Pathological and Some Biochemical Studies on Pregnant Ewes Experimentally Infected with Trypanosoma evansi

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SUMMARY

This study was carried out on three groups of healthy, sexually mature Egyptian pregnant ewes aged between 2-3 years old. The first group included three ewes at 6 weeks pregnancy, the second group included three ewes at 12 weeks pregnancy and the third group included three ewes at 6 weeks pregnancy and was kept uninfect and used as a negative control. Ewes of first and second groups were inoculated intravenously with $10^5$ trypanosomes in 2ml of PBS for each ewe. Blood samples were collected weekly from the ewes for parasitological, hematological and biochemical examinations.

Ewes inoculated at 6 weeks of pregnancy aborted at 16 weeks. While those inoculated at 12 weeks of pregnancy delivered at term, of them, lamb of one ewe was born dead (stillbirth) and the 2 lambs who born from the other two ewes died within seven days after birth, meanwhile the control ewes lambed normally with good healthy lambs.

During pregnancy, there were negative hematological changes, increases in total protein and globulin concentrations and significant decreases in albumin, cholesterol, T₃ (triiodothyronine) and T₄ (thyroxin) values. There were significant changes in HDL values in comparison to the control ones. Pregnant ewes had poor body condition during the course of infection and their lambs born weak, emaciated and died within seven days after parturition, meanwhile the control ewes exhibited normal blood parameters and normal gestation period with healthy newborn lambs.

Histopathological results revealed that the aborted ewes suffered from necrotizing serofibrinous endometritis and focal areas of coagulative necrosis in the placenta and their foeti revealed vacuolar degenerative and necrobiotic changes in most of hepatocytes of the liver. While the delivered ewes showed perivascular and periglandular mononuclear inflammatory cell and neutrophils infiltration and degenerated uterine glands in their uteri. The placenta revealed inflammatory cell infiltration mostly of mononuclear type which was seen scattered throughout hepatic parenchyma of their dead lambs. No characteristic lesions were observed in the ovaries and fallopian tubes of both aborted and delivered ewes grossly and microscopically.
INTRODUCTION

Trypanosomiasis is a major veterinary problem over much of sub-Saharan Africa and is frequently associated with under nutrition (Holmes et al., 2000). It is one of the important diseases of domestic animals causing various reproductive disorders like irregular estrus, abortion, intrauterine infection and neonatal death (Losos and Ikede, 1972; Ikede, 1979 and Lohr et al., 1986).

During the post-infection phase with trypanosomiasis, infected ewes were anorexic, anaemic and lost weight (Reynolds and Ekwuruke, 1988). Whereas, 85% of lambs born to ewes infected with trypanosomiasis at late pregnancy died within 48 hours of birth most probably due to inadequate nutrition during pregnancy (Osuagwu and Aire, 1990).

Trypanosomiasis in pregnant ewes resulted in 16.7% abortion, 100% death and 33.3% neonatal deaths (Edeghere et al., 1992). Gutierrez et al. (2005A) presented an outbreak of abortions and high neonatal mortality attributable to \textit{T. evansi} infection in camels as well as the clinical assessment of the affected animals.

There was little information about the effect of \textit{T. evansi} on the reproductive wastage on pregnant ewes. In addition, most of the studies with trypanosomiasis in the perinatal period were undertaken with cattle and very little information is available on the incidence of trypanosome-induced reproductive wastage in sheep (Gunn, 1983; Ikede et al., 1988 and Akinbamijo and Reynolds, 1992).

The aim of the present investigation is to clarify pathological and some biochemical alterations occurring in the pregnant ewes experimentally infected with \textit{T. evansi}.

MATERIAL AND METHODS

Nine healthy, sexually mature Egyptian pregnant ewes aged between 2-3 years old were chosen from Animal Reproduction Research Institute-sheep farm. Pregnancy diagnosis was done by Ultrasonography (Scanner 200, Vet., Pie, Medical, Netherlands). Trypanosome free status of ewes was tested by mouse inoculation test and by microhaematocrit centrifugation technique (Murray et al., 1977). Ewes were dewormed by subcutaneous injection of ivermectin (Bomectine R product of BoMAC Laboratories, 1% W/V) at 1ml/50 kg body weight for internal and external parasites. \textit{T. evansi} strain was maintained in mice by serial passage.

