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Status of Anaemia, Semen and Ejaculate Charateristics in Experimental Trypanosomosis Infections in Yankassa Rams After Berenil, Novidium and Samorin Treatment

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Abstract: *Forty eight out of 52 matured Yankassa rams were shared into 3 groups A, B and C of 16 animals each and remaining 4 rams served as uninfected and untreated control (group D). The groups A-C were infected with about 1x10^6 of Trypanosoma brucei brucei, T. congolense and T. vivax, respectively. The experimental groups A-C were subdivided into Berenil, Novidium and Samorin treatment subgroups (a-c). T. congolense had sub-acute course of infection than the others, hence, there was no late treatment. Anaemia measured as red blood cell (RBC) counts was more severe in T. vivax followed by T. congolense and T. brucei brucei. After treatment, Samorin had increased percentage RBC counts and body weight gain. In groups A-C, pre-treatment semen volume decreased over post-infection values. Early and late sperm concentration reduced by 35.7% and 24.8% in T. brucei, cessation of sperm release in T. congolense with 19.3% and 67.3% in T. vivax. Testicular circumference increased by 11.6% and 19.7% for early and late (Berenil), 26% and 19.6% for Novidium and 12.4% and 32.2% for Samorin. Novidium group had 46.3% and 40.0% decrease in semen volume, 100% reduction in sperm concentration and sperm motility. The testicular circumference increased by 26% and 19.6%. In Samorin group, semen volume decreased by 69.6 and 11.1%, sperm concentrations were lowered by* 35.7% and 24.8% for *T. brucei*; aspermia in *T. congolense* with *19.3% and 67.3% for T. vivax, and reduction in sperm motility by 100%. Treatment with Berenil had remarkably improved the sperm concentration compared to slight increase in Novidium-early and also on testicular circumference. All infected animals showed varied degrees of gross sperm abnormalities. Nevertheless, none of the drugs could fully regenerate hematological parameters, semen and ejaculate abnormalities to pre-infection levels before death.*

Keywords: *Anaemia, Ejaculate characteristics, Rams, Semen, Trypanosoma species, Trypanocides.*

I. Introduction

Five economically important trypanosome species infective to livestock are the cause of diseases called *sammoré* or *nagana* in cattle; dourine in camel, donkey and horse. Those with predilection for blood stream (haemolymphatic) are *Trypanosoma vivax* (Dutonella) and *T. congolense* (Nannomonas) are infective to cattle, sheep and goats (1). Others are *T. suis* of pigs (Pycnomonnas), *T. equipadum* in dromedaries and *T. brucei* (Trypanozoon); the most tissue invasive parasite. Among the *T. brucei* species, *T. b. brucei* subspecies has bimodal infectivity for both man and animal, while *T. b. rhodesiense* is common in East and South Africa, while *T. b. gambiense* in West and Central Africa are responsible for acute and chronic type of sleeping sickness, respectively.

Some domestic animals (cattle, sheep, pig and dogs) could serve as reservoir of potential human infective subspecies (2). One of the major consequences of trypanosomiasis is reduction in reproductive capacity causing abortion, amenural, irregular estrus cycle in females (3, 4, 5, 6). Infection in male host causes reduction in libido, scrotal edema, and poor semen quality and decreased in volume, oligospermia, azoospermia, and elevated incidence of spermatozoa morphological abnormalities (2, 7, 8, 9). Effect of infection on ejaculate or reaction time and the destruction of germinal epithelium which resulted in poor semen quality (10) are of particular significance to livestock farmers in trypanosome endemic regions, particularly infertility of bull. Study by (11) of *T. congolense* infection in West African Dwarf goats showed significant differences in the duration and intensity of oestrus between the infected and control groups.

Case detection and treatment option is complementary to vector control. For now, there is no new drug in sight since none has been approved after the last drug, Berenil, was patented in 1960. The effect of the drug at

various time of infection determines its effectiveness. The commercially available drugs; Berenil (Diminazine aceturate) and Samorin (isometamedium chloride) are for curative while Novidium (Homidium chloride or bromide) is used for prophylactic treatment (12). Added to the dearth of no new drugs for over 50 years, are problems associated with drug misuse and inherent acquired and induced drug resistance. This has made calls for judicious and effective management of available trypanocides to be very apt.

