Disruption of spermatogenesis in boars sub-clinically infected with *Trypanosoma brucei brucei*

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Abstract

Data from 14 crossbred (Landrace × Large white) boars aged 10–12 months were used to investigate specific germ cells and to what extent Sertoli cells are prone to sub-clinical infection with strain Y58/98 *Trypanosome brucei brucei* and effects on spermatogenesis. Boars were divided into three groups, A, B and C of 5, 5 and 4 animals, respectively. Groups A and B were infected intraperitoneally with $2.8 \times 10^6$ trypanosomes per animal. Group C consisted of intact controls. At stable sub-clinical trypanosomiasis, boars in groups A and B together with two from the controls were weighed, scrotal circumferences were measured and animals were castrated on days 56 and 84 post infection, respectively. Testes were weighed. A portion of a testis was processed for histomorphometric assessment and another portion was used to determine gonadal sperm reserves by haemocytometry. Crude cells were converted to true cells.

Sub-clinical trypanosomiasis was characterised by low live and testes weights, reduced scrotal circumference, scanty parasitemia peaks at long intervals and decreased libido. Histomorphometry of animals infected with *T. brucei brucei* revealed somniferous tubular distortion, denudation and or degeneration of germ cells and Sertoli cells leading to distortion of spermatogenesis. Spermatids and young primary spermatocytes were most prone to, while Sertoli cells and spermatogonia were least affected by sub-clinical trypanosomiasis. There was evidence of regeneration of germ cells from precursor stem cells, resulting in slightly increased gonadal sperm reserves as the post infection period increased. Infected boars may not attain original fertility levels consequently. It was concluded that boars in tropical regions that harbour endemic disease should be maintained under prophylactic conditions.

Keywords: Pig-male reproduction; Trypanosomiasis; Sub-clinical; Infection; Spermatogenesis; Fertility

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1. Introduction

Trypanosomiasis-induced infertility is a major livestock problem in species in tropical areas with endemic disease. Trypanosomes preferentially localise in gonads, where they cause severe lesions. This has been well documented for laboratory animals (Ikede and Akpavie, 1982; Anosa, 1988; Omeke and Onuora, 1992b), goats (Kaaya and Oduor-Okelo, 1980; Mutayoba et al., 1988), sheep (Ikede, 1970; Adenowo, 1989) and cattle (Sekoni, 1994). Similar documentations for pigs have been made. Adverse effects of trypanosomiasis on haematological and biochemical values in infected animals including pigs (Anosa, 1988) is known. Omeke and Onuora (1992a) described in detail the effects of *T. brucei brucei* and *T. congolense* on reproductive capacity of young boars. These trypanosomes comparatively caused severe genital lesions and impaired spermatogenesis. However, it is not yet clear, which specific germ cell types are so prone to effects of trypanosomiasis and to what extent Sertoli cells are affected. Sperm production rate at sub-clinical chronic trypanosomiasis may also be critically affected. It has been noted that quantitative histomorphometry of spermatogenic cells is useful in assessing treatment effects on spermatogenesis (Berndtson, 1977; Russell, 1983). The purpose of the present study was to identify and estimate specific germ cells, which are prone to effects of sub-clinical trypanosomiasis, and effects of these defects on spermatogenesis in boars.

2. Materials and methods

2.1. Animals

Fourteen crossbred (Landrace × Large white) boars aged 10–12 months were purchased from Vom, a tsetse free zone in Plateau State of Nigeria. They were first quarantined for 28 days, dewormed and screened free from haemoprotezoan infection, weighed and separated into three groups (A, B and C) of 5, 5 and 4 animals, respectively. They were stocked in pens according to their groups, in an open ventilated insect-proof house at veterinary demonstration farm, University of Nigeria, Nsukka, and maintained on concentrate feed containing 16.2% crude proteins at a range ration of 2–2.6 kg per animal daily, depending on average live-weight per pen.

