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Observations on placentome diameters in gestating West African dwarf does experimentally infected with *Trypanosoma brucei*

OO Leigh

Department of Veterinary Surgery and Reproduction, University of Ibadan, Ibadan, Nigeria

Correspondence: Tel.: +23480 33266825, E-mail: damilareolufisayo@yahoo.com

Abstract

This study investigated the effect of experimental *Trypanosoma brucei* infection on the placentome diameter (PD) of twenty four gestating West African dwarf does. The does were randomized into 4 equal groups with 'G1' as control while 'G2', 'G3' and 'G4' were intravenously inoculated with 5 x 10^8 *trypanosomes* on days 25, 51 and 101 post breeding (PB), respectively. Real-time trans-abdominal scan was carried out with 3.5-5MHz convex transducer. The differences between the mean PD readings on days 60, 75 and 132 for 'G1' (1.18 ± 0.32, 1.63 ± 0.83 and 2.43 ± 0.69) cm, 'G3' (1.20 ± 0.82, 0.49 ± 3.13) cm and 'G4' (1.19 ± 0.26, 1.65 ± 0.05 and 2.39 ± 1.16) cm, respectively were statistically significant ($P \leq 0.05$). At day 60, the mean value for does in 'G1' (1.18 ± 0.32) cm was significantly ($P \leq 0.05$) higher than for 'G2' (0.88 ± 1.53) cm. At day 75, the mean value for does in 'G1' (1.63 ± 0.83) cm was significantly ($P \leq 0.05$) higher than for 'G3' (0.49 ± 3.13) cm. At day 132, the difference between the mean values of PD for does in 'G1' (2.43 ± 0.69) cm and 'G4' (2.39 ± 1.16) cm was not significant ($P \geq 0.05$). The placenta tissue loss following infection for 'G2' and 'G3' were 25.4% and 69.9% at 36 DPI and 25 DPI, respectively. No values were obtained at days 75 and 132 for does in 'G2' and at day 132 for does in 'G3' either due to abortion or death. These findings indicate that experimental *T. brucei* infection led to reduced placentome diameter during critical periods of increased foetal development.

Keywords: Gestation, Placentome diameter, *Trypanosoma brucei,* Ultrasonography, West African dwarf does

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Introduction

Trypanosomosis has not only been described as the greatest constraint to agricultural development and growth of livestock industry in sub-Saharan Africa (Guy, 1994), it is perhaps the largest single aetiology responsible for the acutely short supply of animal protein to humans in Tropical Africa (Swallow, 2000). Its association with the reproductive system had been reported (Silva *et al.,* 2013). Some of these symptoms/clinical signs in female mammalian livestock include intrauterine infection, abortion and fetal death *in-utero* (Ogwu *et al.,* 1986; El-Hassan *et al*., 1995; Bawa *et al*., 2000; Faye *et al*., 2004).

The activities of the placenta in pregnancy are pivotal for successful gestation. The mammalian placenta is the major determinant of intrauterine growth through its supply of nutrients to the fetus (Fowden *et al*., 2006). The efficiency of the placenta has often been evaluated as fetal grams produced per gram placenta (Wilson & Ford, 2001). Baur (1977) also reported that fetal and placental weight near term were positively correlated. Reductions in determinants of placental efficiency (e.g. size) affects its nutrient transfer capacity (Jones *et al*., 2007) which therefore causes restrictions in intrauterine growth (Fowden *et al*., 2006).

Based on the above, the integrity of the organ placenta during trypanosome challenge raises issues to ponder over. Older literatures observed trans-placental infection, neo-natal deaths, with other

fetal pathogenic effects in trypanosome infected animals (Bawa *et al*., 2000; Faye *et al*., 2004; Al-Qarawi *et al.,* 2003). In a recent report, Leigh *et al*. (2014), acute placentitis characterized by marked vascular congestion and multifocal lymphocytic aggregates were observed in *T. brucei* infected pregnant does.

To the best of our knowledge, the mechanisms leading to these symptoms have not been fully elucidated. This study was designed as a further attempt to investigate, through ultrasonic means, the effects of experimental *T. brucei* infection on the functional unit of the placenta i.e. the placentome. The findings will be important in giving more information about the condition of the placenta during an infection.

