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Semen Collection Reaction Time and Sperm Morphological Abnormalities in Yankasa Rams Experimentally Infected with *Trypanosoma brucei brucei* and *Trypanosoma evansi*

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Abstract: To evaluate the pathological effects of Trypanosomosis on the semen collection, reaction time and spermatozoa morphology of Yankasa rams. Sixteen Yankasa rams aged between 24 to 30 months and weighed between 22-25kg were acclimatized for a period of two months in a clean fly proof house and were adequately fed and given water *ad libitum*. Of the sixteen rams, twelve that were clinically fit for the experiment at the end of the acclimatization period were randomly divided into four groups, I, II, III and IV, each having three rams. Groups I and II were each challenged singly with experimental *T. brucei brucei* (Federe strain) and *T. evansi* (Sokoto strain) respectively, while group III was challenged with mixed *T. brucei brucei* and *T. evansi* parasites (50% of each species in the infective inoculum) and group IV was left as uninfected control. Each infected ram received 2ml of the infected blood containing $2x10^6$ trypomastigotes via the jugular vein. All the infected rams developed clinical signs typical of trypanosomosis at varying pre-patent periods. There was observed scrotal oedema, scrotal degeneration, loss of libido, increased semen collection reaction time and significant increase of spermatozoa morphological abnormalities in the infected rams. The rams especially in groups I and III were all deemed unfit for breeding by the end of the 98 days post infection, while the uninfected rams were healthy and had normal values of sperm morphology throughout the study period. The study concluded that trypanosomosis due to experimental *T. b. brucei* or *T. evansi* or mixed infections (of both parasites) is capable of rendering Yankasa rams infertile due to prolong semen collection reaction time and significant deterioration in sperm morphology.

Key words: Semen · Collection reaction time · Sperm · Morphological abnormalities · Yankasa ram · *Trypanosoma brucei brucei* · *Trypanosoma evansi*

INTRODUCTION

Animal trypanosomosis is one of the most severe constraints to agricultural development in Sub-Sahara Africa and is also an important disease of livestock in Latin America and Asia [1]. It is transmitted by the blood parasites of the genus *Trypanosoma* and can be transmitted cyclically, mechanically and by contact during coitus [2]. The major pathogenic trypanosome species in livestock are transmitted by the tsetse fly (genus *Glossina*) and include; *Trypanosoma congolense, T. vivax, T. brucei brucei* and *T. evansi* [3]. Nagana and related diseases also caused by *T. congolense, T.vivax* and *T.brucei brucei* in cattle have interdicted much of sub-Sahara Africa especially Nigeria. Surra, another disease caused by *T. evansi* is a problem wherever camels are or have been. The severity of the infection is influenced by a number of factors; virulence of the

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different species of trypanosomes, age, nutritional status and the breed are important [4]. Clinically, the effects of trypanosomosis on these animals ranges from anaemia, immunosuppression, depression with inability to rise, pyrexia directly associated with parasitaemia, paleness of mucous membrane, rapid pulse beat, retarded growth, roughness of hair coats, enlargement of peripheral lymph nodes, low milk production, low meat quality, weight loss and reproductive disorders, including degeneration of the hypothalamus, pituitary glands and gonads with consequent disruptions in the secretions and plasma concentrations of the hormones necessary for normal reproductive processes in both sexes [5-8]. In female animals, the effect include; severe genital lesions, temporary or permanent anestrus and abnormal estrous cycles. Additionally, trypanosomal-induced death during pregnancy, abnormal pregnancy, dystocia, abortion, premature birth, low birth weight, stillbirth, transplacental fetal infection, neonatal death and other pathogenic effects on fetuses and offspring have been reported [5, 8]. In the males, the effects include delayed puberty, loss of libido, severe degenerative changes of the genitalia, manifested by the production of very poor quality semen and loss of epididymal and gonadal sperm reserve [3, 5, 9]. Testicular atrophy has also been reported with the presence of dead spermatozoa in the seminiferous tubules of goats [5, 6, 9]. Infection in rabbits with *T. brucei brucei* causes increased scrotal circumference, scrotal inflammation, severe testicular degeneration, abnormal spermatogenesis, aspermatogenesis and incomplete resolution of the genital organs [10]. In *T. vivax* or *T. congolense* infected Yankasa rams, the effects on reproduction ranges from scrotal oedema, poor semen quality or the cessation of semen production, degeneration of the testes, loss of libido as well as infertility [3, 11]. Information on the effect of *T. brucei brucei*, *T. evansi* and mixed infections on the semen collection reaction time and sperm morphology in Yankasa rams is scanty and basically unavailable. Rams are grazed alongside cattle or camel from northern Nigeria towards the southern tse-tse endemic vegetation belts by cattle herdsmen in search of greener pasture thereby exposing them to the risk of trypanosomosis. There is therefore an urgent need to investigate factors that may negatively affect the success of sheep breeding such as reproductive problems and diseases such as sperm abnormalities, lowered fertility, infertility or sterility.