2.2. Infection with trypanosome

Each boar in group A and B was inoculated intraperitoneally with 2 ml suspension of strain Y58/98 *T. brucei brucei* containing $1.4 \times 10^6$ trypanosomes per ml. The strain of trypanosome used was isolated from a naturally infected pig, characterised at Nigeria institute for trypanosomiasis research NITR), Vom, preserved in liquid nitrogen, until thawed and infected into a group of healthy mice. At peak parasitemia, mice were bled by heart puncture using 1–2 mg disodium ethylene diamine tetraacetic acid as anticoagulant. The blood was diluted with phosphate buffered saline solution and its concentration was determined by haemocytometric count, as described by Berndtson (1977). Boars in group C were left as intact controls.
2.3. Experimental observations

All boars were monitored daily for symptoms and clinical features of trypanosomiasis. Boars were regularly tested for libido, when a sow was brought close. Libido was evaluated by the rate of interest shown, including mounting of a sow on heat within 5 min of her introduction to the boar. Chronic trypanosomiasis was assumed by day 42 post infection, when there was loss of libido; fairly stable low rectal temperature, packed cell volume and occasional scanty parasitemia peaks. Animal distress was minimised by the provision of adequate feed, care of the animals and by post castration treatment. Survivor boars in group A and two from the controls were castrated on day 56 post infection, while those in group B and the two remaining controls were castrated on day 84 post infection with trypanosomes. Prior to castration, boars were weighed and scrotal circumference was measured. Testes were immediately weighed after removal. The *Tunica albuginea* was carefully excised and weighed so that parenchyma weight was determined by the difference.

A portion of the parenchyma was placed in a vial and preserved at $-20^\circ$C until used for the determination of spermatid reserves. A second portion of the tissue was fixed in Zenker Formol solution for 24 h, washed in running tap water for 24 h, dehydrated, embedded in paraffin wax, sectioned at 5 $\mu$m, stained with periodic acid Schiff’s (PAS) reagent, counter-stained with haematoxylin (Berndtson, 1977) and utilised for histomorphometric evaluation.

2.4. Histomorphometric evaluation for spermatogenesis

A sample slide from a single region of a testis was regarded as a representative of the entire testis because of the structural uniformity of various regions within a testis (Berndtson et al., 1989). The ratio of spermatogonia, young primary (I$^\circ$) spermatocytes, old I$^\circ$ spermatocytes and spermatids to Sertoli cells were determined from direct counts of nuclei of germ cells and nucleoli of Sertoli cells with visible nucleolus in sections of 20 round seminiferous tubules at stage 1 of the cycle of seminiferous epithelium as was defined by Swierstra (1968) for boars. Mean diameter of the nuclei or nucleoli, based on six measurements, was determined for each type of cells and this was used to convert crude cell counts to true (absolute) cell counts by the application of Abervrombie (1946) formula as follows

$$\text{True cell count} = \frac{\text{Crude cell count} \times \text{section thickness}}{\text{Section thickness} + \text{Nuclear diameter}}.$$  

Germ cell numbers were Sertoli cell corrected by expressing on Sertoli cell basis (Berndtson and Foote, 1997). The ratios of specific germ cells to their progenator cell types (germ cell:germ cell) were calculated from true count per Sertoli cell to enable assessment of spermatogenetic efficiency.

2.5. Quantitative assessment of spermatogenesis

The rate of spermatozoal production was determined by enumeration of homogenization — resistant spermatids in homogenates of testicular parenchyma (Amann and Laambiase, 1969). A known weight (15–20 g) of previously frozen testis parenchyma was thawed,
minced with clean scissors and transferred to a Waring blender microhomogenization vessel containing 25 ml or 0.9% NaCl, 0.05% (v/v) Triton X-100 homogenization fluid for 2 min. The blender was rinsed with additional 200 ml of the fluid that was then added to the homogenate. Samples were stored overnight at 10°C, following, which they were evaluated quantitatively by haemocytometric enumeration of elongated homogenation-resistant spermatids in duplicate by each of four independent enumerators. Evaluators repeated counts, when values recorded differed by up to 10%. Data obtained were converted to estimated daily sperm production per testis (Berndtson, 1977).

