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Research Article

Semen Characteristics of Rabbit Bucks Experimentally Infected with *Trypanosoma brucei brucei* in Zaria, Nigeria

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Abstract: Twenty adult rabbit bucks allocated into two groups of ten each as control group A and infected group B was studied for semen characteristics of rabbit bucks experimentally infected with *T. brucei brucei* for 12 weeks. Group B rabbits were infected with 1 mL of the parasites containing 1×10^6 trypanosomes, while group A bucks were left uninfected. Semen was collected weekly between 9.00-11.00 h and semen samples evaluated for colour, pH, motility, volume, morphology, concentration and spermatozoa live dead ratio. The control bucks had total mean semen volume of 0.52±0.05 mLs, while the infected bucks had mean value of 0.43±0.08 mLs. Semen motility decreased from week six post infection giving mean values of 64.67±4.83% for control group and 45.20±5.79% for infected group. Spermatozoa concentration decreased from five weeks post infection to ten weeks post infection. Control group had highest mean spermatozoa concentration of $165.80\pm23.13\times10^6$/mL at week five and lowest mean of $50.80\pm17.81\times10^6$/mL at week one. Spermatozoa morphology had a mean value of 38.73±1.21% for normal cells and 17.84±1.32% for the infected group. Percentage live sperm cell ratio for control group had live mean values of 59.33±2.21% while infected group had mean values of 48.11±5.86%.

Keywords: Spermatozoa, rabbit bucks, *trypanosoma brucei brucei*

INTRODUCTION

African Animal Trypanosomosis (AAT) also known as Nagana is a Zulu word meaning "to be depressed" (WHO, 2001; Courtin *et al*., 2008). It is a disease complex caused by tsetse-fly transmitted *Trypanosoma congolense*, *T. vivax*, *T. brucei* and *T. simiae* or mixed infections with one or more of these trypanosomes of the family Trypanosomatidae, genus *Trypanosoma* (Kamuanga, 2003; Courtin *et al*., 2008). Animal trypanosomosis in Africa poses one of the most serious veterinary problems in the world causing a major constraint to the agricultural and socio-economic development of tropical Africa (Gasser, 1963; Ilemobade, 1981; Llewelyn *et al*., 1987; Ikede, 1989). While most other animal diseases have been successfully controlled during this century, trypanosomosis continues to pose a major threat to animal production in sub-Saharan Africa (Pepin and Meda, 2001; WHO, 2001; Kamuanga, 2003; Bawa *et al*., 2005), despite the age-long attempts to control it Omotainse *et al*. (2000).

Tsetse fly (Glossina), the vector of trypanosomosis occurs only in Africa and about forty countries in Africa are affected by the disease, where it has contributed to gross reduction in animal protein available to man (Sekoni, 1994). The risk of trypanosomosis in most of these areas prevents farmers from keeping cattle and small ruminants thereby accounting for Africa's low livestock productivity. The present study was designed to determine the effects of trypanosome infection on semen characteristics of rabbit bucks experimentally infected with *Trypanosoma brucei brucei*.

MATERIALS AND METHODS

Twenty domesticated adult rabbit bucks with an average weight of 2.0±0.8 kg were acquired from a rabbitry within Zaria metropolis of Kaduna State. The rabbit bucks were kept in individual fly proof cages and given access to fresh grass, growers mash and water was provided *ad libitum*.

The rabbit bucks were allowed to acclimatize for 14 days thereafter; they were examined, screened, dewormed with Levamisole Hcl (0.1% injection subcutaneously) and treated for other diseases such as *Eimeria staedia* with Embazine ® forte (1g/5kg) before the commencement of the experiment.

The rabbit bucks were randomly assigned into two groups; control and infected consisting of ten rabbit bucks respectively.

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Experimental infection of the animals: Stabilates of *T. brucei brucei* were acquired from the Department of Parasitology and Entomology of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, who also sourced it from National Institute of Trypanosomosis Research, Kaduna state. Before infecting the rabbits, the trypanosomes were maintained by serial syringe passages in white rats, and periodically checked for the viability of the parasite. Blood was obtained from the passaged rats by tail bleeding into normal saline and the parasitaemia adjusted to 1×10^6 trypanosomes per milliliter (mL) by the method of Herbert and Lumsden (1976). Each rabbit in Group B was inoculated intraperitoneally with 1ml of saline diluted blood containing 1×10^6 trypanosomes *T. brucei brucei*, while Group A rabbits served as uninfected control.