MATERIALS AND METHODS

Experimental Animals: Sixteen apparently healthy and intact Yankasa rams aged between 24 and 30 months and weighed between 22 to 25 kg were purchased from Sheme market, an apparently tsetse free zone, in Katsina State of the Nigerian Sudan-Guinea Savannah. The ages of the animals obtained from the market were confirmed by the presence of temporary and permanent incisors as described by Wilson and Durkin [12] that is, by the eruption of permanent incisors at permanent corners of the cheek.

Housing and Screening of Experimental Animals: The purchased animals were housed in an insect-proof animal pen at the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where they were screened for the presence of ectoparasites, endoparasites and haemoparasites. The rams were thereafter treated with Oxytetracycline (Tridax®) intramuscularly, at a dose of 20 mg/kg body weight and Albendazole (Sambezole®, Sam Pharmaceutical Ltd; Animal Care, Nig. Ltd) orally, at a dose of 7.5 mg/kg body weight. The rams were sprayed against ectoparasites with Diazinon (Diaznol®, Animal Care, Nig. Ltd.), at concentration of 2 mL/litre of water.

They were acclimatized for eight weeks and neck-tagged for the purpose of identification.

Acclimatization and Physical Examination of Animals: The rams were fed with wheat offal, ground-nut and cowpea hays, fresh grasses (whenever available) and salt licks. Water was supplied *ad libitum*. During the eight weeks acclimatization period, they were subjected to routine handlings, such as physical examination, determination of the body weight, rectal temperature, scrotal circumference, semen collection and collection of blood samples for screening of haemoparasites and determination of baseline semen and haematological indices. Before commencement of the experiment, the rams were ensured to be clinically free of trypanosomes and other haemoparasites in their blood using buffy coat centrifugation technique [13, 14].

Source of Trypanosomes: Trypanosoma evansi was obtained from an infected camel that was slaughtered at the Sokoto abattior, Sokoto state, while *Trypanosoma brucei brucei* was obtained from the Nigerian Institute for

Trypanosomiasis Research (NITR), Kaduna state, Nigeria, originally isolated from a natural infection in cattle. Both parasites were maintained in Wistar rats by serial passage and were transported to the Protozoology Laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for proper identification using the Giemsa-stained thin blood smear diagnostic technique [15].*Trypanosoma evansi* was identified morphologically by the characteristic free and long flagellum with well develop undulating membrane, subterminal kinetoplast and an elongated and centrally placed nucleus while *Trypanosoma brucei brucei* was identified by the presence of short posterior kinetoplast, long and conspicuous undulating membrane with no free flagellum. Both parasites were sub-inoculated intraperitoneally into ten Wistar rats each and were kept in separate cages. The rats were fed with commercial pelleted feed and water supplied *ad libitum*. Prior to inoculation into experimental rams, blood samples were collected daily from each of the rats to determine the level of parasitaemia using the haematocrit centrifugation technique (HCT) as described by Woo [13] and Biryomumaisho *et al.* [14].

