EFFECTS OF *Trypanosoma brucei brucei* AND *Trypanosoma evansi* ON SCROTAL CIRCUMFERENCE, SEMEN CHARACTERISTICS AND HAEMATOLOGICAL PARAMETERS OF YANKASA RAM (*Ovis aries* Linnaeus, 1978)

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Y.A. Wada[1]*, S.J. Oniye[1], P.I. Rekwot[2] and O.O. Okubanjo[3]
[1]Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria
[2]National Animal Production Research Institute, Ahmadu Bello University, PMB 1096, Shika-Zaria, Nigeria
[3]Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria
*Corresponding author: yunuwad@yahoo.com

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Abstract: The effects of trypanosomosis on the scrotal circumferences and semen characteristics of Yankasa rams were studied for ninety eight days (14 weeks). Sixteen Yankasa rams aged between 24 to 30 months and weighed between 22 to 25 kg 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 were randomLy divided into four Groups: I, II, III and IV, of three rams each. 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 2 mL containing 2 x 10^6 trypomastigotes via the jugular vein. All the infected groups developed clinical signs typical of trypanosomosis at various prepatent periods, with significant (P<0.05) decrease in values of haematological indices. There was a drastic and significant decrease in scrotal circumferences in the infected rams with significant deterioration (P<0.05) in semen quality of all the infected rams, characterized by decrease in volume or cessation of semen production, a significant (P<0.05) decrease in progressively motile sperm, elevated numbers of dead sperm and significant increase (P<0.05) in spermatozoa morphological abnormalities in all 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. However, the uninfected rams were healthy and had normal semen characteristics throughout the study period. The results indicate that trypanosomosis due to experimental *T. b. brucei* or *T.evansi* or Mixed infections (of both parasites) caused significant decrease in scrotal circumference and severe deterioration in semen quality with resultant infertility in Yankasa rams.

Keywords: Scrotal circumference, *Trypanosoma brucei brucei*, *Trypanosoma evansi*, Yankasa rams

Introduction

In tropical Africa, livestock production plays an important role not only for income, food and fertilizer but also for sustaining the livelihoods, security and health of the poor (Liebenehm, 2008). African trypanosomosis is one of the most debilitating diseases, limiting livestock production in Sub- Saharan Africa (Ouma, 2010). The transmission of the disease by tsetse fly, cause anaemia, fever, poor condition and finally terminates in death. Reproductive disorders in both males and females are part of the clinical features of African trypanosomosis, and the major pathogenic forms causing diseases in livestock include *Trypanosoma congolense*, *T. vivax*, *T. brucei* and *T. evansi* (Bossard *et al*., 2010).

Studies in ruminants infected with *T. brucei*, *T. congolense* or *T. vivax* have shown that infection can lead to irregular oestrus cycles and intra- uterine infections with abortions in females (Sekoni, 1994). In males, the infection is characterized by elevated sperm morphological abnormalities (Sekoni, 1994; Okubanjo *et al*., 2014). Increased scrotal diameters, scrotal inflammation with scab formation, peri-orchitis, epididymitis, severe testicular degeneration were among the many pathological changes observed in *T. brucei brucei* infected rabbits (Ikede and Akpavie, 1982). An investigation on the effect of *T. vivax* and *T. congolense* infections on reproduction in Zebu bulls revealed the *T. congolense* to be more pathogenic with progressive elevation of semen ejaculation time and poor semen quality manifested by decreased volume of semen, oligospermia, aspermia, dead spermatozoa, very poor sperm motility and significant sperm morphological abnormalities (Sekoni *et al*., 1990).

