

The effects of *Trypanosoma brucei* infection on post-partum uterine involution

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ABSTRACT

This study investigated the effects of *Trypanosoma brucei* infection on post-partum (PP) uterine involution in the albino rat (*Rattus norvegicus*). Ninety-six rats (80 females and 16 males) were used for the study. The females were divided into 2 equal groups of 40 infected and 40 uninfected. The males which were uninfected were used for mating with the females. From each of the 2 female groups, 5 were humanely sacrificed daily from day 0 to day 7 PP. Body mass (BM), uterine mass (UM), uterine mass as a percentage of body mass (UMPBM), uterine histomorphology, packed cell volume (PCV), level of parasitaemia (LOP) and rectal temperature (RT) were evaluated in the females. Results showed that both the UM and the UMPBM of the infected rats were significantly higher ($P < 0.01$) than those of the uninfected between days 1 and 7 PP. Uterine histomorphology showed that between days 3 and 7 PP, involution was more advanced in the uninfected group. Uterine sections of the infected rats had more glands, which were also larger in size. Endometrial stroma was less cellular in the uninfected rats and the myometrium showed higher nuclei density for myofibrils, which suggests some loss of cytoplasm. The PCV of the infected rats was significantly lower than that of the uninfected ($P < 0.01$) between days 9 and 14 post-infection (PI), while the RT of the infected rats was significantly higher ($P < 0.01$) than that of the uninfected between days 5 and 14 PI. It was therefore concluded that *T. brucei* infection led to a significant delay in PP uterine involution, as evidenced by the higher UM and UMPBM, and the uterine histomorphological findings in the infected rats.

Key words: *Trypanosoma brucei* infection, albino rat, post-partum uterine involution

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Introduction

Post-partum (PP) involution of the uterus in mammals is necessary for the preparation of the uterus for the establishment of the next pregnancy (OGAWA et al., 2001). Invariably, any condition that causes either delayed uterine involution or non-involution of the post-partum uterus will lead to some degree of infertility. Several studies had been carried out on the morphological, histological and chemical changes associated with normal uterine involution in animals, such as cattle (GIER and MARION, 1968; OKANO et al., 1981; OKANO and TOMIZUKA, 1987), pigs (PALMER et al., 1965; OGAWA et al., 2001) and rats (HARKNESS and MORALEE, 1956; WRAY, 1982; WOESSNER and TAPLIN, 1988). However, there are no reports in all the available literature on the possible effects of certain infectious diseases associated with reproductive disorders and infertility on PP uterine involution.

African trypanosomosis is an economically important disease of animals and humans in tsetse-infected areas of Africa (SHAW, 2004). Trypanosome infections had been associated with various pathologic conditions, including irregular fever, anaemia, emaciation or weight loss, impairment of immune function, reproductive disorders, infertility and death if the affected animals and individuals are not treated (HORST, 1996; TAYLOR and AUTHIE, 2004). Several reports have associated trypanosome infections specifically with such reproductive disorders as alterations in the release of reproductive hormones, irregular oestrous cycle, anoestrus, abortion, stillbirths and neonatal deaths (IKEDE et al., 1988; SEKONI, 1994). There has been no report in the available literature on the effects of trypanosome infection on the rate of PP uterine involution of infected female animals. This present study was therefore designed to investigate the effects of *Trypanosoma brucei* infection on PP uterine involution, using the albino rat (*Rattus norvegicus*) as a model.

Materials and methods

Animals. Ninety-six (96) mature outbred Sprague-Dawley albino rats (*Rattus norvegicus*), comprising 80 females and 16 males, were used for the study. Just before commencing the study, all the animals weighed between 140 and 150g, and were aged between 14 and 16 weeks. Throughout the duration of the study, they were housed at room temperature of 28-32 °C in the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, in stainless steel cages. They were fed *ad libitum* with commercial feed (Top Feeds Nigeria Ltd, Sapele). Clean water was also provided freely. The experimental protocol followed the University of Nigeria, Nsukka guidelines for the humane use of laboratory animals for experiments.