This study was aimed at investigating the ability of commercially available anti-trypanosomal drugs (Berenil, Novidium and Samorin) to reverse the clinical deterioration and anaemia assessed as total whole red blood cell (RBC) counts. Also, to determine effects of treatment on testicular circumference, semen quantity and quality among other parameters. Similarly, the course of experimental infections with equal inoculum of *T. brucei, T. congolense and T. vivax* in rams treated at two different time intervals of 4 and 6 weeks post-infection and post-treatment were also evaluated.

II. Materials And Methods

***Experimental Design*:**

Fifty two (52) matured Yankassa rams purchased from open market in Sokoto were acclimatized for 6 weeks. The animals were screened for haemoparasites and de-wormed with bolus of Pyraziquatel. They were treated with teramycine long active, intramuscularly and Ivomec was applied on their skin along the lateral spin (de-ticking). The rams were kept in animal house with net screened fly proof windows and fed with hay, water and salt lick *ad libitum*. Forty eight experimental animals were shared into 3 infected groups (A, B and C) of 16 animals each. The remaining 4 rams served as uninfected and untreated control (group D). All rams in the experimental groups (A, B and C) were inoculated with about 1 x 10^6 of *Trypanosoma brucei, T. congolense and T. vivax* isolates, respectively. Prior to inoculation, the parasites concentrations were estimated using Neumbauer improved bright-lined haemacytometer chamber (Germany). Twelve animals from each experimental group were further shared into 4 sub-groups (a-d) of 4 animals. Each sub-group a-c were treated with one of the three drugs, Berenil, Novidium and Samorin while sub-group "d" served as infected and untreated control. Two animals in each sub-group were treated at 4 and 6 weeks post-infection.

Red Blood Cell Counts (RBC):

Blood samples obtained by vein-section using sterile disposable syringes and needles were collected into ethylene diamine tetra acetic acid (EDTA) containing tubes. Blood samples were diluted in RBC diluents and enumerated in a Neumbauer improved bright-lined hemocytometer chamber (Germany) using hand held tally counter to determine concentration (x10^6) per µl. We compared the RBC counts with the packed cell volume (PCV) data that have been published (17) to assess the status of anaemia.

***Parasitaemia*:**

Blood was drawn by capillary attraction into heparinized EDTA capillary tubes and spun in a Hawksley Haematocrit Centrifuge (BDH, England) at 1,000 revolution per minute for 5 minutes. Parasitaemia was monitored under the 40x10 microscope objective to examine buffy coat region of blood capillary tubes subjected to Haematocrit Centrifugation Technique (HCT) as described (13). Details of the parasitaemia have been published (17).

Semen Collection and Analyses:

Direct current (DC) battery powered Alfred Cox bull and ram electroejaculator probe (Germany) was used in electrical stimulation of rams at 5amp. The probe was inserted into the rectum until animal produces semen because ejaculation time varied between animals (14). The time taken to ejaculate and emit semen (reaction time, RT) was recorded in seconds but not analyzed. Colour of semen was taken as white or slight, moderate and creamy appearance. The volume was read from the graduated collection centrifuge tube. Semen was graded as watery, slightly, moderate and thick turbidity.

A drop of semen was placed on a microscope slide containing Negrosin Stain and thin film made using a second slide. Percentage of stained (live or active) and unstained (dead or inactive) cells were enumerated under x40 of binocular microscope. Samples were kept warm in cooler just before motility test were carried out in a Neumbauer improved bright-lined haemacytometer chamber (Germany) using hand held tally counter to determine sperm concentration (x10^6). Differential counter was used to assess percentage of motile and non-motile sperm cells based on count of 100 cells. Sperm cell motility was graded semi–quantitatively from no motility 0, slow 1 through increasing order up to vigorous motility, 5.

Morphological characteristics of sperm cells were enumerated under x40 magnification. Sperm cells were categorized into normal when it has remained completely intact without any abnormal features. Defective spermatozoa were those with any of the following: detached heads, acrosome, cytoplasmic droplets, mid-piece and the tail, tailless head or headless tail, enlarged head, small head and absence of spermatocore.