Experimental Design: By the end of the eight weeks acclimatization period, four of the sixteen rams that were deemed unfit (due to poor semen characteristics) for the experiment were eliminated. The remaining twelve that were clinically fit (due to good semen characteristics and higher haematological values) for the experiment were randomized into four experimental groups (GI, GII, GIII and GIV) of three rams each, based on their mean packed cell volumes (PCV) and weights. The rams in groups I, II and III were experimentally infected with *T.brucei brucei*, *T. evansi* and mixed inoculum of both parasites, respectively, while those in group IV served as the uninfected control. The experimental protocol and sampling was approved by the ethical committee of Animal Welfare and Integrated Services, Ahmadu Bello University, Zaria.

Inoculation of Experimental Animals: After detection of the trypanosome parasites in the blood of the inoculated rats, they were monitored to their peak value (30 – 40 in buffy coat layer per field). All the infected rats became parasitaemic within 3-14 days post inoculation. The rats were bled using sterilized surgical blades through cardiac (heart) puncture to collect sufficient blood into Bijou bottles, containing 2 mg of Ethylene DiamineTetraacetic Acid (EDTA) for the inoculation of the rams in groups I, II and III. The dosage of *T. evansi* and *T. b. brucei* used for inoculation was estimated using the rapid matching wet-examination technique described by Herbert and Lumsden [16]. A drop of mouse blood was examined under the X40 magnification of a microscope, the number of trypanosomes in each field counted and matched with log figure obtained from a reference table [16].

Group I – each ram was inoculated via the jugular vein with 2 mL of blood containing $2x10^6$ *Trypanosoma brucei brucei.*

Group II – each ram was inoculated via the jugular vein with 2 mL of blood containing $2x10^6$ *Trypanosoma evansi*.

Group III – each ram was inoculated via the jugular vein with 2 mL of blood containing $1x\ 10^6$ *Trypanosoma evansi* and $1x10^6$ *Trypanosoma brucei brucei.*

Group IV - served as the uninfected control, each ram received 2 mL of normal saline.

Observation of Clinical Signs: Clinical signs that were investigated include rise in rectal temperature, weight gain, scrotal diameter, reduced feed intake, loss of body condition, weakness, dullness, roughy hair coat, packed cell volume, haemoglobin concentration, total plasma protein and anaemia.

Semen Collection: Semen collection was done weekly by electrostimulation with the help of an electrical ejaculatory mini tube for small ruminants as described by Morar *et al*. [17] between 9am and 10am.This was to ensure that optimum quality semen was obtained. Each ram was restrained in a standing position and the prepuce thoroughly cleaned using cotton wool soaked in diluted chloroxylenol (0.002%; Dettol^R) to remove dirts and debris. Erection was stimulated by the introduction of the lubricated electroejaculator into the animal rectum. A collection cone with an attached warmed graduated collection tube was then placed over the erected penis. Semen began to flow once the animal has achieved excitation by the stimulatory action of the electroejaculatory device. The impulses consisted in applying the stimulus at an interval of 5 seconds with 5 seconds break [17]. The ejaculates were collected into pre-warmed, sterile and graduated transparent collection tube, labeled and kept in a water bath at a temperature range of

35-37ºC. This was done to prevent temperature changes which may affect the quality of semen, before analysis [18].

Semen Reaction Time: The semen collection reaction time, defined as the time when the electro-ejaculator was first inserted into the rectum of the animal to the time the animal first ejaculates, was determined using a digital stop watch and was recorded in seconds.

Spermatozoa Abnormal Morphology: The percentage live sperm and spermatozoa morphological abnormalities were determined using Eosin-Nigrosin stain technique, applied on a glass slide [19]. The staining mixture consisted of 1% Eosin B and 5% of nigrosin in 3% sodium citrate dehydrate solution. One drop of raw semen was added to one drop of the stain, thereafter it was mixed thoroughly and a fresh smear was made from it. The slide was then examined under a light binocular microscope at X100 magnification. A minimum of 100 cells (both stained and unstained) were counted and the percentage of each estimated. The live-dead staining principle was based upon the observation that Eosin-B penetrated the dead sperms (thereby making them appear pink).While the viable sperm cells repelled the stain and appeared unstained (white).Morphological abnormalities of the spermatozoa that were examined include; Mid Piece Droplets (MPD), incidence of Detached Head (DH), Free Tail (FT), Bent Tail (BT) and incidence of Coiled Tail (CT). These abnormalities were classified and calculated as described by Blom [20] and Sekoni *et al*. [21].