Materials and methods

Twenty-four gestating does with bodyweight range 16.0-17.5 kg were mated same day, following synchronization with double intramuscular injection of 5 mg Lutalyse® (Pharmacia & Upjohn Co. NY) as described by Leigh, (2013). The does were randomly divided into four equal groups (i.e. 'G1', 'G2', 'G3' and 'G4'). 'G1' served as control/non-infected does while 'G2', 'G3' and 'G4' served as experimental/ infected does. The does were kept in separate pens according to their groups in the Small Ruminant Unit of the Department of Veterinary Surgery and Reproduction, University of Ibadan. The animals were fed on commercial feed stuff (15.23% CP) at the rate of 0.25 kg per head/day. Elephant grass and fresh water *ad libitum* were also provided throughout the study. Gestating does were earlier screened negative for trypanosomes via haematocrit centrifugation technique (HCT) and buffy coat method (BCM) as described by Woo (1971).

Trypanosoma brucei (Kaura strain) was obtained from passaged rats in the Department of Veterinary Pathology, University of Ibadan. The parasite was originally obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. Parasitaemia in albino rats was evaluated at 8.4×10^7 trypanosomes per milliliter by haemocytometric method (Lumsden *et al*., 1973) using blood obtained from the tail vein of the rats. The method of Lumsden *et al*. (1973) was modified as further dilution of blood was made with normal saline to obtain an inoculation dose of 4.8×10^5 trypanosomes/ 0.5ml diluted blood.

Does in 'G2' were intravenously inoculated with 4.8×10^5 *T. brucei* on day 25 post breeding (PB) i.e. mid first third of pregnancy, 'G3' on day 51 PB (beginning of second third of pregnancy) and 'G4' on day 101 PB (beginning of last third of pregnancy).

A real-time (B-mode) scanner (SA-600V, Medison Co. Seoul, Korea) equipped with a 3.5-5MHz convex transducer (Trans-abdominal) with Masterhand ultrasound gel (Alpspitz strasse 10-82223 Eichenen, Germany) was used, both to confirm conception PB and to evaluate placentome diameters (PD).

Feeds were withdrawn from gestating does at about 6 pm a day prior to scanning. The does were scanned at 7 am in the mornings before they are served feed. This practice reduced gas accumulation within the intestine as a result of digestion of feeds and gave a clearer picture of the abdominal structures. An area 15 cm anterior to the udder and full width of the ventral abdomen extending to the groin was shaved clean to facilitate scanning. For every examination, the entire ventral region of each pregnant doe was cleaned with cotton wool soaked in antiseptic solution. The animal was well restrained in dorsal recumbency by two assistants and measurements were evaluated on days 60, 75 and 132 of pregnancy. Values of placentome diameter (PD) were read as the mean (Degani, 2001; Lee *et al*., 2005) of two big sizes of PD evaluated on the sonogram.

The placenta tissue loss percent (PTLP) was calculated as: the difference between the mean value of PD at any day PB and that of the corresponding control value, divided by the corresponding control value, and thereafter expressed in percentage.

The study was conducted under strict adherence to the principles of the care and use of farm animals in research, teaching and testing (CCAC, 1993).

Measurements obtained were analyzed using analysis of variance (ANOVA). The Student-t-statistic was also used to test the level of significance of the differences between the parameters at 5% (Elston & Johnson, 2008).

Results

The results of the study (Mean ± S.D) of the placentome diameters (PD) are presented in table 1. At 60 days PB, 36 days post infection (DPI), the PD values for does in 'G1' (1.18 ± 0.32) cm were significantly higher ($P < 0.05$) than does in 'G2' (0.88 ± 1.53) cm. Similarly, at 75 days PB, 25 DPI, the PD values for does in 'G1' (1.63 ± 0.83) were significantly higher ($P < 0.05$) than does in 'G3' (0.49 ± 3.13) cm. The differences in the mean values between other measurements evaluated for PD were not significant ($P \geq 0.05$). However, at 132 days

PB, 32 DPI, the PD values for does in 'G1' (2.43 ± 0.69) cm were not significantly different (P > 0.05) with values of 'G4' (2.39 ± 1.16) cm. PD values were not evaluated at days 75 and 132 PB i.e. 51 and 108 DPI for does in 'G2', and at day 132 PB, i.e. 82 DPI for does in 'G3' because the does either died or aborted before these days. Abortion occurred within 46 and 32 days in does inoculated during first and second thirds of gestation, respectively. table 2 shows that the placenta tissue loss percent (PTLP) within 36 days post inoculation (DPI) (G2) and 25 DPI (G3) were 25.4% and 69.9%, respectively.

