Effect of chemotherapy on elevated ejaculation time and deteriorated semen characteristics consequent to trypanosomosis in zebu x Friesian crossbred bulls

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Key words

Zebu x Friesian bull – *Trypanosoma vivax* – *Trypanosoma congolense* – Semen – Chemotherapy – Infertility – Nigeria.

Summary

The effect of the trypanocide Novidium® on elevated ejaculation time and deteriorated semen characteristics was studied in zebu x Friesian crossbred bulls infected with *Trypanosoma vivax* or *T. congolense*. The bulls were divided into three groups, A, B and C. Groups A and B comprised four bulls each, while group C had two bulls which served as control. Groups A and B were infected with 2 x 10^6 *T. vivax* or *T. congolense,* respectively, while group C served as uninfected control. Blood samples from treated bulls were all negative for trypanosomes four days postchemotherapy. The body temperature of the animals normalized. Clinical signs associated with trypanosomosis, such as anemia, cachexia and ruffled hair coat, disappeared gradually in treated bulls. There was only a marginal improvement in the semen characteristics of a bull infected with *T. vivax* at 24 weeks postchemotherapy. However, all bulls infected with *T. vivax* or *T. congolense* irrespective of chemotheraphy still had poor semen characteristics manifested by all or some of the following: decreased volume of semen, oligospermia, azoospermia and elevated incidence of sperm morphological abnormalities. They were thus unfit for breeding. *T. congolense* was more pathogenic than *T. vivax* in the study. Therefore, chronic trypanosomosis either due to *T. vivax* or *T. congolense* could be an important causative agent of infertility or sterility in zebu x Friesian crossbred bulls.

■ INTRODUCTION

Pathogenic animal trypanosomes such as *Trypanosoma congolense*, *T. vivax*, *T. brucei*, *T. evansi*, *T. simiae*, *T. suis*, *T. equinum* and *T. equiperdum* are still very important economic limitations to the livestock industry in most African countries. *T. vivax* and *T. congolense* are the most important pathogenic trypanosomes in cattle. Most of the studies on the pathogenicity of trypanosomes in sub-Saharan Africa were carried out on the purebred indigenous species of livestock. Many studies have associated trypanosomosis with reproductive disorders in animals (9, 16). In addition, some studies were carried out to find out the efficacy of chemotherapy in the amelioration of the reproductive disorders caused by trypanosomosis (8, 18). A study on the effect of Novidium® chemotherapy on the elevated ejaculation time and deteriorated semen characteristics in purebred zebu bulls showed that chemotherapy was of little beneficial effect, the bulls were still unfit for breeding even at 18 weeks postchemotherapy due to poor semen characteristics (18).

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A previous investigation showed that *T. vivax* and *T. congolense* infections in zebu x Friesian crossbred bulls caused a drastic elevation of ejaculation time and deterioration of semen characteristics. Therefore, the objective of this investigation was to study the effect of Novidium® chemotherapy on the elevated ejaculation time and deteriorated semen characteristics of zebu x Friesian crossbred bulls, an area where there is a dearth of information.

■ MATERIALS AND METHODS

Ten healthy zebu x Friesian crossbred bulls aged between 3 and 5 years and with normal semen characteristics during the previous 18 months were used. The bulls were divided into three groups (A, B and C), completely separated from each other in individual compartments in a mosquito-proof building in order to prevent mixed infections and spread of the disease to non- experimental animals by hematophagous flies. The trypanosomes used in the study were isolated in Northern Nigeria from natural infection in cattle by the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria (12). Bulls in groups A and B were infected with *T. vivax* (strain Y58) and *T. congolense* (strain 2295), respectively, while group C served as control. Groups A and B comprised four bulls per group, while group C comprised two bulls. Each bull was inoculated with 2 ml of blood containing approximately 2 x 10^6 trypanosomes. Animals were examined twice weekly to determine rectal temperature, parasitemia, packed cell volume (PCV) and hemoglobin concentration. Wet blood smears were used to detect the presence of trypanosomes and the parasitemia was estimated by the hematocrit centrifuge technique (21). Total plasma protein was determined according to the method of Coles (5) to evaluate the nutritional status of the animals.