There was reported reduced libido with a higher rate of refusals and production of poor quality semen with some adverse effects on mass motility, percentage of live sperm cells and minor sperm cell morphology in *T. congolense* infected Djallonke rams (Osaer *et al*., 1997). Although numerous works on the effect of *T. congolense* and *T. vivax* has been documented, information on the effect of *T. brucei brucei* or *T. evansi* or mixed infections on the scrotal circumference and semen characteristics of Yankasa rams is scanty and basically unavailable. Although *T. brucei brucei* and *T.evansi* are primarily parasites of cattle and camels respectively, there is the likelihood that animals herded together with cattle or camels, especially Yankasa rams, may become infected with these parasites. Yankasa rams are the most numerous breeds of sheep in the guinea savanna region commanding high demands amongst the populace due to their ceremonial values (naming ceremony), religious importance (used as sacrificial animals during the Eid-El-Kabir festive periods) and social values (e.g. Barbecue). Consequently, there is an urgent need to investigate factors that may negatively affect the success of sheep breeding especially in line with reproductive problems such as lowered fertility, infertility and or sterility. Therefore, this study is aimed at evaluating the effects of trypanosomosis on the scrotal circumferences, haematological and semen characteristics of Yankasa rams.

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, 1984) 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 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 *adlibitum*. 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 (Woo, 1969; Biryomumaisho *et al*., 2013).

Source of Trypanosomes

Trypanosoma evansi was obtained from an infected camel that was slaugtered at the Sokoto Abattior, Sokoto State, while *Trypanosoma brucei brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria, originally isolated from a natural infection in cattle, in Kaduna State. 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 (Hoare, 1972). *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 in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. 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 (1969) and Biryomumaisho *et al*. (2013).

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 were 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 Diamine Tetraacetic Acid (EDTA) for the inoculation of the rams in groups GI, GII and GIII. 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 (1976). 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 (Herbert and Lumsden, 1976).

Group I – each ram was inoculated via the jugular vein with 2 mL of blood containing 2 x 10^6 *Trypanosoma brucei brucei*.

Group II – each ram was inoculated via the jugular vein with 2 mL of blood containing 2 x 10^6 *Trypanosoma evansi*.

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

Reproductive parameters

Reproductive parameters measured were scrotal circumference, semen collection reaction time, semen characteristics (colour), pH, concentration of semen, total sperm output, ejaculate, viability (life/dead%), sperm morphology (normal sperms, head abnormalities and tail abnormalities) according to the method of Zemjanis (1977). Gonadal and epididymal sperm/spermatid reserves were also determined according to the method of Sekoni *et al*. (1981), Rekwot *et al*. (1994), Alabi (2005) and Ogunlade *et al*. (2006).

Determination of scrotal circumference

Scrotal circumference was measured in centimetres (cm) using a flexible measuring tape at the point of the widest scrotal diameter by applying pressure with a hand above

the head of the epididymides thereby gently forcing the testes into the scrotum (Osaer *et al.,* 1997).

Semen collection

Semen collection was done weekly between 9am and 10am, by electrostimulation with the help of a portable battery-powered electro-ejaculatory mini tube for small ruminants as described by Morar *et al*. (2010). The rams were adequately restrained; the prepuce was washed and dried using cotton wool soaked in diluted chloroxylenol (0.002%; Dettol^{R}) to remove dirts and debris. The probe of the electro-ejaculator was lubricated using petroleum jelly and inserted into the animal's rectum and switched on, this resulted in erection and subsequently, ejaculation. Semen began to flow once the animal has achieved excitation by the stimulatory action of the electro-ejaculatory device. The impulses consisted of applying the stimulus at an interval of 5 seconds, with 5 seconds break (Morar *et al*., 2010). The ejaculates were collected into pre-warmed, sterile and graduated transparent collection tube, labelled 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 (Rao, 1971).

Semen evaluation

Each ejaculate was evaluated for volume, motility, pH, concentration, sperm morphological abnormalities and live/dead differential sperm count, to determine the effects of trypanosomes on the seminal parameters. General semen examination was done at the point of collection while the microscopic examination was done at the Fertility Laboratory of the Artificial Insemination Unit, National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika, Zaria, using standard laboratory methods as described by Bitto *et al*. (2000).

General examination

Parameters such as semen collection reaction time (sec), semen volume (mL) and semen colour and semen pH were promptly evaluated.

Semen volume and colour

The volume of semen (mL) ejaculated was measured directly from the calibrated tube used for collection. The semen colour was noted immediately after collection for milky, creamy and watery appearance.

