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Theriogenology
Theriogenology 62 (2004) 1438–1451
www.elsevier.com/locate/theriogenology

Effects of an experimental *Trypanosoma congolense* infection on the reproductive performance of West African Dwarf goats

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Received 7 September 2003; received in revised form 12 February 2004; accepted 15 February 2004

Abstract

Thirty-six West African Dwarf (WAD) goats were used to assess the effects of an experimental *Trypanosoma congolense* infection on their reproductive system. Estrous cycles were synchronised and when confirmed pregnant ($n = 31$), the does were randomly allocated into control and trypanosome-infected groups. After infection, the animals were carefully observed till parturition. Trypanosome infection caused an increase of rectal temperature, a significant drop in PCV (infected: $23.3 \pm 0.3\%$; control: $28.5 \pm 0.4\%$; $P < 0.0001$) and abortions in 27.8% of the infected does. Kids born from infected does had a lower birth weight than kids born from control goats (0.9 ± 0.1 kg versus 1.6 ± 0.1 kg; $P < 0.0001$). Eight out of 13 kids (61.5%) that were born alive from infected does died during their first week of life. Plasma pregnancy-associated glycoprotein (PAG) and progesterone concentrations were lower in the infected animals than in the controls. In general, PAG concentration in does which aborted dropped before abortion. Our results revealed that artificial *T. congolense* infection affected reproductive performance of WAD goats with abortions, premature births and perinatal losses being observed. Neither transplacental transmission of *T. congolense* nor histopathological lesions of the placenta could be demonstrated.

Keywords: WAD goat; *Trypanosoma congolense*; Reproduction; PAG; Progesterone

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doi:10.1016/j.theriogenology.2004.02.007

1. Introduction

In addition to their importance as providers of meat and milk, goats play an important socio-economic role in the farming system of many developing countries. One of the beneficial features of goats is their high reproductive potential. However, due to unfavourable environmental conditions, this potential is not always expressed, goats being affected by reproductive losses. As reported by Shalaby et al. [1], goats also appear to be more susceptible to abortion than other species. In fact, many diseases can reduce the reproductive performance of goats and among these, trypanosomosis can be important in tsetse-infested areas. Reduced kidding percentages in *Trypanosoma congolense*-infected West African Dwarf (WAD) goats were reported by Kanyari et al. [2]. In The Gambia, a higher rate of abortion was reported under high tsetse challenge than under a moderate challenge, this abortion rate being higher in goats than in sheep [3]. In another experimental study, Goossens et al. [4] found that the reproductive performance following artificial *T. congolense* infection was more affected in caprine than in ovine native breeds, resulting in abortions and foetal death. Persistent corpora lutea, abortions, foetal death and neonatal death following trypanosome infection have been described by several authors [5–8]. Low levels of plasma oestradiol and/or progesterone have also been reported following trypanosome infection [7,9,10]. The transplacental transmission of *T. congolense* leading to abortions in East African goats was reported by Ogaa et al. in 1991 [11].

Pregnancy-associated glycoproteins (PAG), also called pregnancy-specific protein B (PSPB), are among the proteins synthesised by mononucleate and binucleate cells of the placenta and can be a useful indicator of both pregnancy and foetal well-being [12–14]. A negative effect of infections, such as toxoplasmosis and listeriosis, on PAG concentrations was reported by Zarrouk et al. [13] in goats. To our knowledge, no studies were carried out to assess the effect of trypanosomosis on plasma PAG or PSPB concentrations during the whole period of gestation or to study its relation with abortion. The aim of this study was to assess the effect of an experimental *T. congolense* infection on the maternal PAG and P4 profiles and outcome of pregnancy in WAD goats.

2. Materials and methods

2.1. Study site

The experiment took place from April 2002 to September 2002 at the coastal station of the International Trypanotolerance Centre (ITC), Banjul, The Gambia. The apparent tsetse fly density is very low (0.062 flies/trap per day) on station (ITC, unpublished data). Most of the flies are *Glossina palpalis gambiensis*, which are likely to be a vector of *Trypanosoma vivax* rather than *T. congolense* infections [15].

2.2. Animals

Thirty-six female WAD goats were used for this study. All animals were vaccinated against Peste des Petits Ruminants (PPR ISRA, Dakar, Senegal) and pasteurellosis

(Pasteurellad® ISRA, Dakar, Senegal) and sprayed with flumethrin (Bayticol®, Suffolk, UK) once every 4 weeks during the dry season and fortnightly during the wet season.

