0.02)	0.89 (0.36)	0.78 (0.28)	0.46 (0.13)	0.41 (0.11)	0.64 (0.27)	0.38 (0.09)
8	74.16[a] (37.56)	4.15[b] (1.36)	4.71[b] (1.56)	10.42[a,b] (4.54)	7.53[b] (2.77)	5.49[b] (1.51)	4.25[b] (1.56)	23.84[a,b] (8.67)
12	344.79 (129.18)	122.26 (49.72)	129.06 (43.44)	146.66 (55.64)	116.40 (27.08)	236.66 (46.41)	179.59 (41.74)	223.46 (43.82)
16	656.00[a] (97.60)	332.53[b] (89.86)	377.06[b] (90.30)	396.26[a,b] (85.32)	428.66[a,b] (87.60)	552.53[a,b] (84.80)	591.20[a,b] (95.26)	691.46[a] (66.06)
20	---	624.66[a,b] (85.44)	639.32[a,b] (107.69)	674.53[a,b] (81.53)	746.66[a,b] (68.26)	883.33[b] (74.88)	747.27[a,b] (83.41)	720.00[a,b] (180.34)
24	---	447.99 (180.69)	638.57 (99.66)	749.00 (109.08)				

[a,b]Different superscripts in a row indicate significant difference among means at $P < 0.05$.

Effect of the parasites on Packed Cell Volume (PCV)

The effect of *T. brucei* on the PCV is presented in Table 5. Compared with the uninfected pregnant control group (group A1), there was a progressive decline ($P < 0.05$) in the PCV values of infected rats with increase in gestational age. In all the infected rats (groups A2, B to H), there was a direct but inverse relationship between the level of parasitemia (Table 4) and the PCV.

Histopathology

The placentae sections collected from pregnant rats that died 23 days post-infection showed degeneration and necrosis of the epithelial (secretory) cells of the uterine glands, and edema of the interglandular lamina propia. The fibrinous exudates within the intervillae spaces of the chorion frondosum contained focally diffuse areas of phagocytic mononuclear giant cells aggregation. In addition, there was mild aggregation of plasma cells, lymphocytes and neutrophils around these chorionic villi (perivillitis). The villi had mildly edematous mesenchymal connective tissue around the fetal blood vessels, which contained both normal and karyorrhectic blood cells (Fig. 4). In some other placentae sections collected from this same group of rats, there was severe necrosis of villi trophoblast cells, with collapse of villi core (mesenchymal connective tissue layer) and the blood vessels within (Fig. 5). These histologic lesions were absent in the placentae sections of the uninfected pregnant rats that were sacrificed after littering.

Table 5. Mean (± SEM) effect of *Trypanosoma brucei* infection on Packed Cell Volume (PCV) of rats.

Post-infection period (days)	Uninfected pregnant control (A1)	Infected non-pregnant control (A2)	Infected during the following days of pregnancy						
			1-3 (B)	4-6 (C)	7-9 (D)	10-12 (E)	13-15 (F)	16-18 (G)	19-21 (H)
0	42.52 (0.28)	43.40 (0.42)	43.60 (0.57)	42.46 (0.42)	42.60 (0.46)	42.66 (0.33)	42.33 (0.43)	42.73 (0.37)	42.80 (0.32)
4	44.00^{a} (0.36)	43.30^{a} (0.47)	43.66^{a} (0.48)	42.06^{a,b} (0.58)	41.60^{a} (0.34)	40.40^{a} (0.44)	38.73^{b,c} (0.66)	37.26^{c} (0.34)	37.86^{c} (0.41)
8	43.36^{a} (0.38)	40.80^{b} (0.53)	42.06^{a,b} (0.45)	40.96^{b} (0.40)	39.60^{b} (0.25)	37.33^{b} (0.62)	36.26^{b} (0.54)	38.60^{b} (0.38)	39.13^{b} (0.16)
12	42.24^{a} (0.33)	38.90^{b} (0.81)	36.33^{b,c} (0.88)	35.53^{b,c} (1.01)	37.73^{b} (0.33)	34.26^{c} (0.71)	36.26^{b} (0.49)	38.20^{b} (0.40)	37.66^{b} (0.21
16	40.36^{a} (0.32)	35.20^{b} (0.64)	32.53^{c} (0.76)	31.66^{c} (0.51)	33.60^{b,c} (0.70)	32.66^{c} (0.70)	33.93^{b,c} (0.54)	35.06^{b} (0.65)	31.73^{c} (0.65)
20	38.32^{a} (0.18)	---	28.53^{b} (0.67)	31.00^{b} (0.88)	27.93^{b} (0.56)	29.42^{b} (0.55)	29.66^{b} (0.72)	33.50^{c} (0.60)	---
24	---	---	27.83 (1.35)	28.00 (0.53)	26.70 (0.61)	---	---	---	---