Statistical Analyses: The weekly mean semen reaction time and sperm morphological abnormalities were represented and compared on multiple line graphs using Microsoft Excel Chart Wizard, 2010.

RESULTS

Clinical Observation: The observed clinical signs among the rams in the infected groups I, II and III were similar and include: intermittent pyrexia, pale ocular membrane, reduced and or selective feed intake, reduced body weight gain, roughy hair coat, loss of body condition, scrotal atrophy, poor semen output, loss of libido, drowsiness and death.

Semen Collection Reaction Time (sec): There was a sharp upward climb ($P < 0.01$) in the mean semen reaction time of rams in all the infected groups, I, II and III in comparison to the control rams which remained within the normal range by the end of the experiment (Figure 1). Rams in groups III (135.5±3.24 sec) and I (105.5±2.55 sec) had the highest mean weekly values of semen reaction time at 91 and 98 days post infection respectively, followed by those of group II (85.13±3.316sec) at 49 days post infection. Those of the uninfected control group (IV) maintained the least semen reaction time throughout the study period (Figure 1). Statistical analysis revealed no significant difference ($P > 0.05$) to exist between infected rams in groups I and III but were significantly ($P < 0.01$) different from those of groups II and IV at the end of the experiment respectively (Figure 1).

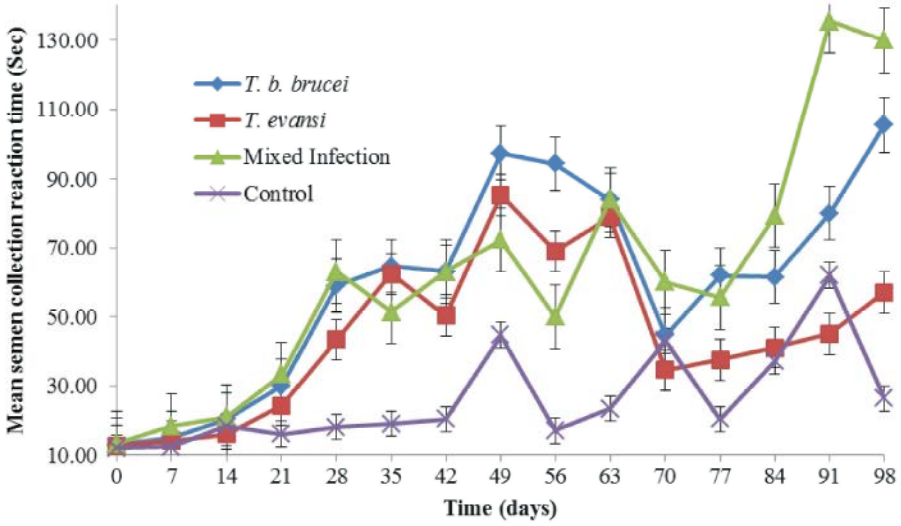

Fig. 1: Mean semen reaction time of Yankasa rams infected with *Trypanosoma* spp

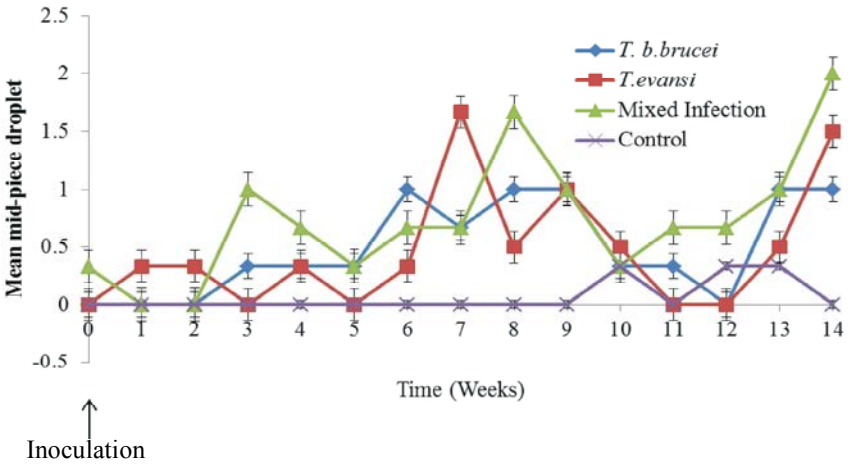

Fig. 2: Mean weekly incidence of cytoplasmic droplets in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T. evansi* (Grp II), or both parasites (Grp III)