Trypanosoma brucei. The trypanosome used for the study was the “Federe strain” of *Trypanosoma brucei brucei*, obtained from the National Institute for Trypanosomosis Research (NITR), Kaduna, Nigeria. The inoculum for each rat was 2.50×10^5 trypanosomes

administered intraperitoneally as 0.1 mL suspension in normal saline. Confirmation of establishment of infection was done starting from day 1 post-infection (PI) using the haematocrit method as described by MURRAY et al. (1977).

Experimental design. Out of the 96 rats, 16 were uninfected males used for mating. The remaining 80, which were females, were randomly divided into two equal groups of 40 each - one group was infected while the other was the uninfected control. The 40 females in the infected group were infected at day 14 of pregnancy. From each of the two groups, 5 rats were humanely sacrificed daily from day 0 PP until day 7 PP, in order to determine the uterine mass (UM) and study the uterine histomorphology. Other parameters determined included body mass (BM), uterine mass as a percentage of body mass (UMPBM), level of parasitaemia (LOP), packed cell volume (PCV) and rectal temperature (RT).

Confirmation of mating and pregnancy. The vaginal plug method (VOSS, 1979), as modified by OCHIUGU et al. (2006), was used in determining successful mating. Briefly, five females were placed in a cage with a male of proven fertility. A vaginal smear of each female was taken on a labeled clean glass slide with the aid of a 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 the copulatory plug) as evidence of successful mating. This procedure was carried out at 12 hour intervals. The day remnants of the plug were found was regarded as day 1 of pregnancy. Thereafter, the females were separated from the males. Changes in body weight were used in monitoring the progress of the pregnancy.

Determination of body mass (BM), Uterine mass (UM), Uterine mass as a percentage of body mass (UMPBM), Packed cell volume (PCV), Level of parasitaemia (LOP) and Rectal temperature (RT). The BM and UM of the rats were determined using an Ohaus weighing balance and a digital electronic balance respectively. The UMPBM was determined by dividing the UM by the BM of respective rats and then multiplying by 100. The rectal temperature was measured using a digital clinical thermometer. The PCV was determined by the microhaematocrit method (COLES, 1986), while the establishment of infection and level of parasitaemia were assessed from day 1 PI using the rapid matching method (HERBERT and LUMSDEN, 1976).

Uterine histomorphology. The method described by DRURY and WALLINGTON (1979) was used for preparation of the uterine tissue for histomorphological examination. Sections of the uteri collected from infected and uninfected rats were fixed in 10% buffered formal saline. Tissues were processed as routinely done with paraffin wax and cut to 5 micron thickness. All sections were stained with hematoxylin and eosin (HE) stain, while selected sections were stained by Price's Giemsa method (LUNA, 1968).

Statistical analyses. Data generated from the study were subjected to the Student's *t*-test. Significance was accepted at $P < 0.01$. The results were presented as means with standard error.

Results

Body mass (BM). The BM of both the infected and uninfected rats increased progressively as pregnancy advanced, and there were no significant differences ($P > 0.05$) between the BW of the infected and uninfected pregnant rats on days 0, 7, 14 and 19 of pregnancy. Also, there were no significant differences ($P > 0.05$) between the post-partum (PP) BW of both the infected and uninfected rats starting from day 0 to day 7 PP.

Uterine mass (UM). The mean UM on day 0 PP was 2.68 ± 0.13 g and 2.40 ± 0.02 g for the infected and uninfected groups respectively, and these mean UM were not found to be significantly different ($P > 0.05$) from each other (Fig. 1). However, from day 1 PP onwards, the mean UM decreased progressively, but the mean UM of the infected rats was significantly ($P < 0.01$) higher than that of the uninfected rats from day 1 until day 7 PP, when the mean UMs for the infected and uninfected rats were 0.60 ± 0.03 g and 0.25 ± 0.02 g respectively (Fig. 1).