Treatment:

Each experimental animal in a subgroup was administered treatment with Berenil (diminazene aceturate) intramuscularly at 7mg/Kg for *T. b. brucei* and 3.5 mg/Kg body weight for *T. congolense* and *T. vivax*. Novidium (mixture of homidium chloride and bromide) was given as 2.5% solution at 1mg/Kg. Samorin (Isometamidium chloride) was used for *T. congolense* and *T. vivax* at 0.25mg/Kg and *T. b. brucei* at 0.8mg/Kg body weight. The trypanocides were reconstituted and administered strictly according to manufacturers' recommended doses. The early (E) and late (L) treatment subgroups were treated at 4 and 6 weeks post-infection (PI), respectively.

III. Results

Gross Clinical Features:

The observed loss in body weight post-infection and post-treatment was significant ($P < 0.05$) for *T. b. brucei* and *T. congolense* groups (data not shown). The body weight apparently recuperated after Berenil treatment. The temperature of experimental animals gradually increased between 38.5°- 42°C and that of control rams were between 37.5°-39.5°C.

Overall, the *T. b. brucei* Novidium-early and late subgroups had the least survival rates 5 and 7 wks PI followed by Berenil-late treatment of (7 wks) PI. Survival rates were more in early Berenil treated *T. vivax* and Novidium treated *T. brucei and T. vivax* followed by Samorin treated *T. vivax.* The *T. brucei*, *T. congolense* and *T. vivax* infected and untreated control subgroups died 6, 4 and 4 wk PI, respectively.

***Parasitaemia*:**

The pre-patent period ranged from 3 to 4 days. Mean weekly pre-treatment parasitaemia had log equivalent values (LEVs) of 2.23, 2.66 and 2.33 for early Berenil, Novidium and Samorin, respectively. There were no detectable parasites in the blood samples before late treatment of *T. b. brucei* infected rams and during post-treatment period before death of experimental animals.

Red Blood Cell Counts:

The infection manifested remarkable decrease in red blood cell (RBC) count ($P<0.05$) post-infection for groups A-C rams. Late Berenil and early Samorin treated *T. b. brucei* sustained high RBC levels as shown on Fig. 1. The RBC counts for Samorin were higher than the Berenil and Novidium early treatment of *T. congolense* (group B) rams as shown on Figure 2.

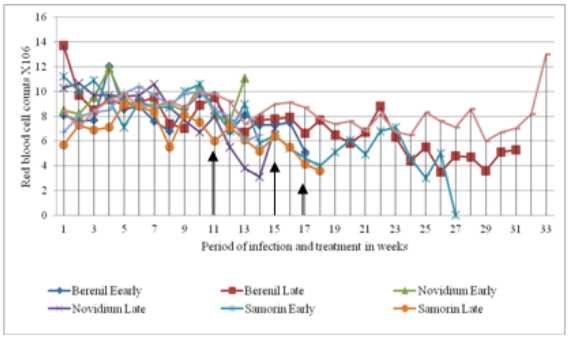

Figure 1: Red blood cell count of *T. brucei brucei* infected rams (Group A). Arrows (↑) indicate early and late treatments.

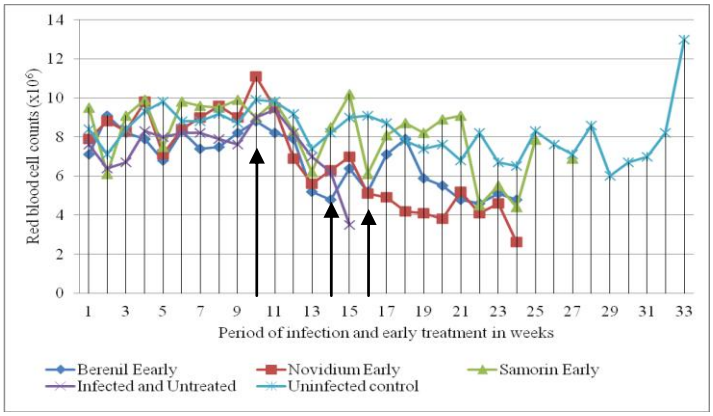

Figure 2: Red blood cell count of *T. congolense* infected rams (Group B). Arrows (↑) indicate early and late treatments.