The pregnant ewes were divided into three equal groups (3 animals each).
The first group included three ewes at 6 weeks pregnancy.

The second group included three ewes at 12 weeks pregnancy.

The third group included three ewes at 6 weeks pregnancy and was kept uninoculated and used as a negative control.

Ewes of first and second groups were inoculated intravenously (jugular vein) with the prepared dose \(10^5\) trypanosomes in 2 ml of PBS for each ewe) according to Damayanti et al. (1994).

All ewes were housed in separate fly proof units within the building to prevent any infection by haematophagous flies and were fed concentrates, fresh green foods (Dara wa), salt licks and water ad libitum.

1- Parasitological, clinical and haematological examinations:

Daily blood samples were taken until the appearance of parasitaemia. Packed cell volume (PCV) for detection of parasitaemia and anaemia was assessed by the dark ground (DG) method (Murray et al., 1977) and scored according to Paris et al. (1982). Thereafter, blood samples were collected weekly from all ewes up to ten weeks. All ewes were kept under strict observations for the appearance of any clinical symptoms (particularly abortions or deliveries) and rectal temperatures were measured weekly throughout the trial.

2- Biochemical examination:

Biochemical analysis of total protein, albumin and cholesterol (mg/dl) using diagnostic kits according to Henery (1968), Domas et al. (1972) and Watson (1960) respectively was done. Serum globulin was calculated by subtraction the value of albumin from the value of total protein according to Doumas and Biggs (1972). Serum high density lipoprotein (HDL) was calorimetrically determined according to Kostner (1976). Quantitative measurements of progesterone was performed using the coated A count progesterone RIA kits, its concentration was determined by solid phase RIA provided by commercially available kit (Diagnostic product) according to Hiller (1990). Triiodothyrinine (T3) and thyroxin (T4) hormones were assayed by radioimmunoassay according to Hollander and Shenkmon (1974) and Britton et al. (1975), respectively.

Statistical analysis was carried out using SASR (SAS Institute, 2000) procedures using Student "t" test according to Snedecor and Cochran (1980).

The aborted and delivered ewes were sacrificed and their genital organs together with internal organs of the aborted foeti and dead lambs were collected for pathological examination.
3- Pathological examination:
Ovaries, fallopian tubes, uteri and placenta of the aborted and delivered ewes and internal organs (liver, lungs, spleen and kidneys) of the aborted foeti and dead lambs were examined grossly. Thereafter, tissue samples from these organs were collected and fixed in 10% neutral buffered formalin, routinely processed in automated tissue processor, embedded in paraffin, sectioned at 3-5 µm, stained with hematoxylin and eosin (H&E) and examined by microscopy (Luna, 1960).

RESULTS
Ewes inoculated at 6 weeks of pregnancy aborted at 16 weeks while those inoculated at 12 weeks of pregnancy delivered at term. However, lamb of one ewe was born dead (stillbirth) and the 2 lambs of the other ewes died within seven days. The control ewes lambed normally with healthy and good condition lambs.

All ewes (control and infected) were chosen pregnant at the start of the study with 100% pregnancy percentages. Lambing % was higher in the control ewes (100%) than the infected ones (50%); the number of lambs born from the control ewes was 3 lambs (one lamb each with 100% lambing) while those born from infected ewes were 3 lambs (one born dead and the other 2 died within 7 days after parturition) with 50% lambing. Fertility % was also higher in control ewes (100%) than the infected ones (50%). The recorded result of prolificacy was higher in control ewes (100%) than that in the infected ewes (50%); the results are represented in table (1).

Table (1) Effect of T. evansi infection on the productivity and reproductive performance of pregnant ewes.

<table>
<thead>
<tr>
<th>Number of ewes</th>
<th>Control (n=3)</th>
<th>Infected(n=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pregnancy %</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>No. of ewes lamped</td>
<td>3/3 (100%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/6 (50%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>No. of ewes aborted</td>
<td>N</td>
<td>3/6 (50%)</td>
<td></td>
</tr>
<tr>
<td>No. of lambs born</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Fertility %</td>
<td>3/3 (100%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/6 (50%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>P , 0.05</td>
</tr>
<tr>
<td>Prolificacy %</td>
<td>3/3 (100%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/6(50%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

P = Probability
Means with different superscripts in the same row indicate significant difference at P<0.05
N = non
1- Parasitological, clinical and hematological findings:

Parasitaemia was generally low-level and often cryptic (Fig., 1). The first case of parasitaemia appeared from the fifth day pi until the end of the experiment. Parasitaemia peaked on day 35 pi when up to 7 parasites / 200 microscopic fields were counted. There were varying increases in body temperature (Fig., 2) which fluctuated throughout the course of infection coinciding with fluctuation in parasitaemia. All control ewes were free from trypanosomes and their temperatures were normal till the end of the study.