Semen was collected once weekly by rectal massage as described by Arthur (3). Ejaculation time, defined as the time between the onset of rectal massage to ejaculation, was recorded at each semen collection. Semen analysis was done according to standard techniques (7, 15). The semen characteristics evaluated included volume, sperm concentration, and incidence percentage of abnormalities (such as detached heads, acrosome, cytoplasmic droplets, midpiece and the tail); they were estimated after the semen was diluted with buffered formal saline. At least 500 spermatozoa per slide under the phase microscope were counted (7, 14, 17). Semen samples stained with eosin-nigrosin were used to determine the morphological abnormalities of the sperm head (11, 14). Five hundred spermatozoa were counted.

Two bulls from each of the infected groups were treated once with Novidium® (homidium chloride, May & Baker) at 1 mg/kg body weight by deep intramuscular injection at the end of the twelfth week postinfection while the remainder were left untreated. Examination of all animals was continued until 24 weeks post-treatment. Student's t-test was used to compare differences between each group and the control. The degree of significance is noted by one, two or three asterisks (*P < 0.05; **P < 0.01 ***P < 0.001).

■ RESULTS

Following treatment with Novidium®, trypanosomes disappeared from the peripheral circulation within five days. Depressed blood parameters such as PCV, hemoglobin, total plasma protein gradually normalized within the next 12 weeks. Clinical signs associated with trypanosomosis, such as lethargy and cachexia, also disappeared within the same period.

Ejaculation time

The preinfection mean ejaculation times in the three groups (A, B and C) of bulls were low. There was a progressive increase in mean values in infected animals that differed significantly from the mean value for the control, which remained low throughout the duration of the study (Table I). At 24 weeks postchemotherapy, all bulls previously infected with trypanosomes (groups A and B) irrespective of chemotherapy (groups A^{+N} and B^{+N}) still had elevated ejaculation times which differed significantly from the control bulls (C) that had low mean values (Table I).

Semen characteristics

The preinfection mean volumes of semen for groups A, B and C bulls were within normal range (Table II). There was a progressive decrease in the mean volumes of infected bulls within 12 weeks postinfection. The mean value for control was normal and differed significantly from the infected groups (Table II). Also, at 24 weeks postchemotherapy, the mean volumes of infected bulls, irrespective of chemotherapy, remained low and differed significantly from the values for the control (Table II).

The mean sperm concentration for the groups (A, B and C) were within normal range prior to infection of groups A and B. There was a progressive decrease in the mean sperm concentration of infected bulls within 12 weeks postinfection. At 24 weeks postchemotherapy, only the treated bulls in the *T. vivax* group had marginal increase (improvement) in sperm concentration. All other bulls (i.e. A, B and B^{+N}) had low sperm concentration (oligospermia), which differed significantly from control. The results are summarized in Table III.

Prior to infection, all the bulls (groups A, B and C) had very low total sperm morphological abnormalities within the range of acceptable values for fertile bulls. Following infection, there was a drastic and progressive elevation of total sperm morphological abnormalities in all infected bulls (groups A and B) within 12 weeks postinfection. Between the eighth and twelfth week of infection, all the infected bulls had 100% of total sperm morphological abnormalities. At 24 weeks postchemotherapy all the infected bulls, both treated and untreated (A, A^{+N}, B and B^{+N}) still had highly elevated sperm morphological abnormalities, which differed significantly from values for the control (C). The results are summarized in Table IV. The preinfection mean values for groups A and B bulls, when their ejaculation times were short and semen characteristics very good, differed significantly from postinfection mean values when their ejaculation times were elevated and semen characteristics had deteriorated. Also, postinfection mean values of groups A and B, and post chemotheraphy mean values of groups A^{+N} and B^{+N} differed significantly from mean values for control.