Semen collection and evaluation: Bucks were on a weekly semen collection regimen for two weeks pre-infection and ten weeks post infection during the course of the experiment using a specially designed artificial vagina for rabbits by IMV Technologies 2911 model. Lubrication with non perfumed petroleum jelly in the end where intromission of the penis occurs is essential (Dorji, 2012). The rabbit doe was restrained firmly in place allowing for the male exhibit his courting behavior. The buck was allowed few false mounts, after which the person with the artificial vagina collected the ejaculate by directing the penis into the artificial vagina. The test tube containing the ejaculate was carefully handled against foreign bodies, contamination and direct sunlight and cold temperatures (Nuti, 2012). The ejaculated sample was subjected to routine semen evaluations such as semen volume, pH, colour, spermatozoa concentrations, motility, live/dead ratio and morphology (Zemjanis, 1970).

The volume of the ejaculate was easily measured according to the method described by Mickelsen *et al*. (1991). Opacity and color of the samples were coded according to Brito *et al*. (2002).

Sperm motility was assessed subjectively for meaningful results (Brito *et al*., 2002). Gross motility was assessed based on the swirling activity observed in a drop of semen on a pre warmed clean glass slide and viewed at a low power magnification (x 40) (Al-Ghalban *et al*., 2004). Concentration of spermatozoa was determined by the use of the improved Neubaeur haemocytometer according to the method of Coffin (1953) and Rekwot *et al*. (1994, 1997).

The live/dead ratio was determined as described by Johnson (1994), Esteso *et al*. (2006) and Koonjaenak (2006). The spermatozoa abnormalities were determined as described by (Koonjaenak, 2006).

Statistical analysis: Data generated on haematological parameters and semen characteristics (colour, volume, pH and spermatozoa concentration were expressed as mean±Standard Error of the Mean (SEM). Data on motility and percent live/dead of the spermatozoa were expressed as percentages and mean±SEM. In all cases, unpaired t-test was used to test for differences between groups using Graph pad prism version 5.0. Values of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Semen characteristics: Mean semen volume of infected and control rabbit bucks are shown in Table 1. There was statistical significant difference ($p<0.05$) in the volume of semen collected in the bucks at weeks 9 and 10. During the course of the experiment the control bucks had a total mean value of 0.52±0.05 mLs, compared to the infected bucks with a total mean value of 0.43±0.08 mLs. The semen volume progressively decreased from week four to week ten post infection, with a mean value of 0.41±0.02 mLs and 0.26±0.10 mLs for the control bucks and infected bucks respectively during the period of decrease.

There was a consistent decrease in the semen motility from week six post infection, with a significant difference of ($p<0.05$) and mean value of 64.67±4.83% for control group and 45.20±5.79% for infected group. The highest motility was at the beginning of the experiment giving mean values 85.30±4.55% and 77.50±9.17% for control group and infected group respectively, while the lowest motility was at the end of the experiment with mean values of 53.00±12.28% for

Table 1: Mean (±SEM) semen volume (mLs) of infected and non infected rabbit bucks pre and post infection

		Control (n = 10)		Infected (n = 10)	
Period	Time (Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	0.48	0.18	0.44	0.19
	2	0.53	0.19	0.56	0.12
Week of infection	3	0.72	0.16	0.67	0.16
post infection	4	0.76	0.17	0.63	0.14
	5	0.49	0.18	0.54	0.19
	6	0.44	0.07	0.54	0.15
	7	0.40	0.07	0.45	0.13
	8	0.35	0.08	0.17	0.06
	9	0.46	0.09^a	0.08	0.05^b
	10	0.42	0.12^a	0.06	0.05^b

[ab] Means in the same row of each parameter with different superscript letters are statistically ($p<0.05$) different

Table 2: Mean (±SEM) spermatozoa motility (%) of infected and non infected rabbit bucks pre and post infection

		Control (n = 10)		Infected (n = 10)	
Period	Time (Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	86.60	3.510	44.25	15.25
	2	73.50	2.240	40.00	9.780
Week of infection	3	85.30	4.550	56.11	7.670
Post infection	4	71.90	8.37[a]	31.00	13.31[b]
	5	73.00	10.81	77.50	9.170
	6	60.00	8.270	37.50	10.81
	7	70.00	9.97[a]	26.00	9.66[b]
	8	62.50	8.51[a]	37.00	6.51[b]
	9	68.50	8.920	61.90	12.51
	10	53.00	12.28[a]	22.00	4.61[b]

[ab] Means in the same row of each parameter with different superscript letters are statistically ($p<0.05$) different