2.3. Experimental design

All the goats were synchronised by two intramuscular administrations 12 days apart of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Estrumate®, Coopers, Burgwedel, Germany) at a dose rate of 125 μg per animal. Twenty-four hours after the second administration of Estrumate®, bucks were introduced for mating. Eleven weeks after introduction of the males, 31 does diagnosed pregnant by the measurement of PAG were allocated to two experimental blocks based on their live-weight: block 1 (14 animals with an average weight of 16.2 ± 1.3 kg) and block 2 (17 animals with an average weight of 23.0 ± 4.6 kg). Goats from both blocks were randomly allotted to two different treatment groups: infected (18 animals) and control (13 animals). Assigned animals were infected by intravenous route with a clone of *T. congolense* (SAT86/CRTA91) isolated from cattle at Satiri, 50 km from Bobo Dioulasso in Burkina Faso. The infective dose was approximately 10^5 trypanosomes.

Experimental animals were weighed once a week up to the end of the experimental period and rectal temperature was taken twice a week up to kidding. The packed cell volume (PCV) was determined from Week 2 before infection (Week −2) till Week 15 after infection (Week 15). The parasitaemia was assessed by the dark ground (DG) buffy coat method [16] and scored according to Paris et al. [17]. Following infection, all animals were bled two times a week until kidding and plasma was extracted for the measurement of P4 and PAG. Placenta samples from does which aborted and samples of liver, spleen and brain from foetuses or kids that died were collected into 10% formalin for histopathological analysis.

Blood from the foetus of goats which aborted were inoculated into inbred white Swiss mice for trypanosome isolation. When infected animals died or were slaughtered if their PCV dropped below 15%, blood from their foetus (1.0 mL) and amniotic liquid (1.0 mL) were also inoculated to mice. In all cases, inoculations were done within 1 h after abortion or slaughter of animals. Inoculated mice were monitored twice a week for 60 days by microscopic examination of a wet blood film from the tail. Overall, 8 mice were inoculated with amniotic liquid from 4 infected does that died or were slaughtered, 8 mice with blood from foetuses ($n = 4$) of does which aborted and 16 mice with blood from the jugular vein of 8 underweight kids born from infected does. Two mice were also infected with 0.2 mL of a stabilate of SAT86 containing 10^5 trypanosomes/mL to assess the infectivity and the pathogenicity of this strain in mice.

After kidding, birth weight of the offspring was recorded and the offspring born from the infected does that survived were checked for trypanosome infections.

2.4. Radioimmunoassay (RIA) for PAG

Pure bovine PAG ($boPAG_{67}$) preparation, purified according to the method of Zoli et al. [18], was used as tracer and as standard. Radiolabelling was carried out using iodine-125 (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the chloramine T method [19]. A rabbit polyclonal antiserum anti-caprine PAG_{55+59} (anti-$cPAG_{55+59}$, AS708) was

used as the first antibody at an initial dilution of 1:30,000. The second antibody precipitation system was composed by a mixture of sheep anti-rabbit immunoglolobulin (0.83%, v/v), normal rabbit serum (0.17%, v/v), polyethylene glycol 6000 (20 mg/mL; Vel, Leuven, Belgium), cellulose microcrystalline (0.05 mg/mL; Merck, Darmstad, Germany) and BSA (2 mg/mL; ICN Biochemicals Inc., Aurora, OH, USA) diluted in Tris buffer (0.025 M Tris and 0.01 M $MgCl_2$; pH 7.5).

Concentrations of PAG in plasma samples were measured by a heterologous RIA derived from the method previously described by Gonzalez et al. [20]. Briefly, sample volumes of 25 μL were mixed with 300 μL of Tris-BSA assay buffer into duplicate assay tubes. Pure stock of bovine PAG (lyophilised powder) was diluted with Tris-BSA assay buffer to give standard curves ranging from 0.8 to 100 ng/mL. Thereafter, 100 μL of each standard concentration was mixed with 200 μL of Tris-BSA and 25 μL of caprine PAG-free serum to make up the standard curve. Zero standard tubes (B0; 300 μL Tris-BSA + 25 μL PAG-free serum) and tubes for assessing non-specific binding (NSB; 400 μL Tris-BSA + 25 μL PAG-free serum) were also prepared. The tracer [^{125}I]boPAG$_{67}$ (25,000 cpm/100 μL) was then added to all tubes. Finally, 100 μL of anti-serum solution (AS708 at 1:30,000) was added to each tube except for those evaluating NSB.