Plates 1 and 2 show the PD evaluated on day 60 as 1.2 cm and 1.0 cm in control and infected does, respectively. Plates 3 and 4 show the PD evaluated on day 75 as 1.5 cm and 0.8 cm in control and infected does, respectively. Plate 5 shows the PD evaluated on day 132 as 2.5 cm in control and infected does.

Table 1: Comparison of mean PD readings during gestation in the groups of does

	Day (s)	Control (G1)	G2 (infected day 25 Post breeding)	G3 (infected day 51 Post breeding)	G4 (infected day 101 Post breeding)
PD (cm)	60	1.18±0.32 [a]	0.88±1.53 [b]	1.20±0.82 [a]	1.19±0.26 [a]
	75	1.63±0.83 [c]	NA	0.49±3.13 [b]	1.65±0.05 [c]
	132	2.43±0.69 [d]	NA	NA	2.39±1.16 [d]

[a, b, c & d] Means in rows and columns with different superscripts are significantly (P<0.05) different

'NA': Values carrying 'NA' could not be resolved either due to large fetal size or abortion

Table 2: Placenta tissue loss percent (PTLP) of WAD does

Group of WAD does	placenta tissue loss (%)	days post inoculation (dpi)
G2	25.4	36
G3	69.9	25

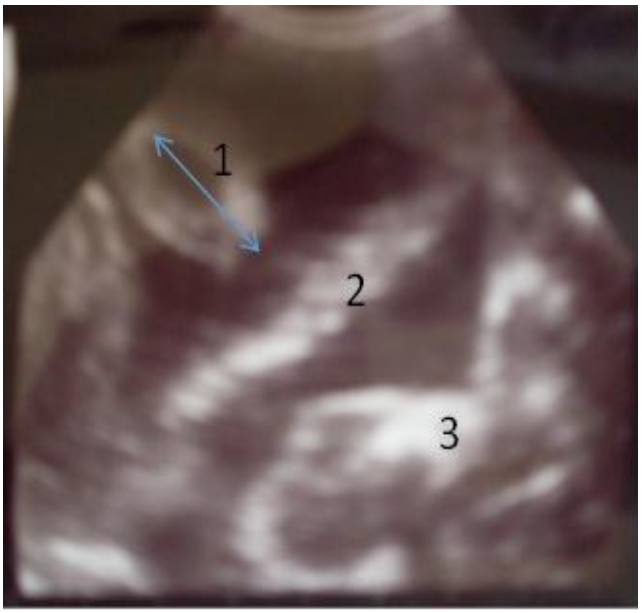

Plate 1: Sonogram showing PD = 1.2 cm (↔) taken on day 60 post breeding in control does. 1: Placentome; 2: Vertebra; 3: fetus

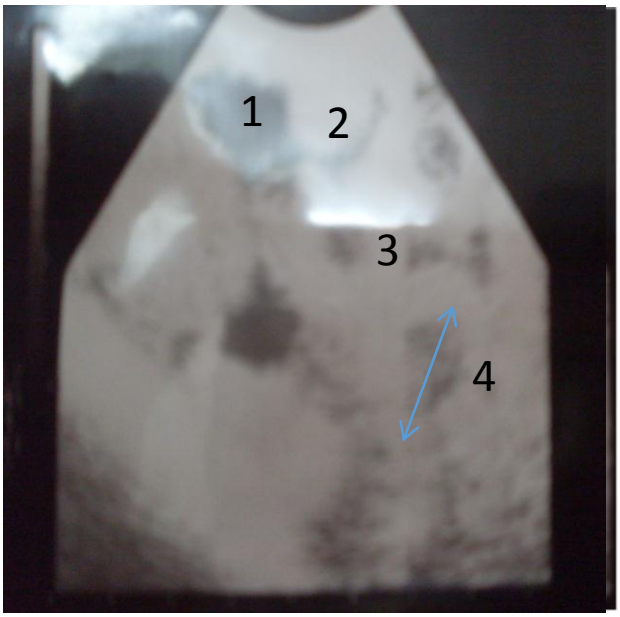

Plate 2: Sonogram showing PD = 1.0 cm (↔) taken on day 60 post breeding in infected does. 1: Amniotic fluid; 2: Fetus; 3 & 4: placentomes