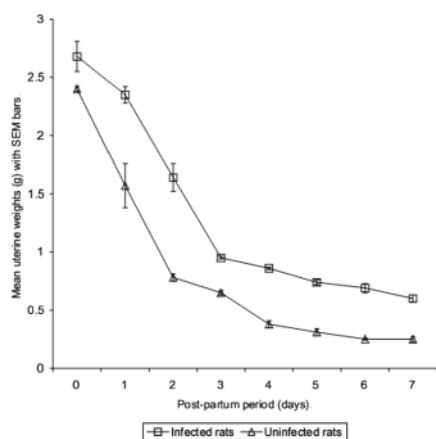


Fig. 1. The post-partum uterine mass of the rat group infected with *T. brucei* when compared with the uninfected rat group

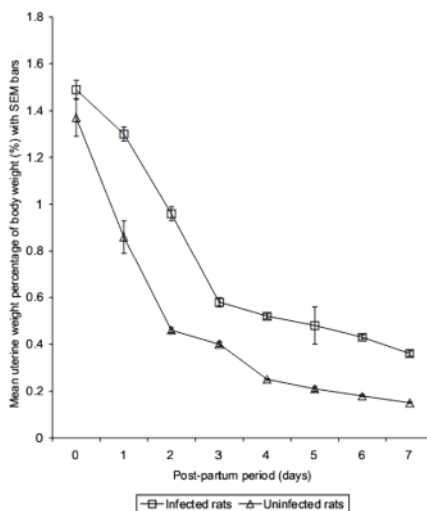


Fig. 2. The post-partum uterine mass percentage of body mass of the rat group infected with *T. brucei* when compared with the uninfected rat group

Uterine mass as a percentage of body mass (UMPBM). The mean UMPBMs of the infected and uninfected rats on day 0 PP were $1.49 \pm 0.04\%$ and $1.37 \pm 0.08\%$ respectively, and there was no significant difference ($P > 0.05$) between these two means (Fig. 2). But from day 1 PP onwards until day 7 PP, the UMPBMs of the infected rats were significantly higher ($P < 0.01$) than those of the uninfected rats, though both decreased progressively (Fig. 2). The mean UMPBMs of the infected and uninfected rats at day 7 PP were $0.36 \pm 0.01\%$ and $0.15 \pm 0.004\%$ respectively (Fig. 2).

Uterine histomorphology. Uterine sections collected from the uninfected rats on day 0 PP showed severely hyperaemic endometrial stroma containing a large number of inflammatory cells in loose connective tissue. The surface epithelium was of tall, pseudostratified, columnar epithelial cells. The scanty inner (cellular) layer of the stroma contained large numbers of polymorphonuclear (PMN) and mononuclear (MN) leukocytes, mast cells and blood cells. The less cellular, outer (basal) layer of the endometrium was oedematous with fibrinous/collagenous deposits (Fig. 3). The uterine glands appeared cystic and contained many dead cells *in situ*. The mode of cell death was apoptotic. There was moderate degeneration of myofibres in the myometrium. Corresponding uterine sections (day 0 PP) from the infected rats were of similar mucosal epithelial cells. The cellular stroma was equally scanty, but the stroma generally had more inflammatory cells than found in the uninfected rats. Fibrinous/collagenous deposits also appeared more numerous. Uterine glands were more cystic, but with less glandular cell death (Fig. 4). Exudation into and degeneration of myometrium fibres also appeared to be more severe than in the uninfected rats. By day 1 PP, the endometrial stroma of the uninfected rats had more inflammatory cells and collagen than on day 0 PP. Hyperaemia was still a feature and erythrophagocytosis was taking place. Glandular epithelial cells showed signs of degeneration and apoptosis. The myometrium was oedematous and myofibre degeneration was more severe than on day 0 PP. For the infected rat group (day 1 PP), the stroma was generally severely hyperaemic. Erythrophagocytosis was active and the exudate was predominantly fibrinous/collagenous. The myometrium was also haemorrhaging and had inflammatory exudates, while myofibre degeneration/cell death was more severe in the infected than the uninfected group. By day 2 PP, sections from the uninfected rats showed that the mucosa epithelium had become simple columnar with a decrease in thickness, while the entire stroma remained intensively infiltrated by PMN and MN leukocytes. The degeneration/death of the glandular epithelial cells was on the increase, just as the glands appeared crowded by fibroblast proliferation around them. Inflammatory exudation into the myometrium was moderate. As for the infected rats (day 2 PP), the mucosa epithelium was still tall and pseudostratified, while the stroma remained hyperaemic, with moderate to severe inflammatory cell infiltrate, but with large amounts of collagen. Fibroblast proliferation was mild, and the uterine glands were still distended but looked normal. By day 5 PP, the uninfected rats had an endometrium in which the