Semen pH

This was determined by inserting a pH meter into the collection tube and the value was then taken.

Microscopic examination of semen

Microscopic examination of semen sample was done under a light microscope in the laboratory to examine the gross sperm motility, the sperm concentration, and sperm morphology.

Gross sperm motility

This was determined according to the methods described by Zemjanis (1977) and Rao (1971) by placing a drop of raw undiluted semen on a pre-warmed slide then cover-slipped and viewed using a field microscope at X40 magnification.The results were scored objectively, using the scoring pattern described by Omalaka (1992) in percentages as presented below:

90-100% Excellent, continuous progressive motility
80-89% Very good, continuous progressive motility
70-79% Good, continuous progressive motility
60-69% Shift continuous progressive motility
50-59% Very active-none-progressive motion
40-49% Shift none-progressive motion
10-19% No motion

Sperm concentration

Sperm concentration was evaluated by visual count under the microscope using improved Neubauer Haemocytometer. The sperm cells were immobilized using a 1% formaldehyde solution prior to counting. The raw semen was mixed thoroughly and filled into the unopette capillary tube with a dilution ratio of 1:10. The diluted semen was thereafter transferred unto the haemocytometer chamber and counted under the microscope. The number of sperm cells counted using the haemocytometer multiplied by a million (10^6) was the concentration per mL of the raw semen.

The total spermatozoa per ejaculate were estimated by calculation as described by Egbuka (1995):

Total spermatozoa per ejaculate = Sperm concentration per mL x Volume per ejaculate

Percentage live- dead spermatozoa

The percentage live sperm and spermatozoa morphological abnormalities were determined using Eosin-Nigrosin stain technique, applied on a glass slide (Michael *et al*., 2009). 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 X40 magnification. A minimum of 100 spermatozoa 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).

Spermatozoa abnormal morphology

Morphological abnormalities of the spermatozoa that were examined included Mid Piece Droplets (MPD), incidence of Detached Head (DH), Bent Tail (BT) and incidence of Coiled Tail (CT). These abnormalities were determined by making a thin smear of the semen sample on clean grease-free glass slide and fixed with buffered formal saline. Semen samples stained with eosin-nigrosin were used to determine the morphological abnormalities of the sperm head. A minimum of one hundred sperm cells were counted per slide using light microscopy at X40 magnification and were classified and calculated as described by Blom (1972) and Sekoni *et al*. (1981).

Data Analysis

The data obtained from the study were prepared for analysis using Microsoft Office Excel, 2010. Weekly mean scrotal circumferences of the experimental groups were represented with a multiple line graph using Microsoft excel chart wizard, 2010. Inter group comparisons of mean semen parameters and sperm morphological characteristic were analysed by One-Way analysis of variance (ANOVA), and where significant differences exist, Duncan Multiple Range Test (DMRT) was used to separate the means. Statistical analysis (ANOVA) was carried out using the Superior Performing Statistical Software (SPSS) for windows, version 20 (2011). The values of $P<0.05$ were considered statistically significant (Steel and Torrie, 1980).

Results and Discussion

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 oedema, scrotal degeneration, poor semen output, loss of libido, drowsiness and death. Rectal temperature was significantly

higher (P<0.05) in all the infected rams in comparison to the control uninfected rams (Table 1). The weights and haematological parameters of all infected rams significantly (P<0.05) decreased in comparison to the values of the uninfected rams (Table 1)