![Figure (1) Parasitaemia in T. evansi infected ewes](image1)

**Tryp. /200 MF = number of trypanosomes / 200 microscopic field at magnification of 400 X**

![Figure (2) Rectal temperature in T. evansi infected ewes](image2)
From the second week pi, infected ewes showed congestion and hyperemic vulva and fat tail. Emaciation was noticed starting from the fourth week pi, then there was loss of wool (became loose and easy detached) and paleness in the mucous membrane of the eyes. As a progression, there was severe emaciation, loss of condition, skin lesions around the neck and in the upper part of the claws, some respiratory affections (nasal discharges and hard breath), lacrimation and edema of the eyelids. These symptoms remained until the end of the experiment. Control ewes remained healthy and in good condition until the end.

Haematological traits are presented in table (2): initial decline in PCV% and in Hb concentrations were marked from the first week till tenth week pi as it revealed mean values from 34.75 ± 0.39 to 21.9 ± 0.26 for PCV and 10.53 ± 0.12 to 6.6 ± 0.05 for Hb concentrations. There were significant lower mean levels of PCV% and Hb concentrations in infected ewes than their respective control ones over the whole observation period.

Table (2) Effect of *Tr. evansi* on the bloods picture of infected pregnant ewes.

<table>
<thead>
<tr>
<th>weeks pi</th>
<th>PCV%</th>
<th>Hb gm/dl</th>
<th>TLC10³/ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>39.5 ± 0.20ᵃ</td>
<td>11.10 ±0.05ᵃ</td>
<td>9.83 ± 1.82ᵃ</td>
</tr>
<tr>
<td>1</td>
<td>34.75 ±0.39ᵇ</td>
<td>10.53 ± 0.12ᵇ</td>
<td>9.2 ± 2.25ᵃ</td>
</tr>
<tr>
<td>2</td>
<td>30.36 ± 1.5ᶜ</td>
<td>9.2 ± 0.05ᶜ</td>
<td>8.8 ± 0.86ᵇ</td>
</tr>
<tr>
<td>3</td>
<td>27.39 ± 0.38ᵈ</td>
<td>8.3 ± 0.12ᵈ</td>
<td>8.4 ± 0.80ᵇ</td>
</tr>
<tr>
<td>4</td>
<td>23.43 ± 1.2ᵉ</td>
<td>6.8 ± 0.23ᵉ</td>
<td>10.4 ± 1.44ᶜ</td>
</tr>
<tr>
<td>6</td>
<td>23.76 ± 1.3ᵉ</td>
<td>7.2 ± 0.4ᵉ</td>
<td>14.4 ± 0.80ᶜ</td>
</tr>
<tr>
<td>8</td>
<td>21.3 ± 0.05ᵉ</td>
<td>6.4 ± 0.05ᵉ</td>
<td>12.7 ± 0.63ᶜ</td>
</tr>
<tr>
<td>10</td>
<td>21.9 ± 0.26ᵉ</td>
<td>6.6 ± 0.05ᵉ</td>
<td>11.5 ± 0.63ᶜ</td>
</tr>
</tbody>
</table>

Values represent mean ± SE
Means with different superscripts in the same column indicate significant differences at P< 0.05
Concerning the whole white blood cell counts, there was a significant decrease (P<0.05) in their means at the second and third week pi in infected ewes followed by significant (P<0.05) increase started from fourth week pi till the end. This rise was paralleled by a significant (P<0.05) rise in the mean proportion and numbers of lymphocytes and a significant decrease (P<0.05) in the proportions and numbers of neutrophils. Neither monocytes nor eosinophils showed changes in their levels throughout the experiment (table, 3).

Table (3) Effect of *Tr. evansi* on the differential white blood counts in infected pregnant ewes.