mucosa surface was becoming villous (outpouching), the entire stroma was intensively cellular and the uterine glands were almost absent in the endometrium (Fig. 5).

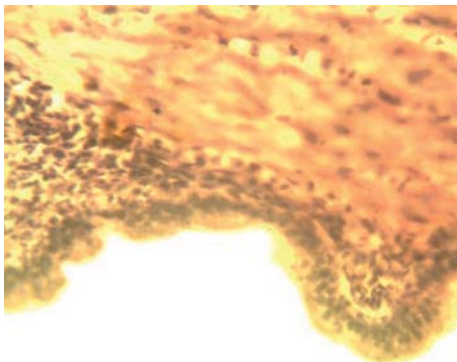


Fig. 3. Uterine section of an uninfected rat on day 0 of parturition showing tall pseudostratified columnar epithelium on top of scanty loose connective tissue stroma. Note the empty intercellular spaces in the basal layer of endometrium; H&E, $\times 400$.

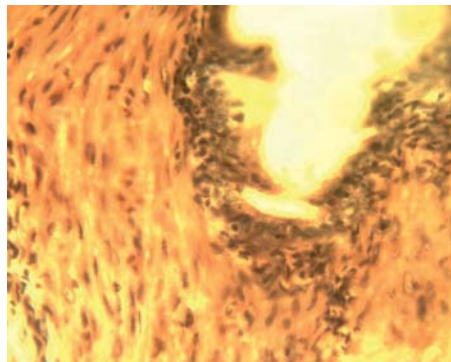


Fig. 4. Uterine section of *T. brucei* infected rat on day 0 of parturition showing pseudostratified columnar epithelium on top of scanty loose connective tissue. Note the heavy collagen deposition in the basal layer of endometrium; H&E, $\times 400$.

The myometrium was markedly atrophied. In the corresponding sections from the infected rats (day 5 PP), the stroma was equally cellular, fibroblast proliferation was still moderate, and large uterine glands with apoptotic epithelial cells were still many and conspicuous (Fig. 6). The endometrium of the uninfected rats showed a mucosa epithelium by day 7 PP that was simple columnar and of average height. The stroma was severely cellular and the uterine glands were almost absent from the inner cellular layer. The myometrium had increased beyond its size at day 5 PP, with moderate hypertrophy of the muscle fibres. As for the infected group of rats (day 7 PP), cellular infiltration into the basal stroma layer was mild; fibroplasia was still moderate and apoptosis of the uterine gland epithelial cells was on-going in the basal endometrial layer. The myofibres of the myometrium also demonstrated hypertrophy, as observed in the uninfected group.

Level of parasitaemia (LOP). Trypanosomes were first detected in the blood of the infected rats on day 5 PI, and from then on the LOP increased progressively from a mean of $0.89 \pm 0.36 \times 10^6$ /mL of blood recorded on day 5 PI until the end of the study (day 14 PI), when it was $4.29 \pm 87.60 \times 10^8$ /mL of blood (Fig. 7).

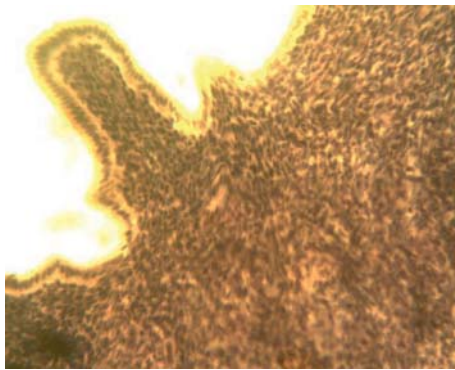


Fig. 5. Uterine section of an uninfected rat on day 5 post-partum showing villous nature of mucosa and highly cellular loose connective tissue stroma; note the absence of uterine glands; H&E, $\times 400$.