Table 3: Mean (±SEM) spermatozoa concentration ($\times10^6$/mL) of control and infected rabbit bucks pre and post infection

		Control (n = 10)		Infected (n = 10)	
Period	Time (Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	50.800	17.81	45.70	17.64
	2	50.800	17.81	45.70	17.64
Week of infection	3	50.800	17.81	45.70	17.64
Post infection	4	109.00	8.46[a]	39.30	23.79[b]
	5	165.80	23.13[a]	87.80	25.26[b]
	6	134.20	9.92[a]	45.40	19.54[b]
	7	118.70	13.51[a]	32.60	8.57[b]
	8	117.50	5.82[a]	42.80	23.45[b]
	9	113.80	19.40[a]	32.00	10.61[b]
	10	143.30	13.80[a]	38.70	30.11[b]

[ab] Means in the same row of each parameter with different superscript letters are statistically ($p<0.05$) different

Table 4: Mean (±SEM) % values of spermatozoa morphological abnormalities of infected and non-infected rabbit bucks pre and post infection

	Control n = 10		Infected n = 10	
Variable	Mean	SEM	Mean	SEM
Normal spermatozoa cells	38.73	1.21	17.84	1.32
Detached heads	9.460	1.57	3.850	0.40
Folded tails	8.430	1.05	3.920	0.54
Bent tails	11.35	0.90	6.360	1.18

control group and 22.00±8.95% for infected group as shown in Table 2.

Table 3 shows spermatozoa concentration which progressively decreased from five weeks post infection to ten weeks post infection, with significant difference ($p<0.05$). Control group had the highest spermatozoa concentration of 165.80±23.13×10^6/mL at week five and lowest of 50.80±17.81×10^6/mL at week one. Infected group had the highest sperm concentration of 87.80±25.26×10^6/mL in the fifth week post-infection. Thereafter, there was a continual decreasing fluctuation with mean values of 45.40±19.54×10^6/mL at week six till the end of the experiment giving mean values of 38.70±30.11×10^6/mL at week ten post infection.

Experimental trypanosomosis also had effects on sperm morphology, with significant difference ($p<0.05$) and consistent fluctuations. The control group had a mean value of 38.73±1.21% for normal cells and 17.84±1.32% for the infected group, detached heads had a mean value of 9.46±1.57% and 3.85±0.40% for control and infected groups respectively. Folded tails had mean values of 8.43±1.05% for control group and 3.92±0.54% for infected group. The mean values for bent tails for the control group was 11.35±0.90% and 6.36±1.18% for infected group. This is illustrated in Table 4.

There was statistical significant difference in the percentage live sperm cell ratio at weeks nine and ten. Control group had live mean values of 59.33±2.21% compared to infected group that had live mean values of 48.11±5.86% as presented in Table 5.

The *Trypanosoma brucei brucei* used in this study caused clinical trypanosomosis in all the infected rabbits showing a marked pathogenicity in consistence with the findings in *Trypanosoma congolense* infected rabbits (Takeet and Fagbemi, 2009), *Trypanosoma congolense* infection in rats (Egbe-Nwiyi *et al*., 2005) and *Trypanosoma brucei brucei* infection in rabbits (Orhue *et al*., 2005). The infection did not cause any death which is presumed to be due to the strain of the parasite used in this work.

The infected rabbit bucks had an average prepatent period of six days post infection which gradually rose to a peak by the 10th day post infection thereby followed by a fluctuating parasitaemia and anorexia. Emaciation in the infected bucks was consistent with the findings of Ogunsanmi *et al*. (1994) and Omotainse *et al*. (1994) which was reported to be as a result of the trypanolytic crisis which occurs in the peripheral blood of the infected host in the early stage of the disease (Seifert, 1996). Ogwu (1983) and Agu and Bajeh (1986) reported an eventual disappearance of parasites from peripheral circulation which was also observed during the course of this experiment 28 days post infection.

Table 5: Mean (±SEM) live spermatozoa (%) of infected and non infected rabbit bucks pre and post infection

		Control (n = 10)		Infected (n = 10)	
Period	Time (Weeks)	Mean	SEM	Mean	SEM
Pre-infection	1	58.00	8.670	58.00	8.670
Week of infection	2	58.00	8.670	58.00	8.670
Post infection	3	68.00	4.670	56.00	6.860
	4	67.00	7.750	66.00	2.210
	5	61.00	7.060	57.00	6.510
	6	66.00	7.770	56.00	7.180
	7	51.00	10.27	30.00	10.43
	8	51.00	10.27	41.90	9.000
	9	54.00	10.13[a]	11.00	7.06[b]
	10	67.00	7.75 [a]	11.00	7.06 [b]