Early Samorin, then Berenil and Novidium subgroups maintained high RBC counts compared to control (group D) uninfected rams (Fig. 3). The differences in RBC counts of *Trypanosoma brucei* infected (group A) BE and NE, NE and SE, and NL and SL treated subgroups were statistically significant by ANOVA ($P<0.05$). There were significant differences in RBC counts between BE and NE treated ($P<0.05$) and between BL and NL treated ($P<0.001$) subgroups.

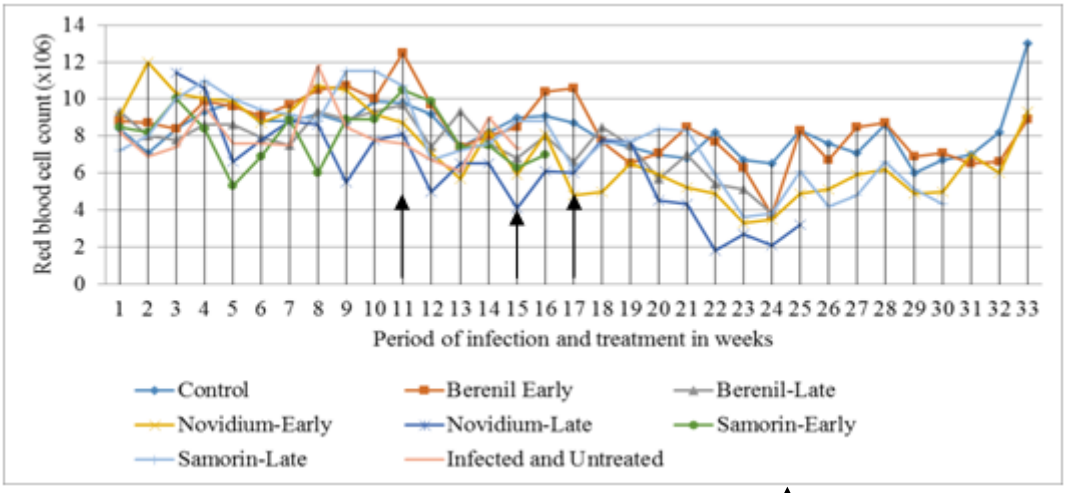

Fig. 3: Red blood cell counts of *T. vivax* infected animals (Group C). Arrows (↑) indicate week of infection for early and late treatments.

Group A (T. b. brucei) Semen and Ejaculate Data:

Testicular circumference increased from 24.9 pre-infection to 27.5 pre-treatment. It further increased to 31.5, 26.5 and 32.0 post-early and 25.5, 20.0 and 27.3 post-late treatment values. Except the TC for early Berenil and late Samorin treatments, which had no remarkable changes, other treatments had significantly increased as shown on Fig. 4. The testicular circumference increased by 11.6% and 19.7% for early and late (Berenil), 26% and 19.6% for Novidium and 12.4% and 32.2% for Samorin. There was significant decrease of 55.6% and 47.3% in semen volume; sperm concentration was lowered by 35.7% and 24.8%; sperm motility decreased by 66.7% and 60% for early and late PI (pre-treatment with Berenil) respectively. Similarly, for early Novidium subgroup, it was 46.3% and 40% decrease in semen volume, and 100% reduction in sperm concentration. In early Samorin treated subgroup, semen volume decreased by 69.6% and 11.1%, sperm concentrations were lowered by 19.3% and 67.3%, sperm motility had 100% decrease as shown on Fig. 5. There were no semen release in late Novidium and Samorin treatments before death of experimental animals. A mean $1.67x10^6$ and $1.41x10^6$ sperm cells count for early and late Berenil compared to the aspermia in Novidium and Samorin treatments (Table 1). The sperm counts for the control animals remained steadily high compared to the infected group.