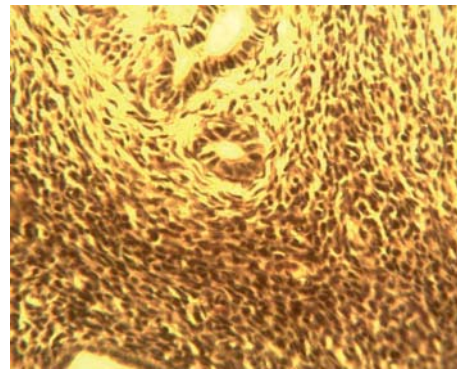


Fig. 6. Uterine section of *T. brucei* infected rat on day 5 post-partum showing low columnar epithelial lining cells of the endometrium and highly cellular stroma; note the fibrous connective tissue around large endometrial glands; H&E, $\times 400$.

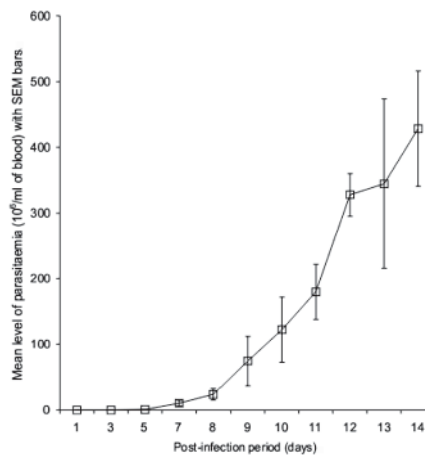


Fig. 7. The level of parasitaemia in pregnant rats infected with *T. brucei* (the rats were inoculated with *T. brucei* on day 14 of pregnancy)

Packed cell volume (PCV). The mean PCV of both groups decreased gradually from their initial values of $45.15 \pm 0.42\%$ and $45.52 \pm 0.45\%$ for the infected and uninfected groups respectively, as pregnancy advanced up to the day of parturition. However, there

were no significant differences ($P>0.05$) between the mean values for the infected and uninfected groups until day 23 of the experimental period (equivalent to day 2 PP or day 9 PI for the infected group) (Fig. 8). The PCV of the infected rats was found to be significantly lower ($P<0.01$) than that of the uninfected group from day 23 of the experimental period until day 28, when the study ended (Fig. 8). From day 24 of the experimental period (equivalent to day 10 PI for the infected group), while the PCV of the infected rats decreased progressively further to its lowest value of $31.00 \pm 1.87\%$ recorded for day 28 of the experimental period (day 14 PI), that of the uninfected group increased to a maximum of $45.80 \pm 1.69\%$ (almost the same as the pre-experimental value) on day 28 of the experimental period (Fig. 8).

Rectal temperature (RT). The mean baseline RT value of the rats in both groups was 37.25 ± 0.13 °C and 37.27 ± 0.12 °C respectively for the infected and uninfected groups (Fig. 9). There were no significant ($P>0.05$) differences between the mean RT value of the two groups until day 19 of the experiment (equivalent to day 5 PI for the infected group) from when the mean RT of the infected rats was found to be significantly higher ($P<0.01$) than that of the uninfected rats, until day 28 of the study, when the RT of the infected and uninfected groups stood at 40.18 ± 0.55 °C and 37.24 ± 0.28 °C respectively (Fig. 9).