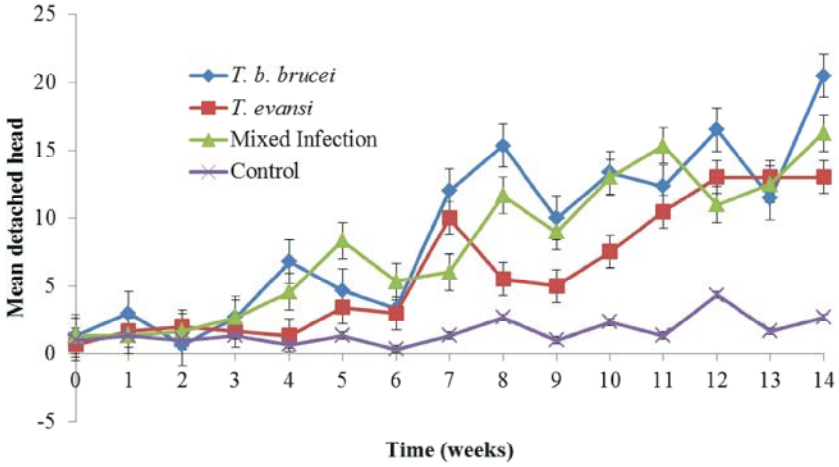

Fig. 3: Mean weekly incidence of detached head in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T. evansi* (Grp II), or both parasites (Grp III)

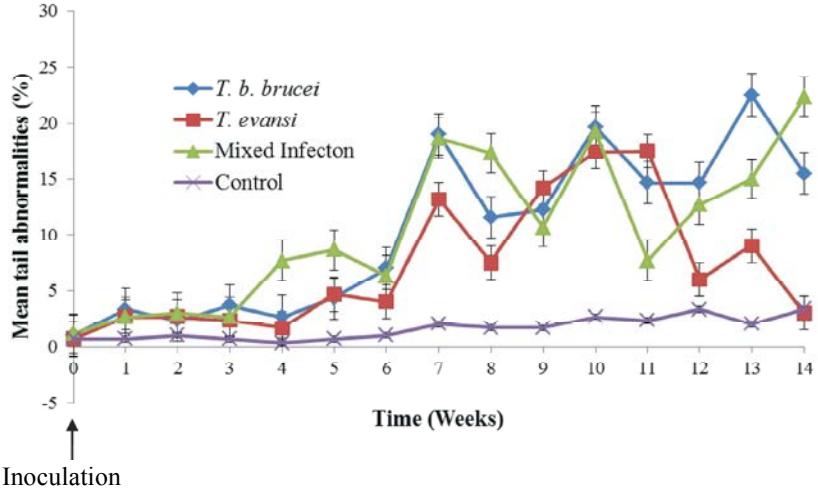

Fig. 4: Mean weekly incidence of tail abnormality in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T. evansi* (Grp II), or both parasites (Grp III)