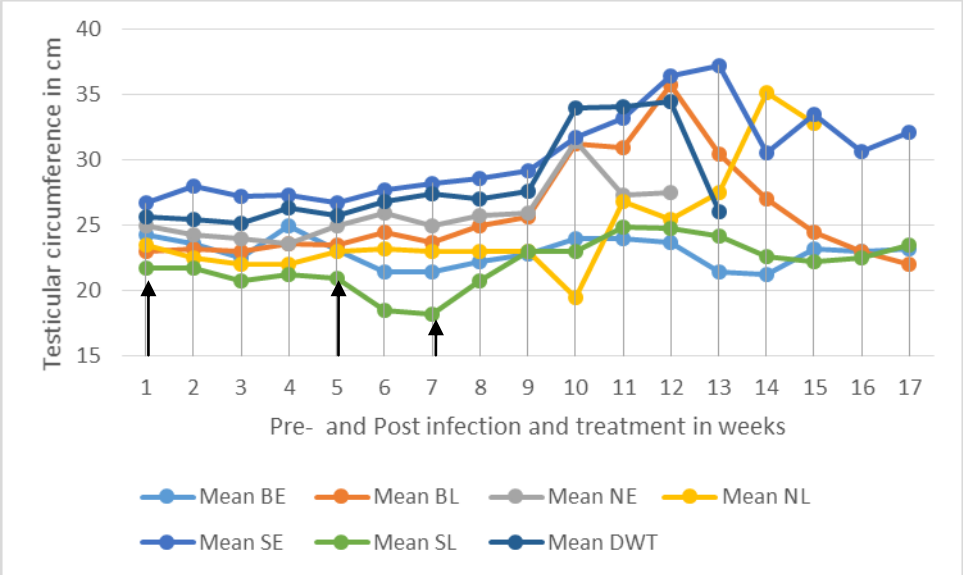

Fig. 4: Testicular circumference of *T. brucei* infected and treated rams. Arrows (↑) indicate early (E) and late (L) Berenil (B), Novidium (N) and Samorin (S) treatments.

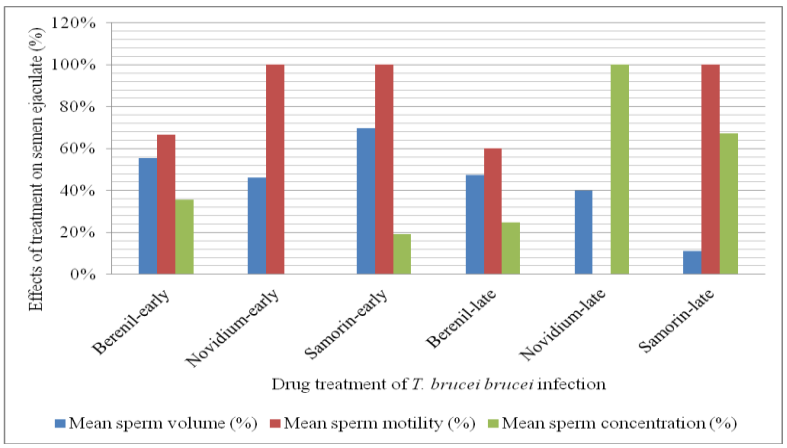

Figure 5: Effects of treatment on decreases in semen characteristics of *T. b. brucei* infected rams (group A)

Table 1: Sperm counts of *T. brucei* infected and treated group-A four weeks to end of experiment

	Weekly post-treatment sperm counts			
Treatment	**14th week**	**15th week**	**16th week**	**17th week**
Berenil early	$1.95x10^6$	$0.86x10^6$	-	$1.67x10^6$
Berenil late	1.13×10^6	$2.33x10^6$	$0.51x10^6$	$1.41x10^6$
Novidium early	-	-	-	-
Novidium late	-	-	-	-
Samorin early	$1.6X10^4$	-	-	-
Samorin late	$0.35x10^6$	-	-	-
Control	$3.1x10^6$	$4.6x10^6$	$2.4x10^6$	$2.3x10^6$

Group B (T. congolense) Semen and Ejaculate Data:

