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Ovarian Weight, Follicle Count and Retrieved Oocyte Characteristics in West African Dwarf Goat Does Experimentally Infected with *Trypanosoma brucei*

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Summary: Trypanosomosis has been described as the single largest disease entity limiting livestock development in sub-Saharan Africa. The effects on ovarian weight, follicle count and retrieved oocyte characteristics in ten West African dwarf goat does (control=5, infected=5) experimentally infected with *Trypanosoma brucei* were investigated. The does were fed with elephant grass and supplement (15.23% CP) daily. Infected does received $4.8x10^5$ *T. brucei* intravenously and thereafter, all does were synchronized using Lutalyse®. The results showed that the differences between control and infected does for ovarian weight (0.68±0.56 g and 0.40±0.09 g) and follicle count (10.50±1.25 and 2.50±1.22), respectively were significant (P<0.05). The difference in retrieved-oocytes-count between control (30, 57.7%) and infected (22, 42.3%) does was not significant (P>0.05). The differences in proportion between control and infected does for well-formed-oocytes (90.5% and 9.5%), completely-denuded-oocytes (30.8% and 69.2%) and proportion per group of oocytes with substantial-investment-of-cumulus (63.3% and 9.1%), respectively were significant (P<0.05). The difference in extensively-denuded-oocytes between control (38.9%) and infected (61.1%) does was not significant (P>0.05). These findings suggest that experimental *Trypanosoma brucei* infection caused reduction in ovarian weight and follicle count, number of oocytes as well as proportion of well-formed oocytes that are capable of supporting embryonic development.

Keywords*: Trypanosoma brucei*, goat does, Lutalyse®, ovarian follicles, oocytes.

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INTRODUCTION

Artificial production of embryos or *in vitro* embryo production (IVEP) is an important procedure/technique capable of boosting animal production in any part of the world. While interest is growing in the technique with goat practitioners, it is a major focus in bovine industries (Camargo *et al.*, 2010). In Brazil, more than 40% of cattle embryos transferred to recipients in 2004 alone consisted of those produced through *in vitro* technique, involving *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culturing (IVC) of immature oocytes (Viana *et al.*, 2010). The oocytes are usually collected through ultrasound guided aspiration or from ovaries post-slaughter (Salamone *et al.*, 2001). IVM is a critical step to *in vitro* embryo production (IVEP/IVP) and the integrity of oocyte morphology at retrieval/collection is a limiting factor (Calder *et al.*, 2005; Pereira *et al.*, 2010). Cumulus-oocytes complexes (COC) with larger follicular diameter, several layers of unbroken cumulus investment and homogenous ooplasm possess greater potential for IVM (Crozet *et al.*, 1995; Gandolfi, 1996). One of the greatest challenges to global animal production is disease. In Africa, trypanosomosis is a major disease entity limiting livestock production and development (Swallow, 2000), with an estimated yearly loss of 4.5 billion USD (Affognon, 2007). *Trypanosoma congolense*, *T. vivax*, *T. brucei*, *T. evansi* and *T. equiperdum* are the most important trypanosomes causing disease in livestock species in Africa (Chitanga *et al.*, 2011). Losses to trypanosomosis have been associated with varying degrees of infertility among other symptoms (Faye *et al.*, 2004; Prashant *et al.*, 2005). The West African Dwarf (WAD) goat has huge economic importance and potential relevance to reproductive biotechnology in Nigeria. This study investigated the effects of experimental *Trypanosoma brucei* infection on ovarian weight, follicle count and retrieved oocyte characteristics in WAD goat does.

MATERIALS AND METHODS

The study was carried out at the small ruminant Unit of the Department of Theriogenology, University of Ibadan, Ibadan, Nigeria. The Unit houses a little over 100 goats, the WAD being the most predominant breed.

Ten (10) adult WAD goat does weighing between 16.0 kg and 17.5 kg were used in the study. The does

were randomly but equally divided into groups 'A' (control) and 'B' (infected). Each group was kept in a separate pen. The does were fed with Elephant grass in the mornings and commercial feedstuff containing 15.23% Crude Protein (CP) at the rate of 0.25kg/head in the afternoons. Freshwater as well as salt licks were provided *ad libitum* throughout the study. None of the animals was however allowed to graze.

Trypanosoma brucei (Kaura strain) was obtained from passaged rats from the Department of Veterinary Pathology, University of Ibadan. The parasite was originally obtained from the Nigerian Institute for Trypanosomasis Research (NITR), Vom, Plateau State, Nigeria.

Parasitaemia in albino rats was evaluated at $8.4x10^7$ trypanosomes per millilitre by haemocytometric method (Lumsden *et al.,* 1979) using blood obtained from the tail vein of the rats. Further dilution of blood was made with normal saline to obtain an inoculation dose of 4.8×10^5 trypanosomes/ 0.5ml diluted blood per doe (Leigh and Fayemi, 2010). This dose was used to infect each doe via the jugular vein.

