Animal Reproduction Science 167 (2016) 109–116

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Changes in some pregnancy biomarkers of Yankasa ewes experimentally infected with *Trypanosoma evansi*

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ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 1 February 2016
Accepted 15 February 2016
Available online 18 February 2016

Keywords:
Obstetric disease
Pregnancy biomarker
Trypanosoma evansi
Yankasa ewe

ABSTRACT

The study was designed to determine the effect of *Trypanosoma evansi* infection on some pregnancy biomarkers of Yankasa ewes (YE). Twenty pregnant YE were assigned into 3 groups (A, B and C) comprising 7 ewes each in groups A and B, while group C comprise 6 YE. Groups A and B were each inoculated with blood containing approximately 1.0×10^6 of *T. evansi* through the jugular vein on days 59 and 110 of pregnancy, representing second and third trimesters, respectively, while group C served as the uninfected control. Progesterone (P_4) and pregnancy specific protein-B (PSPB) of YE in group A were significantly ($p < 0.05$) high at weeks 4 and 12 post infection (pi) respectively, while there was no significant ($p > 0.05$) difference in P_4 and PSPB of YE in groups B. Estrone sulfate (E_1S) significantly ($p < 0.05$) decrease for YE in group A at weeks 2 and 11 pi. However, it was not significantly ($p > 0.05$) different in group B. Cortisol concentration of YE in group A was significantly ($p < 0.05$) decreased at week 12 pi. Conversely, the cortisol concentration of YE in group B significantly ($p < 0.05$) increased at week 3 pi. There was no significant ($p > 0.05$) association among the pregnancy biomarkers of YE in groups A and B throughout the study, except between progesterone and cortisol in group B, which were significantly associated ($r = 0.77$, $p < 0.05$). It was therefore concluded that *T. evansi* infection affects pregnancy biomarkers more at mid pregnancy than at late pregnancy.

1. Introduction

From fertilization to parturition, pregnancy involves a complex process comprising cleavage of the zygote, embryo formation and foetal development (Noakes et al., 2001). During this period, the survival of the developing conceptus is dependent partly on the viability of the placenta and the maintenance of its endocrine function (Sousa et al., 2008). The ruminant placenta nourishes the developing foetus until the process of parturition is completed (Kindahl, 2007). This is achieved through the production, conjugation and release of many pregnancy biomarkers such as hormones (oestrogens, placental lactogens, progesterone (P_4), glycoproteins, prolactins, cortisol); enzymes, growth factors and inhibitors at different periods of gestation (Sousa et al., 2008). Cortisol is not a pregnant-specific hormone. However, during pregnancy, it gradually increases in concentration and doubles towards the end of gestation (Bell et al., 1991). These pregnancy biomarkers are released into maternal circulation during

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http://dx.doi.org/10.1016/j.anireprosci.2016.02.014
0378-4320/

pregnancy where their levels are detected and used to determine the status of pregnancy and obstetric diseases (Karen et al., 2003). Their concentrations are particularly important to clinicians and researchers studying the pathophysiology of pregnancy in ruminants (Breukelman et al., 2005).

Trypanosomosis is an abortifacient disease known to cause early embryonic death and foetal losses during pregnancy (Ikede et al., 1988; Sekoni, 1994). It is caused by *Trypanosoma congolense* (Faye et al., 2004; Abubakar et al., 2015), *T. vivax* (Bawa et al., 2000; Silva et al., 2013) and *T. brucei* (Anene and Omamegbe, 1984; Leigh and Fayemi, 2013), which are the most pathogenic trypanosome. *Trypanosoma evansi* (*Surra*) is less pathogenic compared to the trio, affecting buffaloes, camels and horses (Office of the International Epizootic (OIE), 2012); where abortion has been reported (Gutierrez et al., 2005). *Surra* is not principally a trypanosome of sheep, but disease ranging from chronic to acute infections have been reported (Audu et al., 1999; Dalal et al., 2008). The effect of trypanosomosis on pregnancy biomarkers has not been thoroughly studied in spite of their roles as indicators for pregnancy viability. Apart from Osaer et al. (1998) and Faye et al. (2004), who studied the effect of experimental *T. congolense* infection on pregnancy specific protein-B (PSPB) of Djallonkeí ewes and West African dwarf goats, respectively, no other information is available on similar study in sheep or other ruminants to the best of our knowledge. This study was therefore designed to determine the effect of *T. evansi* on some pregnancy biomarkers of Yankasa ewes. Information generated from the study could provide a better understanding on the pathophysiological mechanism of *Trypanosome* infection-induced pregnancy disorder in ewes. Such information could be explored in designing and developing control measures to reduce reproductive losses associated with the disease thereby increasing sheep productivity.