There was no late treatment as the experimental animals in the group died without treatment (DWT). The mean±stdev of testicular circumference were 25.8±0.06 and 27.5±1.0 at pre- and post-infection. There were significant increases at post-treatment with the three drugs and except Berenil, increased testicular circumference apparently recuperated up to pre-infection levels as shown on Fig. 6. The mean testicular

circumference of control rams had no significant change from start to finish with a range of 21.9-28.9 and a mean±stdev of 24.6±1.2 inches. A decrease of 11.43%, from 0.70±0.14 pre-infection to 0.62±0.17 post-infection in semen volume was recorded. Cessation of semen release occurred at 6^{th}, 9^{th} and 12^{th} week post-treatment with berenil, novidium and samorin, respectively. Sperm counts were lowered by 35.7% and 24.8% for post-infection and post-treatment with Berenil. In Novidium subgroup, the sperm concentrations were moderately and slightly increased, while Samorin treatment effected only moderate improvement. Sperm counts were $7.12x10^4$, $1.18x10^4$ and $4.18x10^4$ before death for the three drugs respectively.

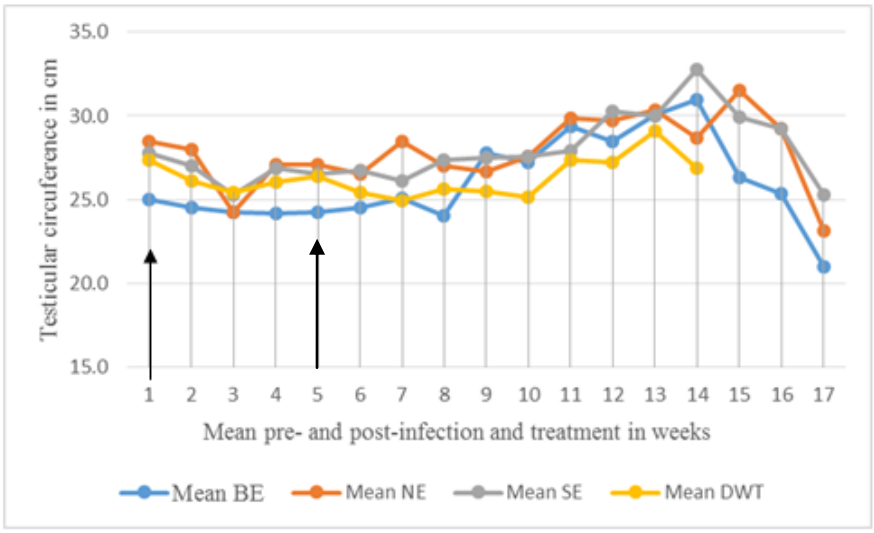

Fig. 6: Testicular circumference of *T. congolense* infected and treated rams. Arrows (↑) indicate infection and early (E) Berenil (B), Novidium (N) and Samorin (S) treatments. DWT represent experimental animals that died without treatment

Group C (T. vivax) Semen and Ejaculate Data:

The mean±stdev of testicular circumference slightly increased from 23.6±0.7 to 24.2±2.2 ($p>0.05$). At Post-treatment the mean±Stdev testicular circumference were 27.4±1.5, 27.0±1.1 and 28.3±3.1 for early, 24.9±2.0, 23.5±2.3 and 26.4±0.9 for late treatment with Berenil, Novidium and Samorin. Bimodal changes in testicular circumferences at post-treatment had significant increases in Samorin and Berenil subgroups ranged from 20.1 to 32.0 and 24.3 to 31.5 cm and Novidium had 26.5 cm (with a range of 25.3 to 28.8) before death as shown on Fig. 7. There was significant decrease in semen volume of 25.35% from 0.71±0.15 pre-infection to 0.53±0.1 post-infection. Berenil and samorin treated subgroups had moderate level of semen volume than the Novidium early treatment as against the sustained high level maintained by Samorin, followed by Novidium and Berenil had the least as shown on Fig. 8.

Post-Treatment Sperm Morphological Characteristics Data:

All the infected animals showed gross abnormalities of defective sperm cells with varying degrees of tail-less head, enlarged head, picnotic head, cytoplasmic droplets, absence of spermatocore, low sperm count and invariably aspermia before death were observed. Overall, none of the three drugs could fully regenerate semen and ejaculate abnormalities to pre-infection levels.