Mean weekly scrotal circumference

The pre-infection mean scrotal circumferences of the rams in the experimental Groups I, II, III and IV were 24.37±1.1 cm, 23.94±1.0 cm, 24.52±1.3 cm and 24.51±0.3 cm, respectively. By day 21 p.i., the mean weekly scrotal circumference of the infected Groups I and III showed a transient sharp increase in scrotal circumference to a value of 24.87±0.1 cm and 24.72±0.2 cm, respectively. There was thereafter, an observed sharp and progressive decrease in scrotal circumference which set in by day 28 p.i. and day 35 p.i. to a value of 22.29±0.2 cm and 22.23±0.1 cm for the infected rams in Groups I and III, respectively. These values decreased up to the end of the experiment (Fig. 1). For rams infected with *T. evansi* (Group II), there was slow and gradual decrease in scrotal circumference from a pre-infection value of 23.94±0.7 cm to 23.27±0.1 cm by the end of the experiment (98 days post infection). There was a statistical significant (P<0.01) reduction in the mean weekly scrotal circumference of all the infected rams in comparison to the uninfected control rams that maintain a relatively normal and increased scrotal circumference (24.64 ±0.3 cm) by the end of the experiment (Fig. 1).

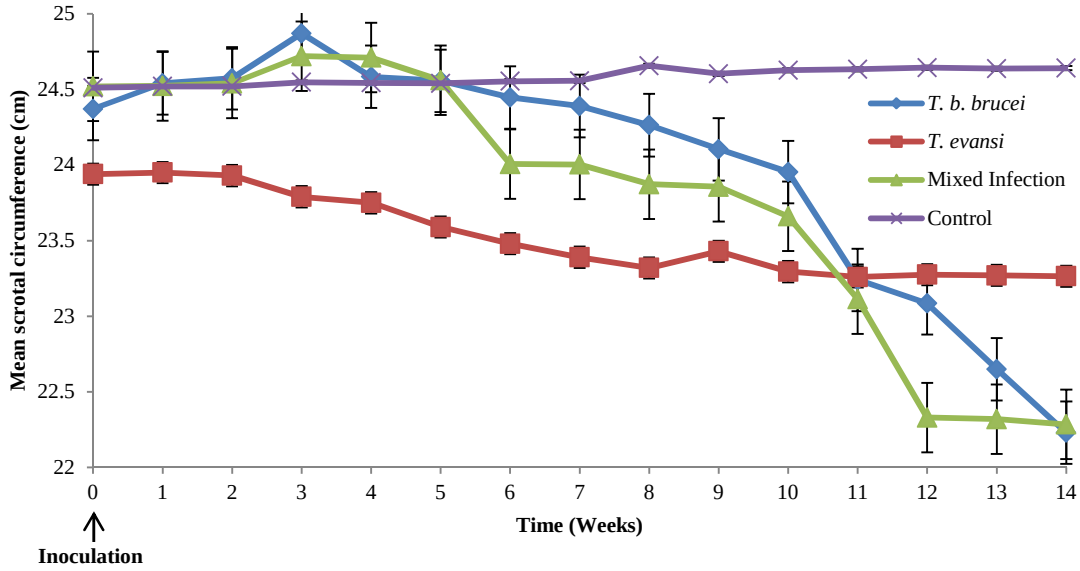

Fig. 1: Mean weekly scrotal circumference of uninfected control yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T. evansi* (Grp II), or both parasites (Grp III).

Semen characteristics

Table 2 is the values for semen characteristics of experimental Yankasa rams. For all the semen parameter studied, the mean values for the infected Groups I, II and III differed significantly (P<0.05) from those of the control Group IV, which remained within normal range throughout the duration of the study (Table 2).

Semen volume and colour (mL)

Rams in the control Group IV had the highest mean semen volume (0.72±0.02 mL), followed by those of Groups II (0.49±0.02 mL), I (0.37±0.03 mL) and III (0.36±0.02 mL), respectively (Table 2). The mean semen volumes of the infected groups were significantly lowers (P < 0.01) in comparison to that of the control group IV. However, the value of Groups I and III were not significantly different (P>0.01) from each other, but differed significantly (P<0.01) from those of rams in Groups II and IV at the end of the experiment (Table 2). The semen colour of all the infected groups differed significantly (P<0.05) from those of the control groups. The colour ranged from milky white in the control, creamy white in *T. evansi*-infected group, to watery colour (seminal fluids) and in *T brucei brucei*-infected rams and those with mixed infection (*T. b. brucei* and *T. evansi*) (Table 2).