After one night of incubation at room temperature, bound and free fractions were separated by the addition of 1.0 mL second antibody precipitation system. After 30 min of incubation, 2.0 mL of Tris-BSA was added and the tubes centrifuged at 1800 × *g* and at 10 °C for 20 min. The supernatant was then discarded and the radioactivity of the pellets assessed using a Multigamma Counter (LKB Wallac 1261; Turku, Finland) with a counting efficiency of 75%. The intra-assay and inter-assay coefficients of variation of the heterologous PAG-RIA have been determined by Gonzalez et al. [20].

2.5. Radioimmunoassay for progesterone

Progesterone (Sigma Chemical Co., St. Louis, MO, USA) was used as tracer and as standard. Progesterone-11-hemisuccinate-2[^{125}I]iodohistamine was prepared by the mixed anhydric method [21]. Purification of the tracer was carried out by HPLC on C-18 reverse phase. Antiserum anti-P4-11-hemisuccinate-BSA was prepared in our laboratory [22] and used as the first antibody at an initial dilution of 1:40,000. Steroid-free serum was prepared by treatment of normal goat sera with charcoal (10 mg/mL; Merck) and Dextran T70 (1 mg/mL; Amersham Pharmacia Biotech). The second antibody precipitation system for progesterone was quite similar to that previously described, being diluted in phosphate-gelatine buffer [11.9 mg/mL Na_2HPO_4, 4.5 mg/mL KH_2PO_4, 9 mg/mL NaCl, 1 mg/mL NaN_3 and 1 mg/mL gelatine; pH 7.0].

A P4-RIA without extraction step was used for the determination of plasmatic P4 concentrations, as described previously by Ronayne and Hynes [23]. Briefly, 50 μL of goat plasma was mixed with 400 μL of citrate buffer [0.10 M citrate and 0.25% BSA containing 100 μL anilino-naphthalene-sulphonic acid (ANS)] at pH 4.0. The standard curve was prepared in a steroid-free serum and ranged from 0.4 to 50 ng/mL P4. A zero standard concentration was also made up by mixing 400 μL of ANS buffer and 50 μL of steroid-free serum. All the tubes were incubated overnight at 4 °C with 100 μL of P4-11-hemisuccinate-2-

Table 1
Intra-assay and inter-assay coefficients of variation (CV) of two quality controls with different concentrations of P4

	Intra-assay		Inter-assay	
	P4 concentration (ng/mL)	CV (%)	P4 concentration (ng/mL)	CV (%)
Quality control 1	3.13 ± 0.26 ($n = 9$)	5.02	2.55 ± 0.69 ($n = 8$)	7.62
Quality control 2	12.72 ± 0.90 ($n = 9$)	5.05	12.41 ± 1.21 ($n = 9$)	10.52

Concentrations are expressed as mean ± S.D. n = number of replicates.

[^{125}I]iodohistamine (25,000 cpm/100 μL) and 100 μL of antiserum anti-P4-11-hemisuccinate-BSA (1:40,000). Afterwards, 1.0 mL of second antibody precipitation system was added to the tubes. After 30-min incubation, 2.0 mL of phosphate-gelatine buffer were added and the tubes centrifuged at 1800 × g for 20 min. The radioactivity of the pellets was assessed in a Multigamma counter.

The intra-assay and inter-essay coefficients of variation of two quality controls were determined for the progesterone-RIA (Table 1).

2.6. Histopathology

A classical histological technique was used. Pieces of placenta tissue or liver, spleen and brain were fixed in formalin 10% and paraffin-embedded sections were cut at 5 μm on a sliding microtome. All sections were stained with haematoxylin and eosin and examined microscopically.

2.7. Statistical analysis

Data are presented as least square means (lsmeans) ± standard error (S.E.) unless stated otherwise. Statistical analyses were carried out using SAS® statistical package version 8 [24]. A General Linear Model (GLM) procedure was used to analyse continuous parameters as repeated measurements using a mixed model. The model included the following main effects: treatment (infected = 18 animals versus control = 13 animals), period (pre-infection, acute and chronic periods), interaction treatment × period, block (1 or 2), animal nested within treatment and block. The pre-infection period covered Weeks −8 to −2 before infection corresponding to Weeks 3–9 of gestation. The acute period covered Weeks 0–6 post-infection corresponding to Weeks 11–17 of gestation. The chronic phase covered Weeks 7–11 post-infection corresponding to Weeks 18–22 of gestation. The animal effect was considered as random.