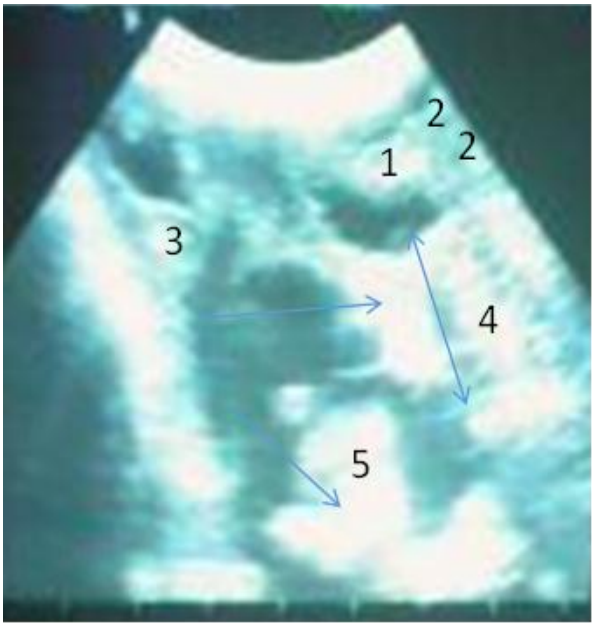

Plate 3: Sonogram showing PD = 1.5 cm (⟷) taken on day 75 post breeding in control does. 1: Fetus; 2: Anterior limbs; 3: Umbilical cord; 4 & 5: placentome

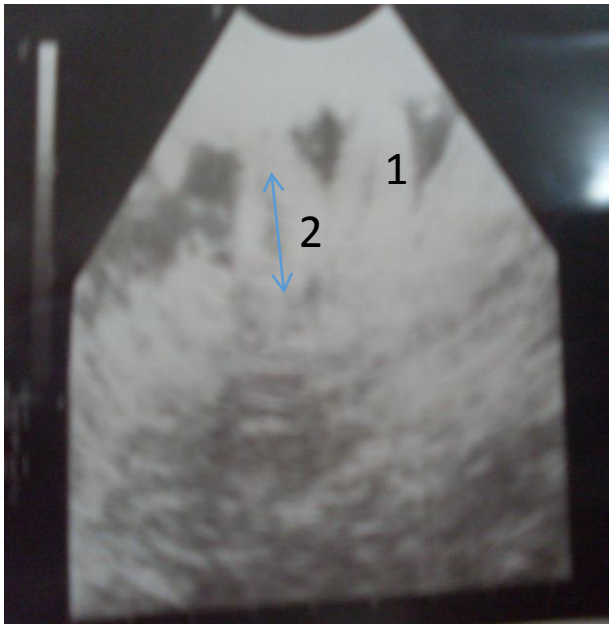

Plate 4: Sonogram showing PD = 0.8 cm (⟷) taken on day 75 post breeding in infected does. 1 & 2: placentomes

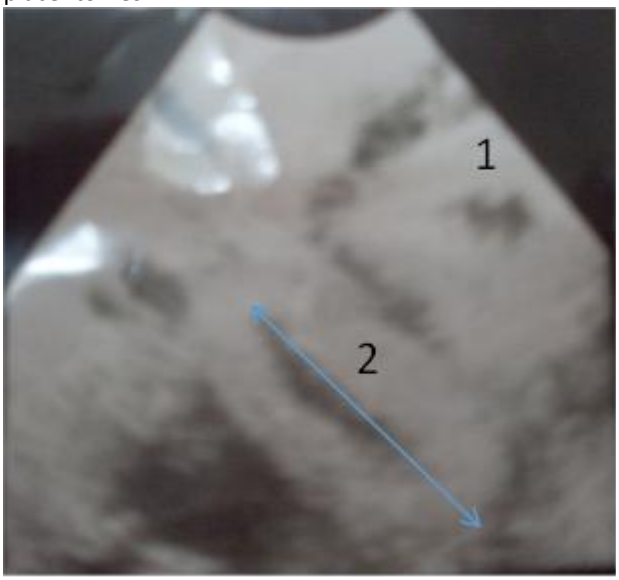

Plate 5: Sonogram showing PD = 2.5 cm (⟷) taken on day 132 post breeding in control and infected does. 1 & 2: Placentomes