Gross forward motility (%)

The percent gross forward (wave pattern) motility of spermatozoa in the semen was significantly higher (P<0.01) in the control Group IV (82.83±1.15%) when compared to all the infected Groups I (55.90±3.43%), II (61.86±3.27%) and III (45.87±3.65%), respectively. Among the infected groups, percent gross forward motility was significantly higher (P < 0.01) in group II in comparison to Groups I and III.

Semen pH

Although, rams infected with *T.brucei brucei* (Group I) and those with mixed infection (*T brucei brucei* and *T. evansi*) (Group III) had a higher mean pH values, there was no significant differences (P>0.01) in the semen pH of all the experimental groups under study (Table 2).

Semen concentration (10^6/mL)

There was high significant decrease (P<0.01) in spermatozoa concentration in millions per mL of all the infected Groups I, II and III when compared to the control Group IV. Among all the infected groups, spermatozoa concentration was lowest in Group III (372.00 ±14 x $10^{6/}$mL) and was highest in Group II (409.00 ±13 x $10^{6/}$mL) (Table 1). However, ANOVA revealed no significant difference (P>0.05) between all the infected Groups I, II and III but were statistically different (P<0.01) in comparison to the control Group, IV (Table 2).

Total spermatozoa output

The total spermatozoa per ejaculate was significantly higher (P<0.01) in the control Group IV (641.52 ±5.00 x $10^{6/}$mL) when compared to all the other infected Groups I (141.00 ±5.00 x $10^{6/}$mL), II (201.00 ±5.00 x $10^{6/}$mL) and III (134.00±4.00 x $10^{6/}$mL). No difference exist (P>0.01) between infected Group I in comparison to Groups II and

III, respectively. However, there was significant different between Groups II and III (Table 2).

Percent dead sperm (%)

The percent dead spermatozoa were highest in Group III (44.62± 3.03%), followed by those of Group I (36.41±2.88%) and Group II (27.71±2.37%), respectively; while those of the control (Group IV) had the least percent (12.14±0.82%) dead spermatozoa at the end of experiment (Table 2).

Table 1: The clinical parameters of Yankasa rams infected with *Trypanosoma* species (mean ± SEM)

Parameter	Group I	Group II	Group III	Group IV	Level of sig.
Rectal Temperature (°C)	39.47 ± 0.47^{a}	38.86 ± 0.70^{b}	39.40 ± 0.48^{a}	38.31 ± 0.21^{b}	< 0.001**
Weight (Kg)	21.43 ± 1.69^{bc}	21.21 ± 1.47^{c}	21.70 ± 1.63^{b}	25.61 ± 0.37^{a}	< 0.001**
Scrotal circum. (cm)	24.16 ± 1.19^{ab}	23.21 ± 1.56^{c}	23.79 ± 1.08^{bc}	24.59 ± 1.47^{a}	0.002**
PCV (%)	21.88 ± 2.39^{c}	23.34 ± 4.40^{b}	22.05 ± 4.09^{c}	34.21 ± 1.70^{a}	< 0.001**
Haemoglobin (g/dl)	7.29 ± 0.80^{c}	7.78 ± 1.47^{b}	7.35 ± 1.36^{c}	11.40 ± 0.57^{a}	< 0.001**
Total protein (g/dl)	4.77 ± 0.67^{c}	5.32 ± 0.59^{b}	4.93 ± 0.58^{c}	6.09 ± 0.50^{a}	< 0.001**

Means with different superscripts across the same row differed significantly; ** = High significant difference exists at P≤0.01; GI (*T.b. brucei*); GII (*T. evansi*); GIII (*T. b. brucei* and *T. evansi* Mixed); GIV (Uninfected Control)

Table 2: The Semen Characteristics of Infected and Control Yankasa Rams (Mean ± SEM)