[ab] Means in the same row of each parameter with different superscript letters are statistically ($p<0.05$) different

There are direct and indirect detrimental effects of trypanosomosis on reproduction in male and female animals. It is well established that most tissues and organs are damaged during the course of infection (Morrison *et al.*, 1981). The reproductive system which is controlled by a well co-ordinated and efficient neuro-endocrine system (Nalbandov, 1976), has been reported in literature to be affected in both natural and experimental trypanosome infections (Isoun and Anosa, 1974; Ikede *et al*., 1988). The hormones involved in reproduction originate in three principal structures which are the hypothalamus, the pituitary and the gonads. Any detrimental effect on any of the organs would result in detrimental effects on reproduction. It has been well established that severe degenerative changes of the interstitial cells of leydig within the testes takes place, and this cells are responsible for the production of testosterone, which is the hormone responsible for libido, anabolic effects and secondary male characteristics (Sekoni, 1991). Therefore, poor libido or lack of libido in all male species with trypanosomosis is a possibility, which can be supported by the observations of Mulligan *et al.* (1970) who reported that chronic *T. congolense* infections in cattle may lead to loss of libido, interference with reproduction and delayed puberty in calves. Waindi *et al*. (1986) found low levels of plasma testosterone in goats infected with *T. congolense.*

Hoogenboezeom and Swanepoel (2001) reported that testicular degeneration might be due to nutritional deficiencies and management related factors. Degenerative changes in the seminiferous tubules, testicular degeneration, low semen output, low spermatozoa concentration, high percentage of dead spermatozoa and spermatozoa abnormalities have also been reported to be caused by heat stress (Kumi-Diaka *et al*., 1981; Sekoni *et al*., 1991; Mamabolo, 1999; Hoogenboezeom and Swanepoel, 2001).

Spermatozoon is produced from the seminiferous tubule of the test is in a complex process known as spermatogenesis (Roosen-Runge, 1977). The process of spermatogenesis is under the control and influence of both the endocrine and certain external factors such as photoperiod and presence of noxious agents (Ortavant *et al*., 1977; MacDonald and Pineda, 1989). Any effect such as invasion of the tissues involved in spermatogenesis by pathogens or other structures regulating the spermatogenic tissues will result in reduction of ejaculate qualities (White, 1933; Noakes *et al*., 2001). Since *Trypanosoma brucei brucei* is tissue invasive, the observed reduction in ejaculate characteristics can be linked to the effects of the infection.

Some pathogenic effects on the male reproductive systems have been reported. The reduced libido, increased scrotal diameters, scrotal inflammations, alopecia, periorchitis, severe testicular degenerations, balanitis, abnormal spermatogenesis in the infected rabbit bucks agrees with the findings of trypanosoma infected animals as reported by Ikede and Akpavie (1982) and Sekoni (1991, 1992), which may be influenced by the effect of trypanosomes on the test is affecting the leydig cell steroidogenesis (Sekoni *et al*., 1988a) through the indirect effects of pyrexia and the accompanying waves of increasing parasitaemia during infection and increased scrotal temperatures (Mutayoba *et al*., 1995).

There were genital lesions within 13-29 days post infection such as alopecia, periorchitis, epididymitis, severe testicular degeneration and haemorhage and deteriorated semen characteristics which agrees with reports by Isoun and Anosa (1974) and Anosa and Isoun (1980).

Agu and Bajeh (1986) reported deteriorated semen characteristics in rams infected with *T. vivax* which was a consistent finding in this study. There was a consequent reduction in the volume of semen collected and also a reduction in the gross motility and concentration of the sperm cells with an increased number of morphological defective sperm cells such as the detached heads, free tails, bent tails and coiled tails which agrees with the findings of Akpavie *et al*. (1986), Sekoni *et al.* (1990a) and Sekoni (1993) who reported aspermatogenesis and various sperm abnormalities in trypanosomosis infected animals.

CONCLUSION

In this study, spermatozoa motility and concentration were significantly reduced, while morphological abnormalities of spermatozoa increased. These characteristics of the ejaculate are not only vital

to the physiological functions of the spermatozoa, which include spermatozoa migration and fertilization, but are also considered in the evaluation of an animal for breeding soundness. This means that either or both natural and experimental *Trypanosoma brucei brucei* infection of bucks will result in reduced spermatozoa migration and fertilization and also the rejection of such bucks as potential breeders.

Conflict of interest: The authors declare no conflict of interest.

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