<table>
<thead>
<tr>
<th></th>
<th>Lymphocyte%</th>
<th>Neutrophil%</th>
<th>Monocyte%</th>
<th>Basophil%</th>
<th>Eosinophil%</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>52.3 ± 6.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.66 ± 6.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>2.66 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>weeks pi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>45 ± 4.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.66 ± 5.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.33 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>2.33 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>49.62 ± 4.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.75 ± 3.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25 ± 1.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>2.07 ± 1.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>51.15 ± 6.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.65 ± 0.63</td>
<td>0</td>
<td>3 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>61.33 ± 3.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.66 ± 1.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.66 ± 2.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>1.66 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>70 ± 5.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.33 ± 6.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.66 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>2.33 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>71.66 ± 3.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.33 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>2.66 ± 0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>64.33 ± 0.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>2.66 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent mean ± SE
Means with different superscripts in the same column indicate significant differences at P< 0.05

2- **Biochemical findings:**

The results are illustrated in table (4). The mean levels of both total proteins and globulins showed significant increase (P<0.05) started from the first week pi till the sixth week pi then the mean values were significantly decreased (P<0.05) within the infected group till the end. Meanwhile, the mean values of albumin showed significant decrease (P<0.05) till the end of the study. Control ewes exhibited normal levels during the study.

Cholesterol mean values in infected ewes showed significant
changes (P<0.05) varied in the different weeks of infection. The mean levels showed significant increase (P<0.05) throughout the first four weeks pi showing maximum increase in the fourth week pi (64.8 ± 0.87) mg/dl, while it showed the minimum decrement levels at 10th week pi (34.93 ± 1.15) mg/dl. The mean values of HDL were significantly higher (P<0.05) than the control ewes overall the study. Within the infected ewes, HDL concentrations showed significant decrease (P<0.05), this decrement started from third week pi. The minimum decrement was recorded at the 10th week pi versus the first week pi.
Table (4) Effect of *T. evansi* on Total proteins, Cholesterol, T₃ and T₄ of Infected Pregnant Ewes.

<table>
<thead>
<tr>
<th></th>
<th>TP g/dl</th>
<th>Alb g/dl</th>
<th>Glob g/dl</th>
<th>Cholesterol mg/dl</th>
<th>HDL mg/dl</th>
<th>T3 ng/dl</th>
<th>T4 ug/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>6.6 ± 0.251</td>
<td>3.3 ± 0.11</td>
<td>3.3 ± 0.11</td>
<td>49.33 ± 0.37</td>
<td>20.4 ± 0.2</td>
<td>132.3 ± 0.14</td>
<td>7.2 ± 0.14</td>
</tr>
<tr>
<td>weeks pi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.45 ± 0.26</td>
<td>2.66 ± 0.08</td>
<td>4.43 ± 0.11</td>
<td>55.53 ± 0.86</td>
<td>43.7 ± 0.21</td>
<td>137.3 ± 0.23</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>7.07 ± 0.06</td>
<td>2.56 ± 0.28</td>
<td>4.3 ± 0.11</td>
<td>54.97 ± 1.52</td>
<td>49.4 ± 0.17</td>
<td>128.3 ± 0.11</td>
<td>5.07 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>8.47 ± 0.18</td>
<td>2.53 ± 0.15</td>
<td>5.66 ± 0.18</td>
<td>49.2 ± 0.39</td>
<td>42.4 ± 0.15</td>
<td>204.2 ± 0.14</td>
<td>8.5 ± 0.23</td>
</tr>
<tr>
<td>4</td>
<td>8.2 ± 0.15</td>
<td>2 ± 0.06</td>
<td>5.7 ± 0.24</td>
<td>64.8 ± 0.87</td>
<td>35.4 ± 0.17</td>
<td>140.3 ± 0.15</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>9.2 ± 0.15</td>
<td>1.73 ± 0.12</td>
<td>7.2 ± 0.08</td>
<td>39.13 ± 3.2</td>
<td>49.6 ± 0.18</td>
<td>79.6 ± 0.24</td>
<td>6.6 ± 0.14</td>
</tr>
<tr>
<td>8</td>
<td>6.72 ± 0.2</td>
<td>1.46 ± 0.08</td>
<td>5.3 ± 0.11</td>
<td>55.7 ± 0.93</td>
<td>34.4 ± 0.11</td>
<td>119.7 ± 0.23</td>
<td>6.2 ± 0.12</td>
</tr>
<tr>
<td>10</td>
<td>7.3 ± 0.42</td>
<td>1.8 ± 0.32</td>
<td>5.46 ± 0.2</td>
<td>34.93 ± 1.15</td>
<td>33.5 ± 0.14</td>
<td>62.6 ± 0.3</td>
<td>3.2 ± 0.14</td>
</tr>
</tbody>
</table>