2.6. Statistical analyses

Data for each variable were subjected to one way analysis of variance according to Steel and Torrie (1980). Where significant treatment effects were found, differences among means were tested by Student–Newman Keul’s method.

3. Results

All infected boars manifested characteristic initial acute leading to chronic symptoms of trypanosomiasis as described previously (Omeke and Onuora, 1992a, b). One boar (A.2) died at the acute phase of the disease. Sub-clinical trypanosomiasis was characterised by inappetence, emaciation, lacrimation, testicular shrinkage with scrotal plaques, scanty parasitemia peaks at long intervals and decreased libido. Control boars appeared healthy and sexually active.

There was significant difference ($P<0.05$) in live weight, scrotal circumference and testes weight of boars castrated at different periods of post infection with trypanosome (Table 1), when compared with the controls, the effects of which decreased with increase in post infection period. Liveweight positively but not significantly correlated with scrotal dimension and testes weight irrespective of treatment effects.

Table 1
Mean (±S.E.M.) live weight, testicular measurement and sperm reserves from boars at different post infection periods with *Trypanosoma brucei brucei*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Board group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected and castrated after 56 days</td>
</tr>
<tr>
<td>Animals evaluated</td>
<td>4</td>
</tr>
<tr>
<td>Liveweight at onset of expt. (kg)</td>
<td>54.6±3.1</td>
</tr>
<tr>
<td>Liveweight at castration (kg)</td>
<td>66.6±3.8a</td>
</tr>
<tr>
<td>Daily weight gain (kg)</td>
<td>0.2a</td>
</tr>
<tr>
<td>Scrotal circumference (cm)</td>
<td>26.4</td>
</tr>
<tr>
<td>Paired testes weight (g)</td>
<td>372.1±9.6a</td>
</tr>
</tbody>
</table>

*Coefficient of correlation*

<table>
<thead>
<tr>
<th></th>
<th>Liveweight vs. Sertoli circumference</th>
<th>Liveweight vs. Testes weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>
| *a* Values in same rows with same letters do not differ significantly at ’$P<0.05$’.
Fig. 1. Representative testicular tissues from trypanosome infected (A) and normal control (B) boars. Note extent of denudation of germ cells and presence of spermatogonia and Sertoli cells.
Table 2
Mean (±S.E.M.) Sertoli and germ cell numbers and daily sperm production in control boars and those infected with trypanosome and castrated 56 and 84 days post infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>Boar group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected and castrated after 56 days days</td>
</tr>
<tr>
<td>Individual testes with disrupted tubules</td>
<td>7/8</td>
</tr>
<tr>
<td>Testes assayed</td>
<td>5</td>
</tr>
<tr>
<td><strong>Cell count (10⁹)</strong></td>
<td></td>
</tr>
<tr>
<td>Sertoli cells per testis</td>
<td>18.6±2.60</td>
</tr>
<tr>
<td>Spermatogonia per Sertoli cell</td>
<td>0.2±0.02</td>
</tr>
<tr>
<td>Young primary spermatocytes per Sertoli cell</td>
<td>1.1±0.04a</td>
</tr>
<tr>
<td>Old primary spermatocytes per Sertoli cell</td>
<td>1.0±0.01a</td>
</tr>
<tr>
<td>Round spermatids per Sertoli cell</td>
<td>0.8±0.03a</td>
</tr>
<tr>
<td><strong>Germ cell:germ cell</strong></td>
<td></td>
</tr>
<tr>
<td>Young primary spermatocytes/spermatogonia</td>
<td>5.4±0.25a</td>
</tr>
<tr>
<td>Old primary spermatocytes/spermatogonia</td>
<td>5.0±0.21a</td>
</tr>
<tr>
<td>Round spermatids/spermatogonia</td>
<td>4.1±0.30a</td>
</tr>
<tr>
<td>Round spermatids/young primary spermatocytes</td>
<td>0.7±0.04a</td>
</tr>
<tr>
<td>Gonadal sperm reserves/testis</td>
<td>12.4±4.1a</td>
</tr>
</tbody>
</table>

a Values in same row will same letters do not differ significantly at *P*<0.05, a,b — *P*<0.05; c,d — *P*<0.01.