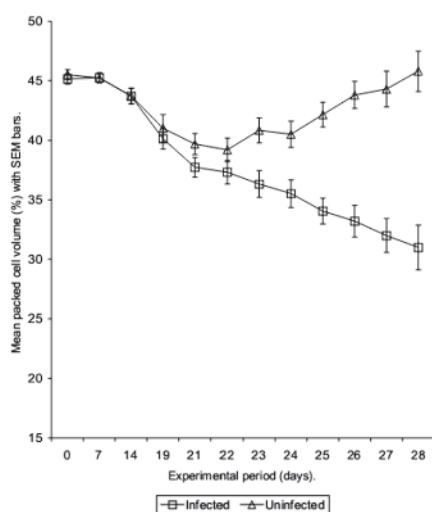


Fig. 8. The packed cell volume (PCV) of the rat group infected with *T. brucei* when compared with the uninfected rat group (during pregnancy and after delivery)

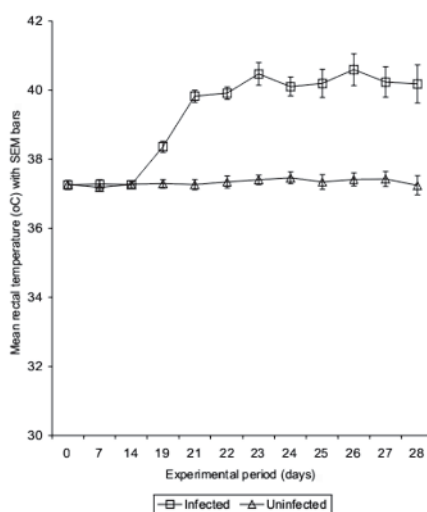


Fig. 9. The rectal temperature of the rat group infected with *T. brucei* when compared with the uninfected rat group (during pregnancy and after delivery)

Discussion

The sharp increase in the mean BM of the mated infected and uninfected female groups was an indication of progressive pregnancy (OCHIUGU et al., 2006). Although emaciation and weight loss are usually associated with chronic cases of *Trypanosoma brucei* infection in some domestic animals (IKEDE, 1983), weight loss was not observed in the infected group, which might be attributable to the acute/subacute nature of the *T. brucei* infection in rats (IKEDE, 1983; NYINDO, 1992).

Rising trypanosome parasitaemia in *T. brucei* infections is usually associated with parasite extravasation, localization and multiplication in tissues with consequent inflammatory reactions in tissues (NYINDO, 1992). The resultant inflammatory exudation into the uterine wall could have contributed remarkably to the significantly higher mean UM and UMPBM values of the infected group, as compared to the uninfected group throughout the PP period of the study (from day 1 to day 7 PP). This is in agreement with a previous report that inflammatory reactions delay uterine involution (SLAMA et al., 1999). Uterine sections of the infected rats demonstrated more collagen deposits than the corresponding sections from the uninfected rats throughout the PP period of study. This observation, which has also been reported from previous studies, was attributed to delayed collagen breakdown in the uterus of the infected rats (HARKNESS and MORALEE, 1956; WRAY, 1982; IKEDE et al., 1988). According to HARKNESS and MORALEE (1956), collagen breakdown in the PP uterus of normal rats is about 85% complete 4 days PP, which is in agreement with the findings in the uninfected group, where the percentage decrease in uterine mass between day 0 and day 4 PP was 84.2% (mean uterine mass on day 0 was 2.40 ± 0.02 g, while on day 4 PP it was 0.38 ± 0.03 g). This is sharply in contrast with what was found in the infected group, where the percentage decrease between days 0 and 4 PP was 67.9% (mean uterine mass on day 0 was 2.68 ± 0.13 g and on day 4 PP, it was 0.86 ± 0.01 g). This finding also corroborates the reports of TAKAMOTO et al. (1998) that uterine mass and collagen decrease rapidly during days 1 to 3 PP. Surely early resolution of the inflammatory uterine environment in the early PP period, and the rapid breakdown of the resultant collagen deposition would naturally influence uterine involution to a great extent.

The fact that endometrial gland density decreased at a faster rate in the uterine sections of the uninfected group, as compared with the infected rats throughout the period of PP study, will tend to suggest that this might have contributed to the significant differences in the mean UM and UMPBM of the infected and uninfected groups. Although the contribution of glands might have been secondary to that of collagen breakdown, a similar observation has been reported for the PP uterine involution in sheep (GRAY et al., 2003). Generally, the significant differences in the mean UM and UMPBM of the uninfected groups in the study must have been due to a concert of factors, the most important of which must have been PP collagen breakdown in the uterus.