The does were considered 3 weeks pregnant when the plasma P4 concentration reached a level equal or greater than 1.4 ng/mL. The infected group was divided into a group which aborted ($n = 5$) and a group which did not abort ($n = 13$). The latter group was further divided into does that kidded in term ($n = 3$) or prematurely ($n = 6$). The remaining four were withdrawn from the experiment before kidding. Thus, the PAG or P4 concentrations of control does were compared with that of infected does that kidded in term or prematurely. For the assessment of the drop in PAG in the different groups during a

given period, the regression coefficient (a) was estimated for each animal from a linear regression ($ax + b$) and analysed.

3. Results

3.1. Temperature and PCV

Trypanosome infection caused a significant increase of rectal temperature ($P < 0.0001$) with means of 38.8 ± 0.04 °C for the infected group compared to 38.4 ± 0.05 °C for the control group.

During the experimental period, trypanosome infection caused a significant drop in PCV ($P < 0.0001$) with a mean of $23.3 \pm 0.3\%$ for the infected group versus $28.5 \pm 0.4\%$ for the control group. As shown in Fig. 1, the lowest mean PCV in the infected group was recorded during the chronic phase of the infection ($15.7 \pm 0.3\%$) with a significant interaction period × infection ($P < 0.0001$).

3.2. Pregnancy in control and infected goats

All 13 does from the control group kidded successfully, giving 18 kids. Five out of the 13 does had twins. In the infected group, 9 out of the 18 infected does kidded giving 13 kids. Four out of the nine does had twins. Three infected does died due to the infection and one was slaughtered when the PCV dropped below 15%. Five out of 18 infected does aborted (27.8%). Abortions occurred between 21 and 59 days following infection (Table 2) with an arithmetic mean of 45.8 ± 11.4 days. Most abortions occurred during the chronic phase of

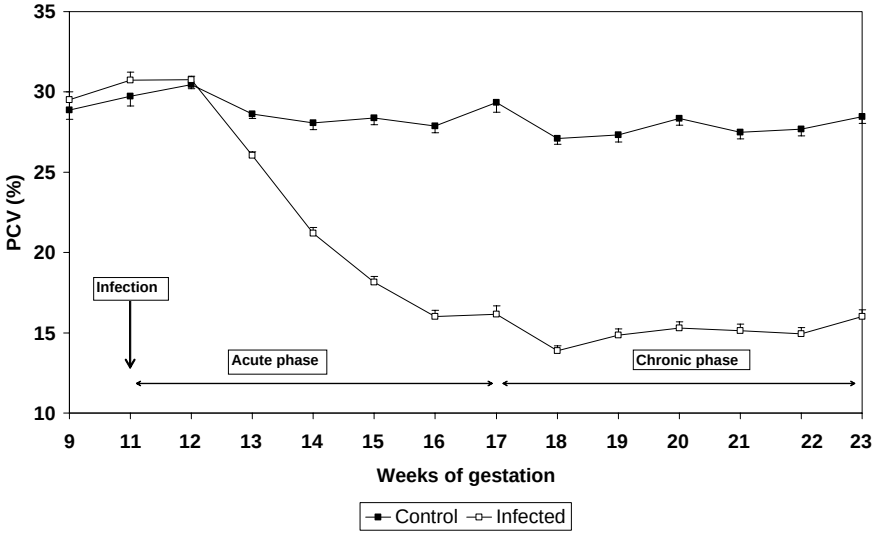

Fig. 1. Mean weekly packed cell volume (% ± S.E.M.) of control and infected WAD does.

Table 2
Rectal temperature (RT), packed cell volume (PCV) and parasitaemia of WAD goats with unsuccessful pregnancies

Goat	Days between infection and abortion	Mean RT from Days 0[a] to A[b] (°C)	RT at Day A[b] (°C)	PCV (%)	Parasitaemia
1924	21	39.15	40.72	25	4+
1947	42	38.80	40.33	12	3+
1921	53	38.70	38.97	17	4+
1918	54	38.94	40.92	14	5+
1916	59	38.79	38.27	11	3+

[a] Day 0 = day of infection.
[b] Day A = day of abortion.

the infection. Three out of the five animals that aborted had fever at the time of abortion but none of them had a prolonged fever before abortion. From those that kidded and irrespective of infection, 9 does (40.9%) had twins and 13 does (59.1%) had a single kid.