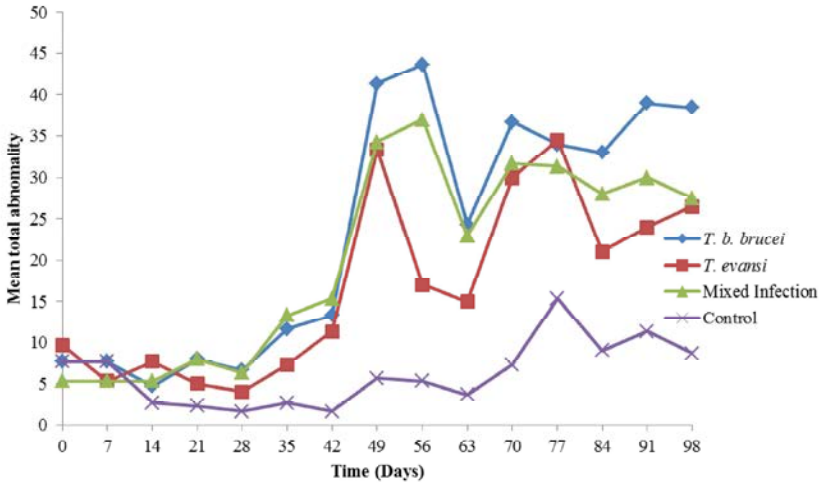

Fig. 5: Mean weekly total sperm abnormalities of Yankasa rams infected with *Trypanosoma* spp. Group I (*T. b. brucei*); Group II (*T. evansi*); Group III (Mixed infection) and Group IV (Control)

Mean Weekly Incidence of Cytoplasmic Droplets (MPD) (%): There was a high rise in the mean weekly percent cytoplasmic droplets (Plate VII) of all the infected Yankasa rams (groups I, II and III) in comparison with the uninfected control (group IV) which had values that remained within the normal range upto the end of the experiment (Figure 2). Statistically, there was a significant increase ($P < 0.01$) in percent cytoplasmic abnormality in the infected rams in comparison to that of the control group, being more pronounced in mixed infection (Figure 2).

Mean Weekly Incidence of Detached Head (Free Head) (DH) (%): The mean weekly percent incidence of detached head of the infected Yankasa rams rose dramatically from the pre-infection values of 1.33 ±0.01% (group I), 0.67±0.07 % (group II) and 1.33±0.01% (group III) to higher significant ($P < 0.01$) values of 20.50±2.13%, 13.00±1.38% and 16.25±2.13% by days 98, 84 and 98 post infection respectively (Figure 3).

Mean Weekly Incidence of Tail Abnormalities (CT) (%): Figure 4 shows the percent incidence of all tail abnormalities of infected and uninfected control Yankasa rams. At the beginning of the experiment, the mean percent tail abnormality of the infected groups I, II and III were 1.00±0.11%, 1.00±0.02 and 1.17±0.01 respectively. There was a drastic and sharp increase in the mean percent tail abnormalities in all the infected groups I, II and III with values 22.50±2.33%, 17.50±2.14% and 22.33±2.33%, observed at days 91 (week 13), 77 (week 11) and 98 (week 14) p.i. respectively. There was significant difference ($P < 0.01$) between the infected groups in comparison to the control group IV (Figure 4).

Mean Total Spermatozoa Abnormalities (%): The mean total spermatozoa abnormalities of all the infected groups I, II and III differed significantly ($P < 0.01$) from that of the control group IV at the end of the experiment (Figure 5). The highest mean percent spermatozoa abnormalities were observed in group I, followed by those of groups III and II. The value was lowest in the control group, IV (Figure 5)

DISCUSSION

Following the development of parasitaemia, all the animals in the infected groups came down with clinical trypanosomosis. Most of the clinical observations made during the course of the disease may be directly attributed to the extravascular invasion by the parasites and resultant tissue lesions. Similar observations were reported by Okubanjo *et al*. [3] and Silva *et al*.[8] The swelling of the scrotum (scrotal oedema) at the early stage of the experiment may be associated with inflammation process (orchitis) of the testes due to invasion by trypanosomes, thereby resulting in the increase in scrotal circumference as well as increase in body temperature. Such inflammatory processes within the testes or scrotum incited by the trypanosomes also resulted in degeneration of the testicular and scrotal tissues leading to decrease in scrotal circumference with resultant decrease in semen quality. Similar observation was reported by Okubanjo *et al*. [3]. Anaemia sequelae to trypanosomosis

in *T. b. brucei*-infected rams and those with mixed infections compared to those of *T. evansi* infected rams, suggests that the *T. b. brucei* strain used in this study was more virulent than the *T. evansi* strain (Sokoto isolate). Fluctuations in PCV and haemoglobin values have been reported in sheep and goats during trypanosomosis [3, 8].