■ DISCUSSION

The doses (number of trypanosomes) at which animals are infected under natural conditions unlike experimental conditions are highly variable and are determined by many environmental factors including the species of the transmitting hematophagous flies. One of the advantages of experimental studies such as this investigation is that the doses of trypanosomes used for the infection of experimental animals could be predetermined. A wide range of doses of trypanosomes (10^4 – 166 x 10^6) have been used for studies on the pathogenicity of trypanosomes on reproduction in animals (2, 4, 6, 10, 12, 13).

The results obtained in this study, even with the low dose of trypanosomes used to infect the bulls, showed that trypanosomosis due to *T. congolense* was more pathogenic than trypanosomosis due

to *T. vivax* for all parameters studied in the zebu x Friesian crossbred bulls. Also, trypanosomosis was more severe in the zebu x Friesian crossbred bulls in this investigation than it was previously reported in purebred zebu bulls (18, 19, 20). However, trypanosomosis due to experimental *T. congolense* infection in trypanotolerant Baoule bulls caused less severe reproductive problems with regards to the deterioration in semen characteristics (4) than in zebu bulls (20) and in the zebu x Friesian crossbred bulls in the present investigation. In Baoule bulls, there was an apparent self cure as normal semen characteristics were restored five to six weeks after the parasites disappeared from the blood (i.e. 13–15 weeks postinfection).

Novidium® chemotherapy, which cleared the peripheral blood of trypanosomes within four days, did not have enough significant

Table I

Mean ejaculation time (seconds) in bulls infected with *Trypanosoma vivax* and *T. congolense* before and after treatment with Novidium® in groups A, B and C

Weeks		Subgroups:	A	A+N	B	B+N	C	Total
PI	PT	Infected:	*T. vivax*		*T. congolense*		No	8/10
		Treated:	No	Yes	No	Yes	No	4/10
		Number:	2	2	2	2	2	10
1	–12		26	20	21	25	15	
4	–8		36**	38**	36**	35**	7	
8	–4		86***	90***	96***	100***	7	
12	0		291***	120***	302***	300***	8	
16	4		240***	105***	320***	330***	14	
20	8		200***	80***	250***	300***	11	
24	12		220***	100***	240***	250***	7	
30	16		230***	100***	250***	300***	10	
34	20		220***	110***	260***	260***	8	
38	24		230***	115***	255***	300***	7	

PI: postinfection; PT: post treatment; +N: bulls treated with Novidium® at 12 weeks postinfection

Significant differences between each group and control group are marked by one, two or three asterisks; * P < 0.05; ** P < 0.01; *** P < 0.001

Number of bulls in each group = 2

Number of observations per group = 20

Table II

Mean volume of semen (ml) in bulls infected with *Trypanosoma vivax* and *T. congolense* before and after treatment with Novidium® in groups A, B and C

Weeks		Subgroups:	A	A+N	B	B+N	C	Total
PI	PT	Infected:	*T. vivax*		*T. congolense*		No	8/10
		Treated:	No	Yes	No	Yes	No	4/10
		Number:	2	2	2	2	2	10
1	–12		6.4	6.5	6.3	6.5	6.5	
4	–8		4. 5*	4.5*	4.0*	4.5*	6.2	
8	–4		4. 0**	4.0**	3.0***	3.5**	7.0	
12	0		1. 0**	1.5**	0.5***	1.5***	7.5	
16	4		0. 5***	1.0***	1.0***	1.5***	7.0	
20	8		1. 0***	1.5***	0.5***	1.7***	7.5	
24	12		0. 5***	1.5***	1.0***	1.5***	7.0	
30	16		0. 6***	1.6***	0.6***	1.4***	6.8	
34	20		0. 4***	1.5***	0.8***	1.2***	7.0	
38	24		0. 5***	1.4***	0.9***	1.3***	7.0	

PI: postinfection; PT: post treatment; +N: bulls treated with Novidium® at 12 weeks postinfection

Significant differences between each group and control group are marked by one, two or three asterisks; * P < 0.05; ** P < 0.01; *** P < 0.001