Infected goats had a significantly shorter pregnancy length than control ones (145.0 ± 2.0 days versus 150.5 ± 1.6 days; $P < 0.05$). Kids from infected goats had a significantly lower birth weight than those from the control group (0.9 ± 0.1 kg versus 1.6 ± 0.1 kg; $P < 0.0001$). Indeed, six out of nine (66.7%) infected does that kidded had kids with a birth weight between 0.6 and 1.0 kg. These six goats with a significantly ($P < 0.05$) shorter pregnancy length (143.3 ± 2.4 days versus 150.5 ± 1.6 days) in addition to a lower birth weight of kids were characterized as having a premature parturition.

Eight kids out of 13 (61.5%) that were born alive from infected does died during their first week of life. Three kids out of 18 (16.7%) born from control animals also died during their first 2 weeks of life.

One of the mice infected with SAT86 died for unknown reasons 1 day after infection. The second mouse became positive 5 days after infection, had a parasitaemia greater than 10^6 trypanosomes/mL 8 days after infection and died at Day 12 following infection. None of the mice inoculated either with amniotic liquid or blood from the foetuses became positive within 60 days of monitoring.

3.3. Histopathology

A total of 22 samples of placenta were collected, 15 from infected does and 7 from controls. Among the infected does, five placenta samples were collected from those that gave birth prematurely, four from those that aborted, four from those that died or were slaughtered before kidding and two from those that had a normal kidding. The histopathological analysis of the placentas did not reveal any degenerative lesions of the epithelial or trophoblastic cells or presence of inflammatory cells in the stroma. There were also no lesions of the blood vessels.

Samples of liver, spleen and brain were collected from 10 foetuses or kids that died after birth. Three of them were from control does and seven from infected does. No specific lesions were detected in the organs of kids born from infected does.

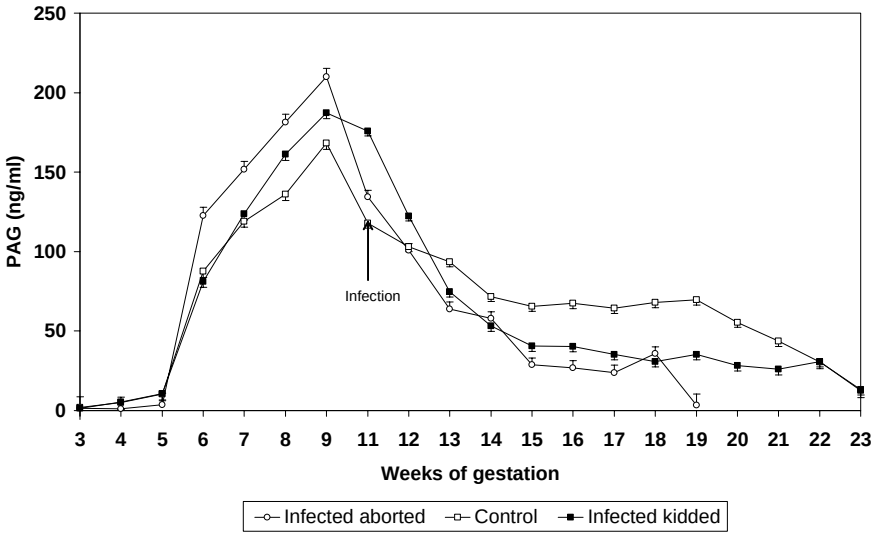

Fig. 2. Pregnancy-associated glycoprotein (PAG) profile (ng/mL ± S.E.M.) of control does compared to that of infected does that kidded successfully or aborted.

3.4. PAG and progesterone profiles

Fig. 2 shows the profile of PAG concentrations measured in control does, infected does that kidded successfully and in infected does which aborted. In general, PAG levels started to increase from Week 3 of gestation and reached a peak at Week 9. Thereafter, the levels decreased gradually until parturition.