2. Materials and methods

2.1. Study site

The study was carried out at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, from July 2013 to February 2014. Zaria is located between latitude 11° and 12° N and between longitude 7° and 8° E at an elevation of 650 m above sea level, within the Guinea Savannah zone of Nigeria (Kershaw, 1968).

2.2. Study animals

The Yankasa ewes used were obtained from local markets around Zaria. The ewes were acclimatized for six weeks during which they were housed in fly-proof pens. They were screened for common ecto and endoparasites and treated with albendazole. Hay and concentrate supplement were given twice a day, while salt lick and water were provided *ad libitum*. The study was approval by the Ahmadu Bello University Committee on Animal use and care.

2.3. Trypanosome parasite

T. evansi was used in this study. The parasite was isolated from a naturally infected camel slaughtered at the Sokoto metropolitan abattoir (Sokoto is about 240 miles from Zaria). Blood from the camel was inoculated into two rats and transported by road to the Protozoology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, where it was maintained by serial passage in mice and rats.

2.4. Study design

Twenty pregnant Yankasa ewes were assigned into 3 groups (A, B, and C) comprising 7 ewes each in groups A and B, and 6 ewes in group C. Their estrus was initial synchronized by two intramuscular administration of Prostaglandin $F_{2\alpha}$ (estrumate®) 11 days apart, and bred with Yankasa rams showing good reproductive capacity. Pregnancy was determined by persistent rise in progesterone (P_4) concentrations and absence of estrus 20 days after breeding. Transabdominal ultrasonography was done using a real time B-mode scanner (Medison SA600 V, Germany) to determine pregnancy at day 45 post breeding. Group A and B were each infected with blood containing approximately 1.0×10^6 *T. evansi* through jugular vein on days 59 and 110 of pregnancy, representing second and third trimesters, respectively, while group C was uninfected and served as the control. The parasitaemia was assessed daily from the time of infection till patency was established. Subsequently, it was assessed weekly. Wet mount and haematocrit centrifugation technique methods as described by Woo (1969) and Murray et al. (1977), respectively were used. Serum was collected from ewes weekly (7 ± 1 days) from the time of infection till few days post-partum and used to determine the pregnancy biomarkers (Progesterone, pregnancy specific protein-B, estrone sulfate and cortisol). These biomarkers were also determined three weeks before infection to establish a baseline.

2.5. Assay for pregnancy biomarkers

The 5 mL of blood was collected and centrifuged at 9000*g* for 5 min to harvest serum. This was stored at −20 °C until enzyme-linked immunosorbent assay (ELISA).

2.6. ELISA assay for progesterone

Progesterone (P_4) was determined using ACCU-BIND® ELISA kits. The procedure was performed following the manufacturer's instruction. It involved pipetting 25 μL of test references and test samples into the antibody coated micro-plate. Fifty microliters of P_4 enzyme reagent was added to all the wells and swirled gently for 10–20 s. P_4 biotin reagent (50 μL) was added to all the wells, swirled again and covered with paraffin paper and incubated for 60 min. After, incubation, the contents of the micro plate were decanted and dried with absorbent paper. Wash buffer (350 μL) was added, decanted and dried with absorbent paper, this was repeated three times. One hun-