Traces of sodium, calcium, potassium and magnesium salts along with Hepes were dissolved in Tridest water at 7.3-7.4 alkalinity and 282 mOsm and kept as stock media. To 25 ml of the stock media, 250 microliters of sodium pyruvate and gentamicin were added along with 0.075 g of bovine serum albumin to make the ready-to-use COCs media at PH 7.3-7.5 (Lonergan *et al.,* 2003; Viana and Camargo, 2007).

Both groups of does were synchronized using an earlier described method (Leigh *et al.,* 2010). Briefly, this involved the intramuscular administration of a double injection, seven days apart, of 5 mg prostaglandin F_2 alpha-Lutalyse$^{(R)}$ (Pharmacia & Upjohn Co. NY). Twelve days prior to treatment with Lutalyse$^{(R)}$ in both groups, does in group 'B' were administered 4.8×10^5 trypanosomes/ 0.5ml diluted blood.

The does (Groups 'A' and 'B') were euthanized on day 23 post Lutalyse$^{(R)}$ administration such that they would be around the first oestrus following the synchronized oestrus. Following laparoscopy, ovaries were severed from the broad ligament with the aid of a sharp knife and transported to the laboratory in labelled Thermo flasks containing phosphate buffered saline- PBS (without calcium and magnesium substituted with 1 ml Gentamycin/litre) at 38.5^0C. The weights of the ovaries were determined immediately post-harvest with the aid of a digital Top Load electronic balance (Lark Inc., Denver). At the laboratory, the ovaries were washed twice in PBS at 38.5^0C. All follicles >2 mm were counted with the aid of a magnifying glass following which follicular fluid was aspirated into 2 ml COCs media in an 18G x 1½ inch needle on plastic disposable syringe. The needle was inserted into the ovarian parenchyma underneath each follicle and by sucking back the air around it, follicular fluid containing oocytes was drained. The content of the syringe was slowly emptied into sterile centrifugation tube and placed in a warm water bath at 38.5^0C for 10 minutes. Afterwards, the top 75% of the follicular fluid was carefully decanted. With the aid of a 20 µl Gilson's pipette set at 10 µl, the oocytes were picked up from the bottom of the centrifugation tubes and washed in a petri dish containing about 2000 µl COCs media. The characteristics of retrieved oocytes were then identified under low magnification microscopy based on investment of cumulus layers (Gandolfi, 1996).

Statistical Analysis

Data were subjected to Student t- statistic (GraphPad, 2000). Values of $P \leq 0.05$ were considered significant.

RESULTS

The results are presented in Tables 1 and 2 as well as Plates 1 and 2.

Ovarian weights and number of follicles counted

Table 1 shows the differences in ovarian weights, number of follicles and number of oocytes between control and infected does. The differences between control and infected does for mean ovarian weights (0.68 ± 0.56 and 0.40 ± 0.09) g as well as number of follicles >2 mm (10.50 ± 1.25 and 2.50 ± 1.22), respectively were significant ($P<0.05$).

Retrieved oocytes and their characteristics

Table 2 shows the differences in the characteristics of cumulus-oocyte complexes retrieved from the ovaries. Among the 21 well-formed oocytes, 19 (90.5%) were obtained from control does while 2 (9.5%) were obtained from infected does ($P<0.05$). Among

Table 1: Ovarian weight and number of follicles (Mean±S.D) counted in WAD goat does experimentally infected with *T. brucei*.

Parameter (s)	Control	Infected
Weight of ovary (g)	0.68 ± 0.56	0.40 ± 0.09*
Number of follicles visible on ovaries (i.e. follicle count)	10.50 ± 1.25	2.50 ± 1.22*

*P < 0.05.

Table 2: Characteristics of oocytes retrieved from the ovaries of WAD goat does infected with *T. brucei.*

Characteristics of oocytes	Control	Infected
Well-formed oocytes (fair/substantial investment of cumulus cells - SIC)	19 (90.5%)	2 (9.5%) *
Oocytes with extensively denuded cumulus cell layers (ED)	7 (38.9%)	11 (61.1%)
Completely denuded oocytes (CD)	4 (30.8%)	9 (69.2%) *
Total	30 (57.7%)	22 (42.3%)
Proportion of SIC/group	63.3%	9.1%

*P < 0.05.

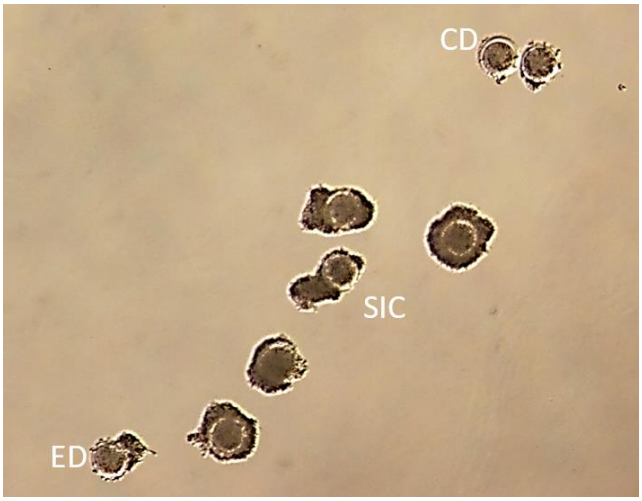

Plate 1: Cumulus oocyte complexes from control does suspended in selection media showing 1 completely denuded (CD), 2 extensively denuded (ED) and 5 with substantial investment of cumulus cells (SIC). (x100).