Parameter	GI (*T. b. brucei*)	G II (*T.evansi*)	G III (Mixed)	G IV (Control)	Level of sig.
Volume (mL)	0.37 ± 0.03^{c}	0.49 ± 0.02^{b}	0.36 ± 0.02^{c}	0.72 ± 0.02^{a}	< 0.001**
Progressive motility (%)	55.90 ± 3.43^{c}	61.86 ± 3.27^{b}	45.87 ± 3.65^{d}	82.83 ± 1.15^{a}	< 0.001**
pH	6.98 ± 0.14	7.26 ± 0.16	7.01 ± 0.16	7.24 ± 0.08	0.233
Sperm concentration ($x10^{6}$/mL)	381.00 ± 15.00^{b}	409.00 ± 13.00^{b}	372.00 ± 14.00^{b}	891.00 ± 19.00^{a}	< 0.001**
Total sperm/ejaculate ($x10^{6}$/mL)	141.00 ± 5.00^{bc}	201.00 ± 5.00^{b}	134.00 ± 4.00^{c}	641.43 ± 5.00^{a}	< 0.001**
Dead sperm (%)	36.41 ± 2.88^{b}	27.71 ± 2.37^{c}	44.62 ± 3.03^{a}	12.14 ± 0.82^{d}	< 0.001**
Total abnormality (%)	43.51 ± 2.48^{a}	35.94 ± 2.16^{b}	40.80 ± 2.15^{ab}	8.57 ± 0.55^{c}	< 0.001**

Means with different superscripts across the same row differed significantly. W.H.O, 1992 Standard values for fertile rams: <20% abnormality: < 25% dead sperm; ** = High significant difference exists at P≤0.01; GI (*T.b. brucei*); GII (*T. evansi*); GIII (*T. b. brucei* and *T. evansi* Mixed); GIV (Uninfected Control)

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 (Table 1). The highest mean percent spermatozoa abnormalities were observed in Group I (43.51±2.48%), followed by those of Group III (40.80±2.15%) and finally Group II (35.94±2.16 %). The value was lowest (8.57±0.55%) in the control Group, IV.

In the present study, the *T. brucei brucei*-infected rams had more severe form of anaemia (as evident by significant decrease in PCV), followed by those with mixed infections. The fact that PCV and Hb values decreased sharply in periods of high parasitaemias, but maintained a gradual decrease during the periods of low parasitaemias, showed a direct relationship between anaemia and parasitaemia. Deficiency of haemoglobin in red blood cells decreases their oxygen carrying capacities leading to symptoms of anaemia. Low value of PCV is an indication of low mean red blood cells count that are responsible for carrying oxygen to body tissues which aid in the characterization of anaemia. Fluctuations in PCV and haemoglobin values have been reported in sheep and goats during trypanosomosis (Shehu *et al*., 2010; Ogbaje *et al*., 2011; Silva *et al*., 2013). Decreases in plasma protein during trypanosomosis are suggestive of increased protein breakdown or urea loss, haemodilution and serum extravasation (Anosa and Isoun, 1974); which could cause oedema in the infected sheep. 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. Such lesions in the skin, skeletal muscles and testicles gave rise to severe oedema, which on palpation elicited painful reactions in affected animals as was reported by Okubanjo *et al*. (2014).

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. Similar observations were reported by Bandyopaghy *et al*. (2007) and Okubanjo *et al*. (2014).

Decreased sperm outputs accompanied by increased semen abnormalities could be attributed to impairement of the ultrastructure of the spermatogenic cells by the trypanosomes resulting in degeneration of the seminiferous tubules within the testes. The consequence is a disruption of the process of spermatogenesis manifested by poor semen quality and increased spermatozoa abnormalities as observed in the present study. The increase in dead spermatozoa in all the infected rams may be due to depletion of cellular nutrients and oxygen as trypanosomes require these for their movement, multiplication and growth thereby denying the sperm cells access. The decrease in the sperm concentration is most likely due to degeneration in testicular size, decreased testicular parenchyma and decrease in the volume of seminiferous epithelium and diameter of the seminiferous tubules where spermatogenesis occurs.

Conclusion

Trypanosomosis due *T. b. brucei*, *T. evansi* and mixed infections results in scrotal degeneration, manifested by poor quality semen and severe sperm morphological abnormalities in Yankasa rams.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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