From Week 11 (period of trypanosome inoculation) to Week 19, the drop in PAG concentrations was significantly faster ($P < 0.05$) in does that aborted than in controls (slope: -2.1 ± 0.5 versus -0.8 ± 0.3, respectively). During the same period, the drop in PAG was also significantly faster ($P < 0.001$) in infected does that kidded successfully (slope: -2.7 ± 0.4) than in controls (slope: -0.8 ± 0.3). Within the infected group, the drop in PAG was not significantly different between does that aborted and those that kidded successfully between Weeks 11 and 19, although there was a sharp drop in PAG levels between Weeks 18 and 19 within the infected, aborted group (Fig. 2).

From Weeks 11 to 13 of gestation, the drop in PAG was significantly faster ($P < 0.05$) in infected does that kidded successfully (slope: -7.9 ± 2.4) than in control does (slope: -1.6 ± 1.2). During the same period, the drop in PAG was also significantly faster ($P < 0.05$) in does that kidded prematurely (slope: -6.5 ± 1.9) than in control ones (slope: -1.6 ± 1.2). Consequently, during the acute phase, infected does that kidded prematurely had a significantly lower PAG concentration than does that kidded normally either infected or not (Table 3). During the chronic phase, a significantly higher PAG concentration was observed in control does than in infected females that kidded normally or prematurely.

Between Weeks 11 and 13 of gestation, PAG concentration was significantly higher in does carrying twins compared to those with a single foetus (133.8 ± 10.2 ng/mL versus 95.6 ± 9.1 ng/mL; $P < 0.05$).

Table 3
Plasma pregnancy-associated glycoprotein (PAG) concentration (ng/mL) in WAD goats during different phases of the infection and according to the outcome of the pregnancy

	Pre-infection	Acute phase	Chronic phase
Control	90.6 ± 6.3^{a}	88.1 ± 4.2^{d}	61.4 ± 4.5^{g}
Infected (normal kidding)	147.9 ± 12.6^{b}	90.3 ± 8.8^{e}	36.6 ± 9.4^{h}
Infected (premature kidding)	95.9 ± 9.6^{c}	65.6 ± 6.2^{f}	29.2 ± 6.9^{i}

Concentrations are expressed as lsmean ± S.E.
Superscript letter b statistically different from a ($P < 0.0001$) and c ($P < 0.01$); f statistically different from d ($P < 0.01$) and e ($P < 0.05$); g statistically different from h ($P < 0.05$) and i ($P < 0.0001$).

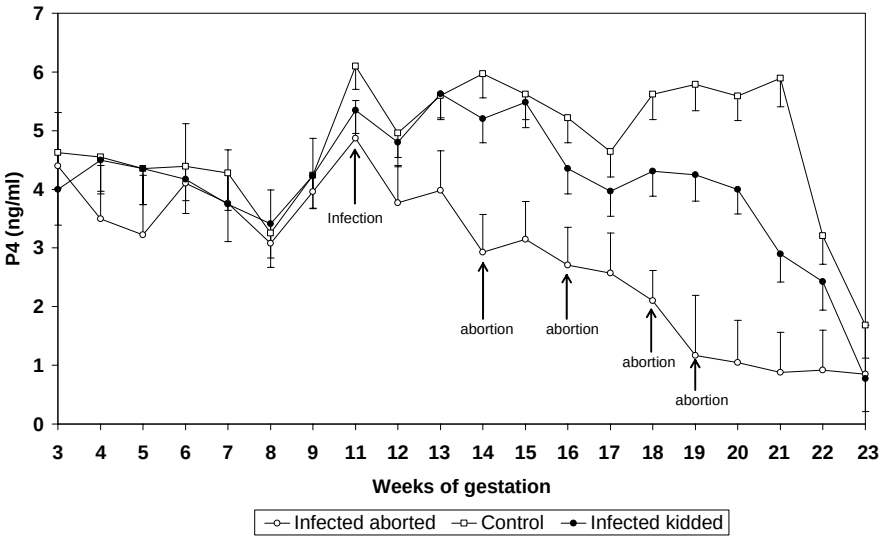

Fig. 3. Progesterone profile (ng/mL ± S.E.M.) of control does compared to that of infected does that kidded successfully or aborted.