Results of the LOP, which showed a consistent increase from day 5 PI onwards to day 14 PI, is characteristic of *T. brucei* infection in rats and is in agreement with earlier reports of EGBE-NWIYI et al. (2003) and IHEDIOHA et al. (2007).

The gradual decrease in the PCV of both groups as pregnancy advanced and also during the peri-parturient period, when the infection was not yet fully established in the infected rats, could be attributed to the stress associated with late pregnancy and the loss of blood during parturition, which is usually associated with the haemo-endothelial type of placenta found in rats (SWENSON, 1984; COLES, 1986; McDONALD, 1989; ARTHUR et al., 1996). After parturition however, the further significant decrease in the PCV of the infected group could only be a consequence of anaemia induced by the *T. brucei* infection. Anaemia is the most important clinico-pathologic finding in *T. brucei* infection, and it occurs in association with high levels of trypanosomes in the blood (NYINDO, 1992; HORST, 1996; TAYLOR and AUTHIE, 2004). In comparison, the PCV of the uninfected group gradually increased from its reduced level during the peri-parturient period to almost the pre-pregnancy level at the end of the study, which is a typical occurrence in normal animals, when the stress of pregnancy and parturition is over (SWENSON, 1984; COLES, 1986).

The significantly higher RT recorded in the infected group when compared with the uninfected group, from day 5 PI until the end of the study, was indicative of pyrexia induced by the *T. brucei* infection. Pyrexia is a general clinical finding in cases of trypanosomosis and usually correlates with the presence of high levels of trypanosomes in blood (IKEDE, 1983; NYINDO, 1992; HORST, 1996; TAYLOR and AUTHIE, 2004).

Based on the results of the study, it was concluded that *T. brucei* infection in the rats led to a significant delay in uterine involution, as evidenced by the significantly higher UM and UMPBM and the uterine histomorphological findings in the infected rats.

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SAŽETAK

Istraživanje je provedeno s ciljem određivanja učinka protozoona *Trypanosoma brucei* na postpartalnu involuciju maternice u albino štakora (*Rattus norvegicus*). Za istraživanje je rabljeno 96 štakora (80 ženki i 16 mužjaka). Ženke su bile podijeljene u dvije jednake skupine tako da je 40 ženki pripadalo kontrolnoj, a 40 invadiranoj skupini. Neinvadirani mužjaci su poslužili za parenje. Iz svake skupine bilo je eutanazirano pet ženki i to svaki dan (od nultog do sedmog dana). Za svaku ženku bila je određena tjelesna masa, masa maternice, odnos mase maternice i tjelesne mase, histološki nalaz maternice, ukupan broj krvnih stanica, razina parazitemije i rektalna temperatura. Rezultati su pokazali da je u invadiranih štakorica maternica bila značajno teža kao što je bila i značajno veća masa maternice u odnosu na tjelesnu masu ženke ($P < 0,01$) u razdoblju od prvog do sedmog dana nakon partusa. Histološkom pretragom dokazano je da je involucija maternice u razdoblju od trećeg do sedmog dana bila izraženija u neinvadiranih ženki što je dokazano i većim brojem povećanih žlijezdi. Stroma endometrija neinvadiranih životinja sadržavala je manje stanica. U miometriju je dokazana veća gustoća jezgara u miofibrilima što govori u prilog gubitku citoplazme. Ukupan broj krvnih stanica u invadiranih štakorica bio je značajno manji u odnosu na neinvadirane ($P < 0,01$) u razdoblju od devetog do četrnaestog dana nakon invazije. Invadirane štakorice imali su višu tjelesnu temperaturu ($P < 0,01$) u razdoblju od petoga do četrnaestoga dana. Na temelju postignutih rezultata zaključuje se da invazija vrstom *Trypanosoma brucei* dovodi do značajnog kašnjenja involucije što je potvrđeno većom masom maternice, većom masom u odnosu na tjelesnu masu te histomorfološkim nalazom u invadiranih ženki.

Ključne riječi: *Trypanosoma brucei*, invazija, albino štakor, involucija maternice