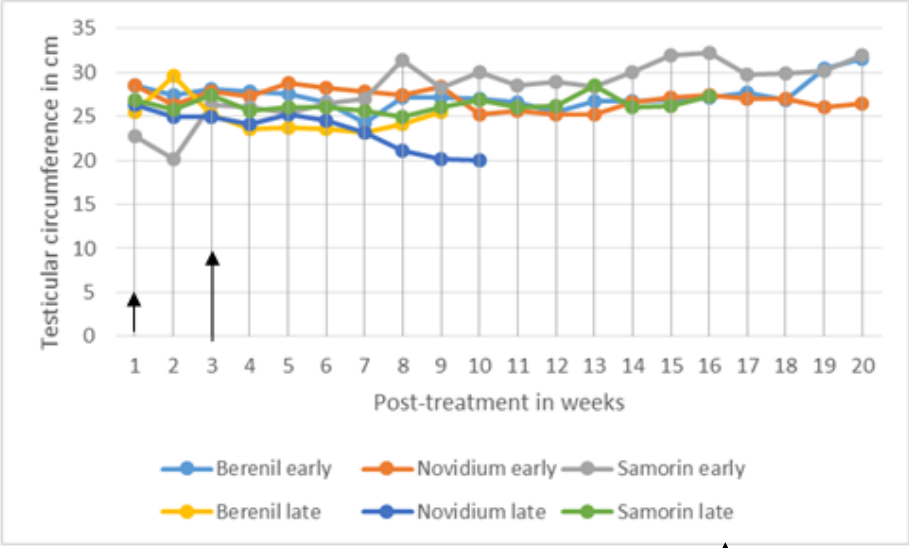

Fig. 7: Testicular circumference of *T. vivax* infected and treated rams. Arrows (↑) indicate infection, early and late treatments.

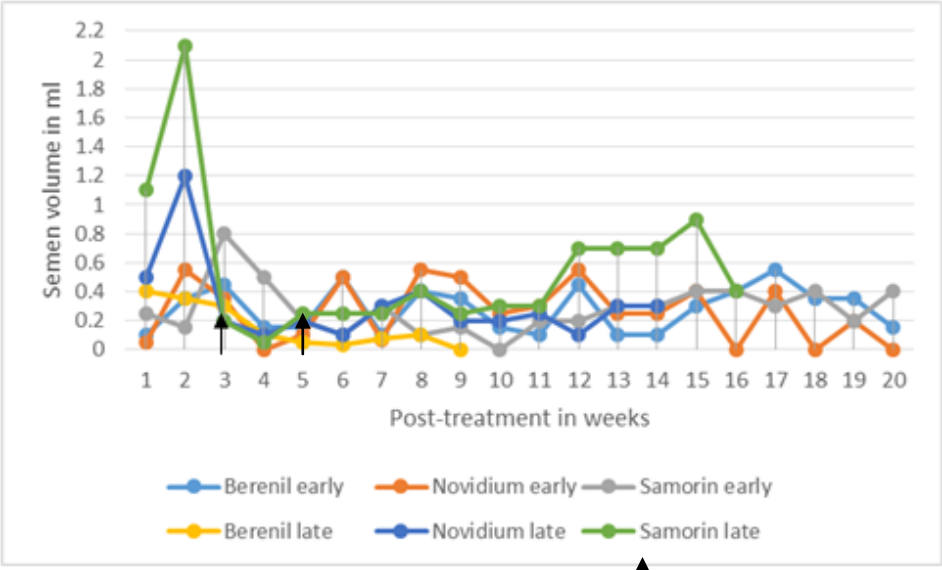

Fig. 8: Semen volume of *T. vivax* infected and treated rams. Arrows (↑) indicate early and late post treatment with Berenil, Novidium and Samorin treatments.

IV. Discussion

Effect of chemotherapeutic treatment using Berenil, Novidium and Samorin on clinical, ejaculate and semen characteristics were studied. Comparison of early with late treatments were made for *T. brucei brucei, T. congolense and T. vivax* isolates syringe inoculated into matured rams that fully established infections within 3-4 days. Apparent absence of trypanosomes in the blood prior to late treatment of *T. brucei brucei* was a clear demonstration of both fluctuating parasitaemia and possible tissue invasiveness of the species are well documented in the literature. The parasites caused various obvert clinical signs leading to terminal course of the disease, which resulted in the death of all infected rams. It was very obvious that *T. congolense* parasite appeared more pathogenic and more devastating than the other two species. This finding tallied with early observation made by (15) among experimental infected bulls.