Table 4
Plasma P4 concentration (ng/mL) of WAD goats during the different phases of the infection and according to the outcome of the pregnancy

	Evolution of pregnancy	Pre-infection	Acute phase	Chronic phase
Control	Normal kidding	4.3 ± 0.1^{a}	5.6 ± 0.1^{e}	5.5 ± 0.1^{i}
Infected	Normal kidding	3.6 ± 0.3^{b}	4.6 ± 0.2^{f}	4.1 ± 0.3^{j}
Infected	Premature kidding	4.2 ± 0.2^{c}	4.6 ± 0.2^{g}	4.4 ± 0.2^{k}
Infected	Aborted	3.7 ± 0.2^{d}	4.0 ± 0.2^{h}	3.1 ± 0.4^{l}

Concentrations are expressed as lsmean ± S.E.
Superscript letter a significantly different from b ($P < 0.05$); e significantly different from f ($P < 0.001$), g ($P < 0.0001$) and h ($P < 0.0001$); f significantly different from h ($P < 0.05$); g significantly different from h ($P < 0.05$); i significantly different from j ($P < 0.0001$), k ($P < 0.0001$) and l ($P < 0.0001$); k significantly different from l ($P < 0.01$).

After 3 weeks of gestation, the P4 concentrations were already high (between 4 and 5 ng/mL), remaining more or less constant up to the period of infection (Fig. 3). Between Weeks 11 and 21 of gestation (Weeks 0 and 10 post-infection), there was a drop in P4 concentrations in infected animals (which either aborted later on or kidded) whilst P4 concentrations were maintained in controls.

During the period of infection, the P4 concentration was significantly lower in infected animals (aborted, giving birth prematurely or kidded normally) than in controls (Table 4).

4. Discussion

This study describes the PAG and P4 profiles of WAD goats during pregnancy. In non-infected WAD goats, the PAG concentration increased significantly in Week 6 of gestation and reached a peak at Week 9. In Brazilian Moxoto and Caninde goats the peak PAG concentration was reached in Week 7 [25] whilst in Spanish dairy goats the highest concentration was reached between Weeks 8 and 12 of gestation [26]. After the peak, PAG dropped rapidly in WAD goats until Week 14 and thereafter more slowly until parturition. A similar pattern was reported in dairy goats by Gonzalez et al. [26] whilst in Moxoto goats, following the peak, PAG decreased slowly until Week 21. In Brazilian Caninde goats, following the peak, PAG concentrations remained relatively constant until Week 21. PAG pattern in WAD goats was also different from those obtained in ewes [27] and cows [28,29]. Differences in PAG patterns described in healthy goats from distinct origins might be due to genetic differences among different breeds or to different environmental conditions.

Between Weeks 11 and 13 of gestation, goats giving birth to multiple foetuses had higher PAG concentrations than those that delivered one single foetus. A higher PAG concentration between Weeks 15 and 19 of gestation was also reported in Brazilian Moxoto goats bearing multiple foetuses [25].

Overall, in the control group, there was no tendency for a great variation of P4 concentration during pregnancy and this is in agreement with the findings of Humblot et al. [30] and Sousa et al. [25] in Alpine and Brazilian native goats, respectively. In uninfected WAD goats, from a mean concentration of 4.6 ± 1.1 ng/mL at Week 3 of gestation, P4 concentration increased slightly until Week 11 (6.1 ± 2.6 ng/mL) and remained at this level until kidding.

During our study, the effects of an experimental *T. congolense* infection on the secretory profiles of PAG and P4 and on the outcome of pregnancy in WAD goats were also assessed. An abortion rate of 27.8% was recorded following trypanosome infection. Abortions occurred mainly at the beginning of the chronic phase of the infection. Embryonic mortalities and abortions following trypanosome infection are widely described in domestic ruminants [6–8,31,32].

Trypanosome-infected does that had underweight kids also had a significantly reduced pregnancy length that is indicative of a premature kidding. These underweight kids did not survive more than 1 week. Premature kidding and perinatal losses have been reported by Elhassan et al. [33] and Okech et al. [34] in *T. vivax*-infected ewes and cattle, respectively.

During our study, trypanosome infection affected both P4 and PAG concentrations. The infection coincided with the descending phase of the PAG concentration in all groups but the decline was more pronounced in infected does. In general, the drop in PAG and P4 concentrations preceded the occurrence of abortion. During the acute phase of the infection, infected goats that had weak kids had significantly lower PAG and P4 concentrations than control ones. Osaer et al. [31] also reported a tendency for a low PSPB concentration during early gestation in Trypanosome-infected pregnant ewes. Similar observations were made by Zarrouk et al. [13], who reported a slow and marked decrease of PAG in goats following *Toxoplasma gondii* and *Listeria monocytogenes* infections.