^{a,b,c}Different superscripts in a row indicate significant difference among means at $P < 0.05$.

Discussion

This study investigated the reproductive performance of female albino rats infected with *Trypanosoma brucei* at different stages of gestation and its effects during the neonatal period. For animals that were infected between days 1-9 of gestation, the study showed that the length of gestation was significantly ($P < 0.05$) extended (Fig. 1). The histologic structure of the chorionic villi in infected pregnant rats that died 23 days post-infection showed that the villi were fully developed, with moderate to severe necrosis of the trophoblast cells and partial to absolute collapse of the villi core (mesenchymal tissue) and blood vessels, leaving necrotic debris (Fig. 4 and 5). These are indications that pregnancy in this group of rats was generally advanced before fetal death in the late stages of gestation, with variable degree of fetal resorption (Genest, 1992). The prolonged gestation was most probably why the pregnant rats had not littered. According to Roberts (1971), in all species of animals, a markedly shortened or prolonged gestation period is frequently associated with the disease of the genital organs. *Trypanosoma brucei* is known to invade many tissues of the body, including the genital organs (Mutayoba *et al*., 1988; Sekoni, 1994) and in the present study might have caused an extension of the gestation period. Although the contribution of parasite-induced alteration in reproductive hormones secretion in the pathogenesis of prolonged gestation have been noted (Ikede *et al*., 1988; Mutayoba *et al*., 1988), it is possible the extension of gestation period in the present study is the result of placental insufficiency. Anemia is the most important clinicopathologic feature of animal trypanosomosis (see Table 5), and it has been reported that relatively short degree of underperfusion with hypoxia will lead to stasis and intervillous fibrin deposition (Redline, 1999) as was observed in the placentae sections of pregnant rats that died of trypanosomosis 23 days post-infection in this study (Fig. 5). The placentae affected by maternal underperfusion will generally show degeneration and necrosis of villi shell trophoblastic cells which probably explains the decreased weight of placenta for the gestational age (Williams and O'Brien, 2000); a condition that will retard the development of the fetus (De Wolf *et al*., 1980) and consequently prolong gestation (Katsulov *et al*., 1982; LeBlanc, 2001). The similarity between the gestation length of animals infected from days 10-21 of pregnancy and the uninfected pregnant control group could be explained by the fact that in the former group pregnancy had fully established and was functionally able to sustain the development of the fetus, at a time when the negative effect of infection on pregnancy was at its minimum.