Though, the mechanism of increased semen collection reaction time or declined libido was not investigated the present study, it may be attributed to testicular degeneration with consequent damage to Leydig cells (responsible for testosterone production) within the testes by the parasites. Testosterone plays an important role in optimal functioning of the testes and initiation of sex drive. Though, testosterone assay was not done in the present study, there are reports of reduced testosterone levels associated with trypanosomosis in bulls, sheep and goat [22, 24]. High levels of testosterone are necessary for normal testicular and epididymal functions. Impairment of the Leydig cells by the trypanosomes may be responsible for alteration in testosterone level, hence the observed decline in libido and increase in semen collection reaction time. Decrease in sperm outputs accompanied with increased semen abnormalities could be attributed to testicular degeneration resulting in poor semen quality and increased spermatozoa abnormalities. The percentage of spermatozoa abnormalities in all the infected rams far exceeds the upper limit of 20% recommended for good reproductive potential and fertility in either normal mating and or artificial insemination for rams [25, 26]. This implies that the rams may be rendered infertile and unfit for breeding.

CONCLUSIONS

In conclusion, trypanosomosis due *T. b. brucei*, *T. evansi* and mixed infections adversely affects the spermiogram resulting in increase in semen reaction time and spermatozoa morphological abnormalities in Yankasa rams. The severity of the infection was greater in *T. brucei brucei* infected rams and those with mixed infection than with *T. evansi*, hence an important cause of infertility in Yankasa rams.

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REFERENCES

1. Soudre, A., S. Ouedraogo-Kone, M. Wurzinger, S. Muller, O. Hanotte, A.G. Ouedraogo and J. Solkner, 2013. Trypanosomosis: a priority disease in tsetse challenged areas of Burkina Faso. Tropical Animal Health and Production, 45(2): 497-503.
2. Steverding, D., 2008. The history of African trypanosomiasis. Parasite Vector, pp: 1-3.
3. Okubanjo, O.O., V.O. Sekoni, O.J. Ajanusi, A.J. Nok and A.A. Adeyeye, 2014. Testicular and epididymal pathology in Yankasa rams experimentally infected with Trypanosoma congolense. Asian Pacific Journal of Tropical Diseases, 4(3): 185-189.
4. Awobode, H.O., 2006. The biochemical changes induced by natural human African trypanosome infections. African Journal of Biotechnology, 5(9): 738-742.
5. Sekoni, V.O., 1994. Reproductive disorders caused by animal Trypanosomiasis: A review. Theriogenology, 42: 557-570.
6. Bezerra, F.S.B., H.A. Garcia, H.M. Alves, I.R.S. Oliveira, A.E. Silva, M.M.G. Teixeira and J.S. Batista, 2008. *Trypanosoma vivax* in testicular and epidydimal tissues of experimentally infected sheep. Pesquisa Veterinária Brasileira, 28: 575-582.
7. Batista, J.S., C.M.F. Rodrigues, R.G. Olinda, T.M. Silva, R.G. Vale, A.C. Câmara, R.E. Reboucas, F.S. Bezerra, H.A. García and M.M.G. Teixeira, 2012. Highly debilitating natural *Trypanosoma vivax* infections in Brazilian calves: epidemiology, pathology and probable transplacental transmission. Parasitology Research, 110: 73-80.
8. Silva, T.M., R.G. Olinda, C.M. Rodrigues, A.C. Camara, F.C. Lopes, W.A. Coelho, M.F. Ribeiro, C. Freitas, M.M. Teixeira and J.S. Batista, 2013. Pathogenesis of reproductive failure induced by *Trypanosoma vivax* in experimentally infected pregnant ewes. Veterinary Research, 44(1): 1-5.
9. Mbaya, A.W., C.O. Nwosu and H.A. Kumshe, 2011. Genital lesions in male red fronted gazelles (*Gazella rufifrons*) experimentally infected with *Trypanosoma brucei* and the effect of melarsamine hydrochloride (Cymelarsan®) and diminazeneaceturate (Berenil®) in their treatment. Theriogenology, 16: 721-728.