Values represent mean ± SE
Means with different superscripts in the same column indicate significant differences at P< 0.05
Hormonal analysis:

Progesterone levels (Fig., 3) showed normal pattern till part-
urition or abortion in both control and infected ewes.

Concerning T₃ and T₄, the mean values were significantly de-
creased (P<0.05) from sixth week pi till the end of the study and The
minimum decrement was at the tenth week pi. (Table 4).

3-Pathological findings:

Gross and microscopical find-
ings of the ovaries of both aborted
and delivered ewes revealed the
presence of normal ovarian struc-
tures (C.L. of pregnancy in aborted
ewes and regressing C.L. in del-
ivered ewes), while the fallopian
ubes showed no characteristic les-
ions.

Aborted ewes:

Uterus:

Gross findings:

The endometrium appeared
swollen, congested and covered by
mucinous clear or turbid exudates.

Microscopical findings:

Uterine alterations consisted of necrotizing serofibrinous endo-
metritis characterized by desquam-
ated endometrial epithelium with
the exception of small areas which
were lined by low columnar degener-
ated epithelium. Endometrial st-
roma showed mild infiltration of
inflammatory cells mostly of mon-
onuclear type in the stratum com-
compactum mostly subepithelial (Fig., 4) meanwhile, moderate infiltration with lymphocytes, histocytes, neutrophils and eosinophils were seen in some areas of the stratum spongiosum which extended deeply between and around the uterine glands (Fig., 5). Diffuse edema dispersing the uterine structures from each other and congested blood vessels were observed (Fig., 6). Focal areas of coagulative necrosis were seen in both stratum compactum and spongiosum of the stroma in 2 out of 3 ewes in addition, calcification was noticed in one ewe (Fig., 7). Most of the uterine glands appeared degenerated, destructed and their epithelium showed necrobiotic changes. Moreover, the lumina of some glands revealed cellular remnants (Fig., 6 & 8). Periglandular and perivascular fibroblastic proliferation together with the presence of fibrin threads in some areas of the endometrial stroma were also observed.

The myometrium appeared loose with widely separated muscle fibers (Fig., 9) and the perimetrium showed focal edema and sometimes infiltrated with lymphocytes.

Placenta

Macroscopical findings:
The placenta showed various degrees of edema and sometimes gray and white areas of necrosis were evident. Some areas of the cotyledons showed caseated material.

Microscopical findings:
Focal areas of coagulative necrosis were noticed (Fig., 10). Moreover, the villi revealed degenerative and necrobiotic changes in their tips, edema, congested blood vessels and mild infiltration of inflammatory cells mostly of mononuclear type (Fig., 11).

Aborted foeti

Gross findings
The foeti revealed general septicemia and the liver was the most organ affected. The liver appeared pale, enlarged and showed tiny pinpoint yellow necrotic foci.

Microscopical findings:
Most of the hepatocytes revealed vacuolar degenerative and necrobiotic changes. Lymphocytes, histiocytes, neutrophils and eosinophils were seen scattered throughout the hepatic parenchyma (Figs., 12 & 13).

Delivered ewes

Uterus

Gross findings:
The endometrium showed mild degree of congestion and edema.

Microscopical findings:
Endometrial epithelium was lined by low columnar or cuboidal epithelium with partial desquamation. Endometrial stroma revealed perivascular and periglandular mononuclear inflammatory cell and neutrophils infiltration, edema and congested blood vessels. Some
uterine glands showed secretory activity with mild dilation of their lumen meanwhile degenerated glands with vacuolation and pyknotic nuclei in their epithelium were seen. In addition, periglandular fibroblastic proliferation was observed in few glands.

There were no characteristic alterations observed in the myometrium and perimetrium of these ewes.

Placenta

Macroscopical findings:
The placenta appeared edematous, congested and covered with purulent exudates.