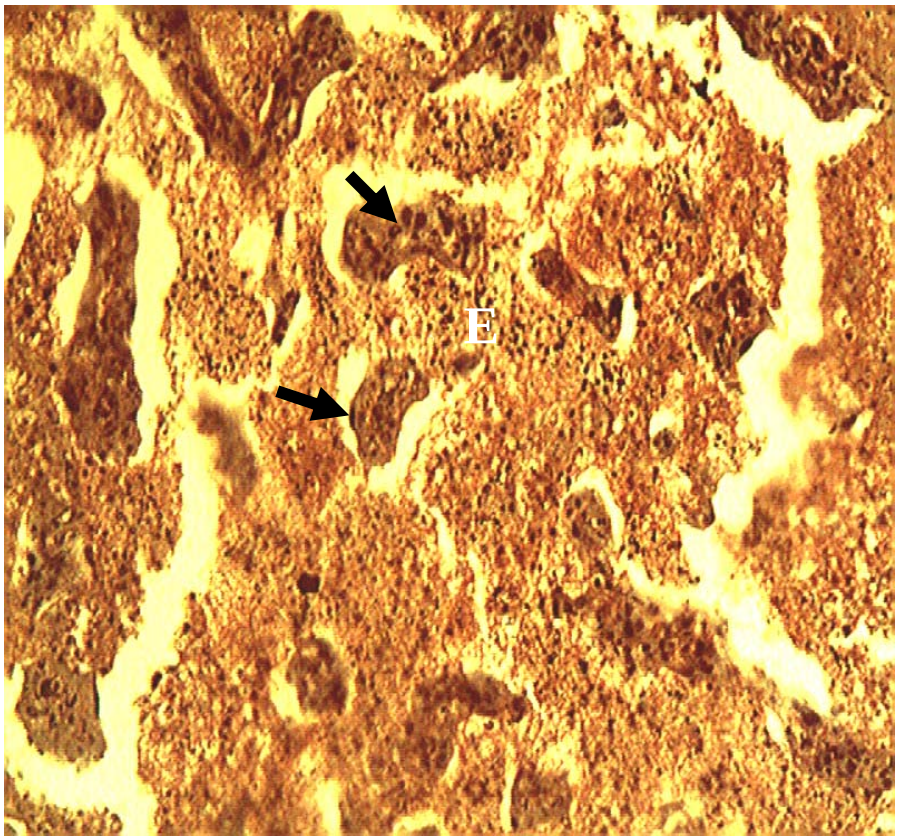

Figure 4. Chorionic villi of placenta 23 days post-infection showing normal trophoblast cells (arrows) around foetal blood vessel; and fibrinous exudates containing mononuclear leucocytes (E). H & E stain X200.

The group of rats infected between days 1-9 of pregnancy had longer survival time ($P < 0.05$) than the infected non-pregnant control group (Fig. 2). This is, however, contrary to the general belief that under pregnancy state, disease conditions are more devastating to animals. The reason for the increase in survival time among the infected pregnant rats is unknown but may be related to the ability of this group of animals to initiate a down-regulation of the interferon-gamma (IFN-γ) cytokines (which stimulates parasitic growth) and up-regulation of interleukin-4 (IL-4) which is important in the control of parasitemia through the promotion of antibody production (Bakhiet *et al*., 1996; Mertens *et al*., 1999). This, by extension, enhanced the survival time of this group of rats. There is evidence that the reproductive hormones, precisely progesterone (which is high during pregnancy), estrogen and chorionic gonadotropins, either individually or in combination, favor IL-4 production by T lymphocytes (Kuklina and Shirshev, 2004). Although the present experiment was not extended to include measurement of plasma levels of these cytokines, the significant reduction ($P < 0.05$) in the level of parasitemia among the long surviving pregnant animals, compared with the infected non-pregnant control group (Table 4) attests to this relationship between level of parasitemia and survival time.

The infection resulted in significant ($P < 0.05$) increase in partial fetal resorption in the groups infected during the first trimester of pregnancy (between days 1-6). Total fetal resorption occurred only in 1 out of 15 animals infected on days 1-3 of pregnancy, suggesting that total fetal resorption is not a common feature of *T. brucei* infection. Unlike the reports of Beck (1967) and Barr *et al.* (1970) who stated that the greatest number of resorptions occurred in the cervical ends of the rat uterine horns, the present study showed that fetal resorption caused by *T. brucei* infection occurred at random in the uterine horns without predilection for any anatomical location.

The results of this study showed that although infection with *T. brucei* neither caused abortion nor interfered with conception, the reproductive indices like live-birth index, post-implantation survival index and gestation index were significantly ($P < 0.05$) reduced especially when the animals were infected in the early stages of pregnancy (Table 3). Even though it was only the group infected on days 1-3 of pregnancy that had a significantly ($P < 0.05$) reduced post-implantation survival index, virtually all the groups infected on the first half of gestation (days 1-9) had a significantly ($P < 0.05$) reduced live-birth index. These coincided with the groups that

had prolonged gestation and placentae lesions.