Seminiferous tubules of testes from boars infected with trypanosome were distorted and shrunk. They showed extensive denudation and or degeneration of spermatogenic cells (Fig. 1). These lesions precluded identification of stages of cycle of seminiferous epithelium and count of specific germ cell types and Sertoli cells in severely damaged testes.

Data obtained from cell counts of round seminiferous tubules are shown in Table 2. The information obtained does not absolutely represent extent of damage due to sub-clinical trypanosomiasis on testes of boars since some severely damaged testes were not assayable. From the available information, spermatids and young primary spermatocytes were most reduced in number, when compared with those of the controls. Spermatogonia were least affected among germ cells. Sertoli cells were minimally reduced in number consequently. Germ cell numbers and ratios and gonadal sperm reserves increased in those testes of boars castrated after 84 days post infection with trypanosome than those castrated earlier, but those differences were not statistically significant.

4. Discussion

Clinical features including low body and reproductive organ weight, scanty parasitemia peaks at long intervals, depressed libido and debilitation noted in the study were reminiscent of observations made in pigs raised in testes endemic tropics (Ilemobade and Balogun, 1981; Sekoni, 1994). It has been highlighted that trypanosomes preferentially localise in nutrient-rich gonads (Ashman and Seed, 1974; Joshua et al., 1985) during which they
deplete blood nutrient and physically damage testicular parenchyma (Omeke and Onuora, 1992a,b). In a sub-clinical state, there may be apparent ‘self cure’ when trypanosomes merely sequestrate preferentially in the brain and muscle of the host only to relapse when nutritional and immunological status of the host is lowered (Onah and Uzoukwu, 1991). Because of the extensive damage of the gonads and other endocrine organs, there is low biosynthesis of steriodogenic hormones that are essential for spermatogenesis (Risbridger et al., 1981). The effects are worsened in situations where trypanosomiasis-induced lesions fail to completely resolve (Ikede and Akpavie, 1982) probably because the sub-clinical infection is not noticed in a herd or because lesions formed may have calcified (Omeke and Onuora, 1992a). On the other hand, positive correlation shown between live weight and scrotal circumference and testes weight lends credence to the hypothesis that irrespective of treatment effects, measurement of body and reproductive organs remain useful in assessing reproductive potential of male animals.

This study has also shown that trypanosomes primarily exert effects on those more tender germ cells involved in meiosis and maturation. This is why spermatogenesis is adversely disrupted. There are no other clear reasons, why spermatogonia and nonproliferating Sertoli cells are fairly resistant to damage by trypanosomes. Cytotoxic agents are also not able to kill spermatogonia and Sertoli cells (Meistrich, 1982; Berndtson and Foote, 1997) as they kill other germ cells. Advantages of resistance of spermatogonia and Sertoli cells to insult due to sub-clinical trypanosomiasis include the likelihood of regeneration of germ cells from precursor stem cells and re-establishing spermatogenesis that was previously disrupted. This has been supported by an increase in proliferating germ cells leading to increase in gonadal sperm reserves of trypanosome infected boars, after a long period of post infection. Nevertheless such animals would not attain previous fertility levels since losses to the number of Sertoli cells are not regained at puberty. It has been observed (Dym and Fawcett, 1971; Steinberger and Steinberger, 1971) that Sertoli cells establish upper limit for sperm production. Thus, testes, whose Sertoli cells are partially reduced do not attain original fertility levels. On the other hand, defects in other accessory organs and other cells of reproduction occur following animal infection with trypanosomes. In male animals, fertility is adversely impaired (Sekoni, 1994). It may be concluded that necessary prophylactic measures are prerequisite for efficient reproductive performance of boars in endemic tropics.

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References