Number of bulls in each group = 2

Number of observations per group = 20

positive effect in ameliorating the elevated ejaculation time and poor semen characteristics several weeks after the termination of the investigation. Results from studies previously carried out in laboratory animals, small ruminants and pure breed zebu bulls agree with the present investigation, which revealed that trypanosomosis caused severe genital lesions which resulted in deteriorated semen characteristics (1, 2, 8, 20). Previous studies in rabbits (8) and pure breed zebu bulls (18) corroborate the findings of the present study, which has shown that resolution of lesions induced by trypanosomosis was protracted and chemotherapy with trypanocides were ineffective, while very poor semen characteristics persisted even at 18 weeks postchemotherapy. Persistence of poor semen

Table III

Mean spermatozoa concentration (x 10^6/ml) in bulls infected with *Trypanosoma vivax* and *T. congolense* before and after treatment with Novidium® in groups A, B and C

Weeks		Subgroups:	A	A^{+N}	B	$B+^{N}$	C	Total
PI	PT	Infected:	*T. vivax*		*T. congolense*		No	8/10
		Treated:	No	Yes	No	Yes	No	4/10
		Number:	2	2	2	2	2	10
1	–12		1 651	1 600	1 661	1 650	1 656	
4	–8		1 011**	1 500**	1 431*	1 400*	1 751	
8	–4		451***	350***	742***	800***	1 836	
12	0		201***	450***	157***	100***	1 871	
16	4		151***	650***	100***	150***	1 851	
20	8		170***	700***	201***	250***	1 860	
24	12		200***	710***	150***	200***	1 850	
30	16		150***	700***	140***	180***	1 900	
34	20		170***	650***	120***	100***	1 950	
38	24		150***	640***	100***	80***	1 900	

PI: postinfection; PT: post treatment; +N: bulls treated with Novidium® at 12 weeks postinfection

Significant differences between each group and control group are marked by one, two or three asterisks; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Number of bulls in each group = 2

Number of observations per group = 20

Table IV

Mean percentage total sperm morphological abnormalities in bulls infected with *Trypanosoma vivax* and *T. congolense* before and after treatment with Novidium® in groups A, B and C

Weeks		Subgroups:	A	A^{+N}	B	$B+^{N}$	C	Total
PI	PT	Infected:	*T. vivax*		*T. congolense*		No	8/10
		Treated:	No	Yes	No	Yes	No	4/10
		Number:	2	2	2	2	2	10
			6	5	5	6	6	
1	–12		48	50	41	46	5	
4	–8		100	100	100	100	4	
8	–4		100	100	100	100	4	
12	0		100	100	100	100	5	
16	4		100	100	100	100	6	
20	8		75	80	81	70	6	
24	12		70	75	75	75	6	
30	16		65	75	80	70	7	
34	20		70	70	81	75	6	
38	24		70	75	80	78	7	

PI: postinfection; PT: post treatment; +N: bulls treated with Novidium® at 12 weeks postinfection

Number of bulls in each group = 2

Number of observations per group = 20

characteristics even at 24 weeks (168 days) postchemotherapy as obtained in this study, which by far exceeded the duration or period for spermatogenesis in the bovine which is about 10 weeks, i.e. 60–70 days (15), could imply that trypanosomosis may be able to inflict permanent damage to the spermatogenic epithelium and consequently cause the stoppage of spermatogenesis.

■ CONCLUSION

Trypanosomosis had a very severe and devastating effect on reproduction in zebu x Friesian crossbred bulls within a few weeks following infection, and Novidium® chemotherapy was ineffective in reversing the deteriorated semen characteristics at even 24 weeks postchemotherapy. Therefore, chemotherapy may be of little remedy in ameliorating the associated infertility caused by trypanosomosis, especially when the genital lesions are very severe. The findings of this study may be of particular interest in pathogenic trypanosome endemic regions of Africa, especially sub-Saharan Africa, where exotic breeds of livestock are routinely imported for the crossbreeding and upgrading of the low productivity of indigenous breeds.