It is not clear why there was no significant change in the litter size at birth between the control and rats infected between days 1-6 of gestation (Table 1) despite significant fetal resorption in the latter group. However, the prominent reduction ($P < 0.05$) in the litter size and litter weight observed at days 7, 14, and 21 postpartum in all the infected animals may be the direct consequence of malnutrition arising from inadequate milk production in the sick mother rats. It could also be attributed to imperfect match between the pups and their foster mothers. The present study therefore shows that although animals infected with *T. brucei* may conceive and deliver, the post-natal survival and productivity of their offspring are seriously compromised.

Finally, despite the occurrence of placentitis in the infected animals, there was no evidence of trans-placental infection. This finding agrees well with the report of Gillet and Herman (1976) in mice and Maudlin *et al*. (2003) in cattle. However, sporadic cases of trans-placental infection of trypanosomes have been reported in humans (Olowe, 1975; Rocha *et al*., 2004), sheep (Ikede and Losos, 1972) and mice (Ijagbone and Agbede, 2000). It has been suggested that trans-placental transmission, even though a rare phenomenon, is dependent on the strain of trypanosome involved (Andrade, 1982). It could also be associated with such factors as intercurrent infection and specie of animal.

In conclusion, trypanosomosis caused by *T. brucei* has a very serious negative implication on female reproduction, affecting all the indices of reproduction and the general productivity of infected animals.

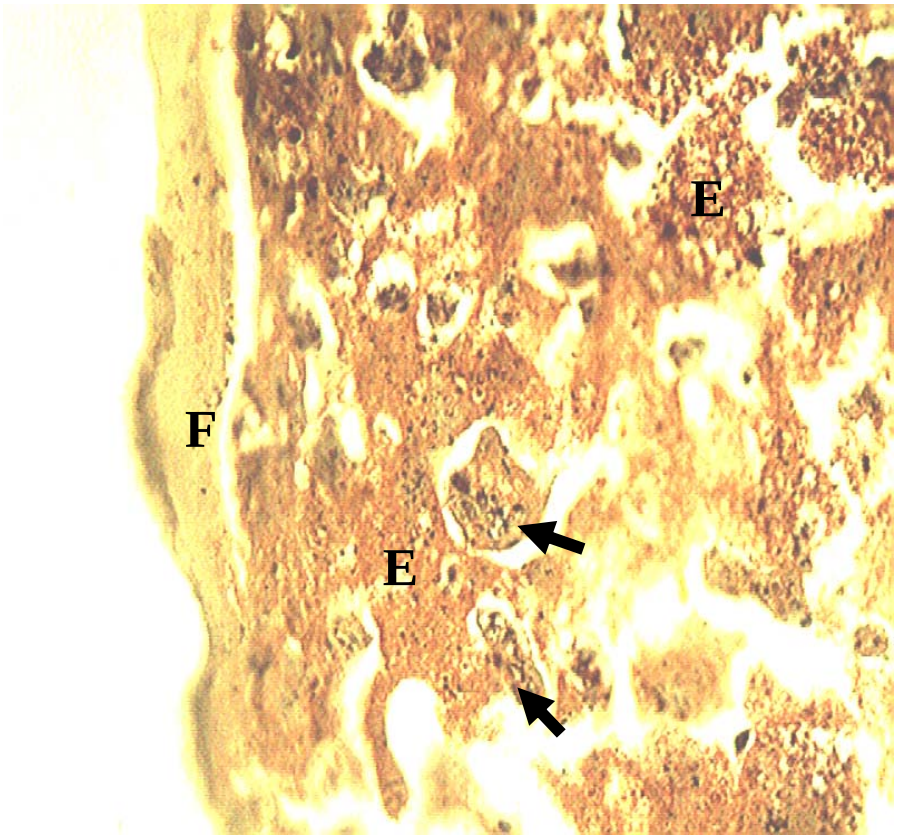

Figure 5. Placenta of pregnant rat, 23 days post-infection showing necrosis of chorionic villi surrounded by fibrin (arrows); intervilli exudate containing mononuclear phagocytes (E) and fibrinous exudation into the supraglandular layer (F). H & E stain X200.

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