Progesterone is essential for the establishment and maintenance of pregnancy in ruminant species. In Djallonke ewes, well known for their trypanotolerance, trypanosome infection did not have any significant effect on plasma P4 concentrations [31]. In contrast, in the present study, a decline of P4 concentrations was observed in infected WAD goats, both in the group which kidded and in that which aborted. This decline could explain at least partially the lower reproductive performance of infected WAD goats when compared to infected Djallonke ewes, confirming earlier observations of Goossens et al. [4]. Further investigations on endocrine parameters during pregnancy have to be carried out in the caprine species in order to compare the magnitude of alterations in more or less trypanotolerant breeds.

In our study, four out of five does that aborted had a PCV below 20% and three out of five had fever at the time of abortion. In field conditions, Rowlands et al. [35] demonstrated that when detected parasitaemic, cows with a mean PCV below 22% had a higher abortion rate than cows with PCV above 22%. Mechanisms leading to abortion and premature birth following trypanosome infection are not yet fully understood. They can result either from systemic disorders like hyperthermia or anaemia, either from alterations of more specific pathways, as suggested by different authors. A first mechanism was described by Tilton et al. [36], who postulated an activation of the foetal hypothalamo-hypophyso-adrenal axis by factors, such as pyrexia or stress, resulting from trypanosome infection with subsequent release of corticosteroids which in turn induce placental secretion of $PGF_{2\alpha}$ to reduce progesterone synthesis. The finding that there is a high and persistent concentration of prostaglandin metabolites in non-pregnant trypanosome-infected goats presenting a low P4 level [37] prompted the suggestion that prostaglandin could be responsible for the cycle and pregnancy disturbances frequently observed in trypanosome-infected females.

Another mechanism was proposed to explain the progressive decrease in P4 concentrations in pregnant goats infected by *T. gondii* and *L. monocytogenes*. Engeland et al. [38,39] suggested that a disturbance of luteotropic activity due to a decrease in placental lactogen secreted by the binucleate cells could explain the drop in progesterone. More recently, Quinn et al. [40] postulated that abortion and foetal resorption following apicomplexan parasitic infections could be immune mediated. According to these authors, intracellular protozoan parasites stimulate Th1 cytokine responses with production of interferon γ and tumor necrosis factor α that can be deleterious to the foeto-placental unit.

PAGs are synthesised by mono- and binucleate cells of the ruminant placenta [41,42]. They are considered as good indicators of the foeto-maternal well-being [12,14]. The decrease in PAG concentrations following trypanosome infection may indicate a modification of placental activity in terms of protein synthesis. Reports on the effects of

trypanosome infections on placenta are very scarce. Ogwu et al. [43] reported minor histological lesions, such as more infiltrations with mononuclear cells of placentas from heifers experimentally infected with *T. vivax*. On the other hand, other protozoan parasites, such as *T. gondii* or *Neospora caninum*, were reported to cause focal necrosis in cotyledonary villi of the placenta [44] or multifocal necrotising placentitis [45], respectively. Transplacental passages of *T. congolense* and *T. vivax* were reported by Ogaa et al. [11] and Elhassan et al. [33], respectively. However, no transplacental passage of trypanosomes could be demonstrated during this study. This was also confirmed by the absence of histopathological lesions in the placental binucleate cells and in samples of organs collected from foetuses or kids.

From our experiment it can be concluded that experimental *T. congolense* infection during pregnancy affected goat's reproductive performance with abortions (27.8%), premature birth and perinatal losses. The infection also led to lower PAG and P4 concentrations. Neither transplacental transmission of *T. congolense* nor lesions of the placenta could be demonstrated.

Acknowledgements

This study was carried out with financial support of the Belgian Directorate General for Development Cooperation (DGDC, Brussels). The authors gratefully acknowledge the technical personnel of ITC for their help in various ways and Ms. Annie Locko and Ms. Sophie Dubois for their help in the PAG- and P4-RIA. The authors are grateful to Dr. Issa Sidibé and Dr. Zacharia Bengaly of CIRDES for kindly providing the trypanosome clone. Many thanks to Dr. Abdou Fall for his help in various aspects of the statistical analysis.

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