Discussion

The placentome represents the functional unit of the placenta through which the fetus is nourished and it increases in diameter within certain limit to accommodate changing requirements of the fetus/feti during intrauterine life (Noakes *et al.,* 2001; Lee *et al.,* 2005; Leigh & Fayemi, 2010). This report is consistent with the findings in this study with 'G1' and 'G4' but not with 'G2' and 'G3'. With 'G2', although the days 75 and 132 PB PDs' were not obtained due to death or abortion, the result showed a reduction in PD during the day 60 PB evaluation. This reduction, occurring about 36 DPI covers 50% and 20% of the first and second (mid) thirds of gestation in the WAD goat, respectively. The implication of this is that it is likely to affect placental nutrient transport and exchange, which are capable of contributing to the incidence of embryonic death as well as inducing intrauterine growth restriction (IUGR), with its associated factors, such as high incidence of perinatal morbidity and mortality (Barker *et al.,* 2010). With 'G3', the result at day 60 PB suggest that *T. brucei* which was inoculated ten days earlier had not caused any noticeable change in the PD. However, by 25 DPI, the day 75 PB PD showed significant ($P < 0.05$) reduction. The observations with 'G3', especially at day 60 PB may suggest that under the conditions of the study, the parasite required longer than ten days before it may produce obvious change in the PD. Also, the observation at day 75 PB indicated grave consequences on the gestation that are comparable to 'G2' does. The result also showed that the proportion of abortion and/or death were higher in 'G2' compared to 'G3' and 'G4' where neither abortion/death was recorded. This observation may be connected on one hand with the age/maturity of the placenta in response to the infection, the younger placenta being more vulnerable and *vice versa*. Histologically, the cells and tissues of the placenta are known to mature through the phases of gestation, until the mesenchyme core of the villi become compact with fibrin deposits (Ahokas & McKinney, 2008). These changes confer strength and culminate in enhanced

defense mechanism to the placenta. This may also be responsible for the observation with 'G4', indicating that the placenta during the last two thirds of pregnancy was better equipped for parasite clearance (Fretes & Kemmerling, 2012), compared with the younger placenta of goats in 'G2'. In small ruminants, placentation begins around day 22 of gestation, reaching its peak at about day 80 (Wilson, 2002). Inoculation of *T. brucei* about 3 days post placenta initiation as it occurred in 'G2' does is likely to have overwhelmed the conceptus, leading to an abortion. Though, the more matured placenta of 'G3' does was less vulnerable to abortion, it succumbed just after day 75 PB. The reduced resistance to abortion may be due to placenta tissue loss beyond tolerable limit. It is interesting to note that as immature as the placenta of 'G2' was, prior to parasite inoculation, only 25.4% of placenta tissue was lost within 36 DPI whereas, with 'G3', 69.9% was lost within 25 DPI. Again, prior to when measurements were taken, does in 'G4' had been inoculated with trypanosomes for about 32 days whereas 'G3' had been for about 25 days only. These observations may be related to the level of activity of the organ. By day 75 PB, the pregnancy had entered the period of hypertrophy, a critical period of greatest fetal vulnerability which is between day 60 and 120 post conception (Osuagwuh, 1992). During this period, which covers 71.43% of the second third of gestation for the goat doe, the nutrition of the pregnant dam is very crucial, the gestating animal requiring more energy in terms of nutrients, and thereby placing increasing demand on the placenta (Fowden *et al.,* 2008). Therefore, infection at such a period may interfere with physiologic processes in the dam which may affect the placenta, and as such, have deleterious effects on gestation. These effects, depending on their gravity, could abruptly terminate gestation, and may on the other hand be responsible for the abortion observed in this study as well as in the report of Faye *et al.* (2004). In general, the hyperactivity of the placenta at such periods of crucial demand is likely to have increased its vulnerability. The result of 'G4' does suggest that the parasite had no obvious effect on the PD, as against what was observed with does in other groups. The age/maturity of the placenta during the last third of gestation may be responsible for this observation. In the sheep however, it has been reported that once the placenta has reached its maximum size, it declines thereafter until term (Ehrhardt & Bell*,* 1995). This was not the case with the WAD does in the current study. Since the goat and the sheep are small ruminants, with some similarities in placentation and function, this deviation requires further investigation; otherwise, the difference between species may simply be adduced.

It is concluded from the present study that experimental infection with *Trypanosoma brucei* caused significant reduction in placentome diameter during the first and second thirds of gestation, and that WAD does were more vulnerable during the mid, at which period, placenta tissue loss was highest.

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