Status of anaemia was assessed by RBC counts and percentage PCV, is one of the clinical outcomes of trypanosomiasis that has been widely reported (16). The RBC outcome of T. b. brucei infected group compared very much with that of post-treatment PCV values reported by (17). It has been suggested that the mechanisms of red cell destruction in trypanosome infections are complex and may vary with the species of trypanosomes, the host and the stage of infection (18). Among the major causes of anaemia in African trypanosomosis at the early stage is said to be due to direct parasite consequence and late stage as a result of erythrocyte hemolysis and erythrophagocytosis are underlining factors (19). In this study, we found that the ability to recover from

observed decreases in RBC counts was more effective in Berenil treatment, followed by Samorin and Novidium. There were apparent loss in bodyweight and condition recorded among the experimental animals were far more than the uninfected control rams.

The acute course of infection was exemplified by 62.5% death of animals in group B infected with *T. congolense* before late treatment. This corroborated finding of others that *T. congolense* was more pathogenic than *T. vivax* reported by Sekoni *et al*. (8); in bulls by Sekoni *et al*. (20, 21) and *T. brucei*. This finding was also in agreement with (15) that recorded improvement on reduced sperm motility and concentration, an increased percentage of dead spermatozoa and abnormal sperm morphology. The authors were of the opinion that recovery was faster in *T b. brucei* than *T. vivax*. Observed abnormalities in spermatozoa are similar to those observed in Zebu by (9, 10). None of the three drugs either administered at early and late (4 and 6 weeks) post-infections could restore the gross semen and ejaculate characteristics. Whether the drugs also have synergistic effect on sperm characteristics; concentration, volume and motility as observed for diminazine aceturate and ceftriaxone by (22) remained unknown.

Our observations in semen and ejaculate characteristics were similar to those observed in Yankassa rams (23) and rabbits (24) had reported drastic and progressive deterioration in semen quality in all infected rams manifested by a decrease in volume or cessation of semen production, oligozoospermia, a sharp decrease in progressively motile sperm, elevated numbers of dead (eosinophilic) sperm and 100% morphological abnormalities of sperm in most animals. The rams were all deemed unfit for breeding by 3 weeks post-infection. Observed changes in sperm morphology, testicular circumference and semen reaction time may be due to reported underlying factors described (25). Among which are necrosis of the interstitial tissue characterized by destruction of cellular structures. Severe testicular degeneration manifested by loss of tissue architecture and infiltration with macrophages, neutrophils, lymphocytes and plasma cells. Threat of Trypanosomosis remains major impediment to actualizing the important contribution of small ruminants (26) serving as source of meat protein and cash income in sub-Saharan Africa still remain. Attempt to apply chemotherapy in the management of small ruminants to improve health and productivity should target both sexes. It was observed from this study, the inability of any trypanocides to restore clinical signs of anaemia, semen and ejaculate quality and quantity to normal pre-infection levels. The finding are similar to that of (27, 28, 29) abnormalities in pregnancy of experimental infected animals.

Inference drawn from the findings of this study, support the conclusion that prompt management of the disease is to ensure first and foremost prevention of infection by avoiding animal-fly contact. The need for regular routine surveillance to engender early case detection accompanied with effective chemotherapeutic intervention cannot be underscored. It appeared that Samorin and Berenil administered much early after infection may assuage the anaemia, ejaculate and semen abnormalities and the general poor body conditions associated with the disease. More importantly, early treatment is the panacea to prevent death particularly, when a virulence trypanosome species is involved as may be the case with the *T. congolense* used in this study.

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Conflict Of Interest

This is to state that the authors has no affiliation with any of the manufacturers of the drugs used in this project. The project was funded from grants from International Foundation for Science (IFS) to H.I. Edeghere. Hence, there is no conflict of interest whatsoever by any of the authors who were all staff of NITR, a Public owned non-profit research institute. Conclusion and inference made were based purely on data generated and analysed following our research design and protocol approved by the funding agency and the institute.

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