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Résumé

Sekoni V.O., Rekwot P.I. Effet de la chimiothérapie sur le temps d'éjaculation augmenté et caractéristiques du sperme détérioré par une trypanosomose chez des taureaux croisés zébus x Frisons

Les effets du trypanocide Novidium® sur le temps d'éjaculation augmenté et les caractéristiques du sperme détériorées ont été étudiés chez des taureaux croisés zébus x Frisons infectés par *Trypanosoma vivax* ou *T. congolense*. Les taureaux ont été divisés en trois groupes, A, B et C. Les groupes A et B comprenaient quatre taureaux chacun, alors que le groupe C comprenait deux taureaux qui ont servi de témoin. Les groupes A et B ont été infectés respectivement par 2 x 10^6 de *T. vivax* ou de *T. congolense,* alors que le groupe C a servi de témoin non infecté. Les échantillons sanguins des taureaux infectés ont tous été trouvés indemnes de trypanosomes, quatre jours après la chimiothérapie et les animaux ont retrouvé leur température corporelle normale. Les signes cliniques associés à la trypanosomose, comme l'anémie, la cachexie et le pelage piqué, ont progressivement disparu chez les taureaux traités. L'amélioration des caractéristiques des spermatozoïdes d'un taureau infecté par *T. vivax* n'a été que marginale 24 semaines après la chimiothérapie. Toutefois, les caractéristiques des spermatozoïdes de tous les taureaux infectés par *T. vivax* ou *T. congolense* ont continué d'être médiocres, que les animaux aient reçu ou non un traitement chimiothérapique. Les caractéristiques suivantes ont toutes été présentes, ou seulement en partie, chez les animaux : réduction du volume du sperme, oligospermie, azoospermie et une incidence élevée de spermatozoïdes à morphologies anormales. Ils ont été ainsi jugés inaptes pour la reproduction. *T. congolense* a été plus pathogénique que *T. vivax* dans cette étude. Par conséquent, la trypanosomose chronique à *T. vivax* ou à *T. congolense* peut être un agent important à l'origine de l'infertilité ou de la stérilité des taureaux croisés zébus x Frisons.

Mots-clés : Taureau zébu x Frison – *Trypanosoma vivax* – *Trypanosoma congolense* – Sperme – Chimiothérapie – Infertilité – Nigeria.

Resumen

Sekoni V.O., Rekwot P.I. Efecto de la quimioterapia sobre el tiempo de eyaculación elevado y características de semen deteriorado como consecuencia a una tripanosomosis en cruces de toros Cebú x Friesian

Se estudió el efecto del tripanocida Novidium® sobre el tiempo de eyaculación elevado y la deterioración de las características del semen en toros de cruces cebú x Friesian, infectados con *Trypanosoma vivax* o *T. congolense*. Los toros se dividieron en tres grupos, A, B y C. Los grupos A y B incluyeron cuatro toros cada uno, mientras que el grupo C incluyó dos toros control. Los grupos A y B fueron infectados con 2 x 10^6 *T. vivax* o *T. congolense* respectivamente, mientras que el grupo C sirvió como control no infectado. Cuatro días post quimioterapia, las muestras de sangre de los toros tratados fueron todas negativas para tripanosomas. La temperatura corporal de los animales se normalizó. Los signos clínicos asociados a la tripanosomosis, como anemia, caquexia y pelo hirsuto, desaparecieron gradualmente de los toros tratados. Hubo solo una mejoría marginal en las características del semen de un toro infectado con *T. vivax* 24 semanas post quimioterapia. Sin embargo, todos los toros infectados con *T. vivax* o *T. congolense*, independientemente de la quimioterapia, presentaron características pobres de semen, correspondientes a uno o todos los puntos siguientes: disminución del volumen de semen, oligoespermia, azooespermia e incidencia elevada de anomalías morfológicas del espermatozoide. Por lo tanto, fueron inadecuados para la reproducción. En el presente estudio, *T. congolense* fue más patogénico que *T. vivax*. Por lo tanto, la tripanosomosis crónica debida a *T. vivax* o *T. congolense* podría ser un agente causal importante de infertilidad o esterilidad en los toros de cruces cebú x Friesian.

Palabras clave: Toro cebú x Friesian – *Trypanosoma vivax* – *Trypanosoma congolense* – Semen – quimioterapia – Infertilidad – Nigeria.