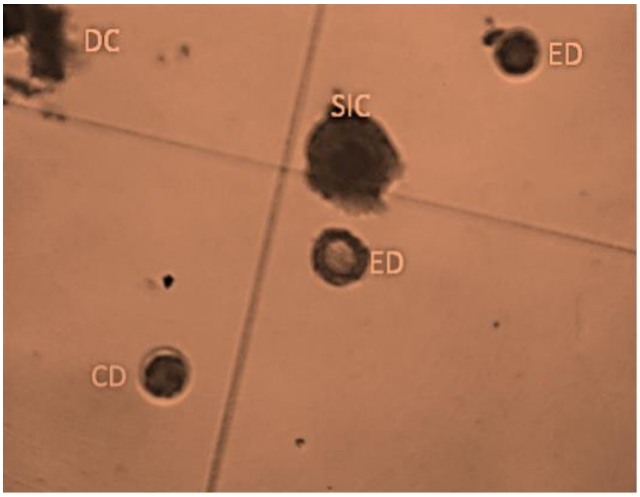

Plate 2: Cumulus oocyte complexes from infected does suspended in selection media showing 1 completely denuded oocyte (CD), 2 extensively denuded (ED), 1 with substantial investment of cumulus cells (SIC) and detached cumulus cells (DC) (x150).

extensively denuded oocytes, the difference between control (7, 38.9%) and infected (11, 61.1%) was not significant (P>0.05). For completely denuded oocytes, the difference between control (4, 30.8%) and infected (9, 69.2%) was significant (P<0.05). The difference between total number of oocytes counted: control (30, 57.7%) and infected (22, 42.3%), was not significant (P>0.05). The difference in the proportions per group, of oocytes with substantial investment of cumulus cells between control (63.3%) and infected (9.1%) does was however significant (P<0.05). Plates 1 and 2 shows the characteristics of retrieved oocytes from the ovaries of control and infected does, respectively. Plate 1 shows 1 completely denuded oocytes (CD), 2 extensively denuded oocytes (ED) and 5 oocytes with substantial investment of cumulus cells (SIC) while Plate 2 shows 1 CD, 2 ED and 1 SIC and some detached cumulus (DC) cells.

DISCUSSION

The mean ovarian weight of control does compare well with those reported for other goat breeds (Islam *et al.*, 2007; Haque *et al.*, 2016) whereas that of infected does was lower (P<0.05). The reduction in ovarian weight as shown in Table 1 may suggest concomitant reduction in physiological activities within the ovary and may be responsible for the observed reduction in numbers of ovarian follicles (P<0.05) as well as oocytes (comparative). Folliculogenesis and oocyte production are components of fertility in the female animal. A reduction in these, therefore, is suggestive of some level of infertility caused by experimental infection with *T. brucei*. An earlier study (Rodriguez *et al.*, 2013) reported key ovarian disorders such as follicular degeneration and reduced weight in goats experimentally infected with *Tyrpanosoma vivax*, similar to findings of the present study. According to these authors, the ovarian findings appeared to be involved in the aetiology of anestrus/infertility observed in the goats. Earlier, Wittmaack *et al.*, (1994) also observed that dams with poorly formed follicles often have poor oocyte recovery and cleavage in in vitro fertilization cycles and are therefore of reduced value for embryo production.

The characteristics of retrieved oocytes in the present study further clarified the infertility caused by *T. brucei*, in that <1 out of 10 well-formed oocytes, and >60% of extensively and/or completely denuded oocytes were obtained from infected does. Similarly, the proportion of well-formed oocytes to retrieved oocytes for infected does was lower (P<0.05) compared to that in control does as shown in Table 2 as well as Plates 1 and 2. Although, it is not known whether the low proportion of developmentally viable oocytes will be sufficient to achieve reproduction still, the finding suggest that experimental *T. brucei* infection led to an increase in the proportion of developmentally non-viable oocytes as well as reduction in the proportion of viable oocytes. This finding is perhaps worrisome due to the nature of the difference (P>0.05) in total retrieved oocytes between control and infected goats. Even though, the result showed a comparative reduction in retrieved oocytes of infected goats, the proportion of that, that could support embryo generation, as assessed by morphological characteristics, describes the toll of *T. brucei* on the reproductive usefulness of infected animals. Since the goal of *in vitro* embryo production is to generate numerous oocytes that are developmentally viable, infected animals may not be selected for the purpose since they are less likely to produce oocytes capable of supporting embryonic development (Crozet *et al.*, 1995). The infection may therefore lead to rejection of animals by *in vitro* embryo production industries. These findings add to existing literature on symptoms of infertility such as

anoestrus (El-Hassan *et al.*, 2005) and hypogonadism (Petzke *et al.*, 1996) that had been reported in trypanosomosis.

It is concluded that experimental *T. brucei* infection led to reduced ovarian weight, follicle count and proportion of developmentally viable cumulus oocyte complexes in West African dwarf goat does.

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