10. Ikede, B.O. and S.O. Akpavie, 1982. Delay in resolution of trypanosome induced genital lesions in male rabbit infected with *Trypanosoma brucei* and treated with Diminazene aceturate. Research in Veterinary Science, 32: 374-376.
11. Sekoni, V.O., 1992. Effect of *Trypanosoma vivax* infection on semen characteristics of Yankasa rams. British Veterinary Journal, 148: 501-506.
12. Wilson, R.T. and J.W. Durkin, 1984. Age at permanent incisor eruption in indigenous goats and sheep in semi-arid Africa. Small Ruminant and Camel Group. Group Document No. SR C3, pp: 451-455.
13. Woo, P.T.K., 1969. The haematocrit centrifuge technique for the detection of trypanosomes in blood. Canadian Journal of Zoology, 47: 921-923.
14. Biryomumaisho, S., E.K. Rwakishaya, S.E. Melville, A. Cailleau and G.W. Lubega, 2013. Livestock trypanosomosis in Uganda: parasite heterogeneity and anaemia status of naturally infected cattle, goats and pigs. Parasitology Research, 112(4): 1443-1450.
15. Hoare, C.A., 1972. *Trypanosomes of mammals*: a zoological monograph. Oxford and Edinburgh, pp: 1-3.
16. Herbert, W.J. and W.H.R. Lumsden, 1976. *Trypanosoma brucei*: a rapid "matching" method for estimation of the host parasitaemia. Experimental Parasitology, 40(3): 427-431.
17. Morar, I.A., I.S. Groza, M. Borzan, Emike Páll, V.R. Neagu., A.L.R. Pop, C. Mate and Emilia Groza, 2010. Studies regarding collection, evaluation and conservation of European Mouflon (*Ovis ammon musimon*) Semen. Lucrări Stiinłifice Medicinã Veterinarã, 2: 15-24.
18. Rao, A.R., 1971. Changes in the morphology of sperm during their passage through genital in bull abnormal and impaired spermatogenesisPh. D. Thesis. Stockholm.
19. Michael, A.J., C. Alexopoulos, E.A. Pontiki, D.J. Hadjipavlou-Litina, P. Saratsis, H.N. Ververidis and C.M. Boscos, 2009. Effect of antioxidant supplementation in semen extenders on semen quality and reactive oxygen species of chilled canine spermatozoa. Animal Reproduction Science, 112: 119-135.
20. Blom, E., 1972. The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram. Attidel VIII. Simposio Int. di Zootechnia. Milan, pp: 125-139.
21. Sekoni, V.O., B.K. Gustafsson and E.C. Mather, 1981. Influence of wet fixation, staining techniques and storage time on bulls sperm morphology. Nordisk Veterinary Medicine, 33: 161-166.
22. Adamu, S., M.Y. Fatihu, N.M. Useh, M. Mamman, V.O. Sekoni and K.A.N. Esievo, 2004. Effect of Experimental *Trypanosoma vivax* infection on serum testosterone levels in zebu bulls: a preliminary report. Journal of Veterinary Science, 6: 14-17.
23. Okubanjo, O.O., V.O. Sekoni, O.J. Ajanusi, I.A. Lawal and I.D. Jatau, 2008. Effects of *Trypanosoma congolense* infection on some reproductive Parameters in Yankasa Rams. Proceedings of Annual Congress of the Nigerian Veterinary Medical Association. 20th-24th October, 2008. Owerri, Imo State, Nigeria, pp: 125-127.
24. Waindi, E.N., S. Gombe and D. Oduor-okelo, 1986. Plasma testosterone in *Trypanosoma congolense* infected Toggenburg goats. Archives of Andrology, 17: 7-17.
25. Zemjanis, R., 1977. Collection and evaluation of semen. In: *Diagnostic and therapeutic techniques in animal reproduction*. The Williams and Wilkins Co. Baltimore, pp: 242.
26. Oyeyemi, M.O. and B.S. Okediran, 2007. Testicular parameters and sperm morphology of chinchilla rabbits fed with different planes of soybean meal. International Journal of Morphology, 25(1): 139-144.