Microscopical findings:
Placenta showed edema, congestion and mild infiltration of inflammatory cells mostly of mononuclear type.

Dead lambs

Macroscopical findings:
As those observed in aborted foeti, in addition the lambs appeared weak and emaciated.

Microscopical findings:
Focal areas of inflammatory cell infiltration mostly of mononuclear type were seen in the hepatic parenchyma.

Fig. (4): Uterus of aborted ewe showing desquamated epithelium except some areas appeared degenerated and mild infiltration of inflammatory cells mostly of mononuclear type in the stratum compactum mostly subepithelial (H&E; X100).
Fig. (5): Uterus of aborted ewe showing infiltration of mononuclear inflammatory cells and neutrophils (arrows) between and around the uterine glands (H&E; X100).

Fig. (6): Uterus of aborted ewe showing diffuse edema dispersing uterine structures from each others, congested blood vessels and degenerated glands revealing cellular remnants in their lumina (H&E; X100).
Fig. (7): Uterus of aborted ewe showing areas of coagulative necrosis and calcification in the endometrial stroma (H&E; X40).

Fig. (8): Uterus of aborted ewe showing degenerated and destructed glands (H&E; X40).
Fig. (9): Uterus of aborted ewe showing loose and widely separated muscle fibers of the myometrium (H&E; X100).

Fig. (10): Placenta of aborted ewe showing area of coagulative necrosis and mild infiltration of mononuclear cells (H&E; X100).
Fig. (11): Placenta of aborted ewe showing degenerative and necrobiotic changes in the tips of the villi, edema, congested blood vessels and mild infiltration of inflammatory cells mostly of mononuclear type (H&E; X100).

Fig.(12): Liver of aborted fetus showing vacuolar degenerative and necrobiotic changes in most of the hepatocytes and infiltration of lymphocytes, histiocytes, neutrophils and eosinophils throughout the hepatic parenchyma (H&E; X100).
DISCUSSION

Trypanosomiasis induces loss of body condition in pregnant and lactating ewes, animals infected during gestation had lambs with low birth weights and poor performance and caused foetal and neonatal losses in West African Dwarf sheep (Akinbamijo and Reynolds, 1992).

The low-level cryptic parasitaemia observed in this study is characteristic of chronic T. evansi infection and agrees with observations of Onah et al. (1996) in sheep and Azza (2001) in buffalo calves. The low level of parasitaemia in T. evansi infection is ascribed to the fact that it is predominantly a tissue dwelling parasite where sequestrated in internal organs (Azza, 2001).

Inoculated ewes were febrile and anaemic. These two symptoms have been widely reported hallmarks in diagnosis of trypanosomiasis (Holmes et al., 2000). Pyrexia indicates metabolic disorders due to the presence of circulating trypanosomes and their by-products as stated by Audu et al. (1999). The fluctuation in temperature coinciding with parasitaemic rises is due to the phenomenon of antigenic variation as reported by Edwards et al. (1956). Emaciation, loss of wool, skin lesions, respiratory affe-
ctions and lacrimation of the eyes observed in our study were also noticed by Karram et al. (1991), Sakr et al. (1991) and Agag et al. (1993) in *T. evansi* infected dromedary camels in Egypt; Cresenico et al. (1994) in *T. evansi* infected cattle and Azza (2001) in *T. evansi* infected buffalo calves. Anaemia is one of the most finding in animal trypanosomiasis and its nature is difficult to elucidate. The present study revealed that the mean Hb concentration values and PCV% appeared lower than those of the control ones and the decline started with the appearance of trypanosomes in the circulation which was considered as an indicative of anaemia. These results come in agreement with the previous observations of Karram et al. (1991) in *T. evansi* infected camels in Egypt; Onah et al. (1996) and Audu et al. (1999) in *T. evansi* infected sheep; Sharma et al. (2000) in trypanosomiasis of goats; Mdachi et al. (2005) in *T. evansi* infected horses and Hilali et al. (2006) in *T. evansi* infected buffalo calves. The drop in Hb concentrations and PCV% might be due to the cumulative effects of hemodilution, extravascular hemolysis and dishemopoiesis as recorded by Richardson and Kendall (1963) and Dargie et al. (1979) or might be due to the by-products of the circulating trypanosomes which could play a significant role in the process of hemolysis and consequently the fall in PCV% as stated by Audu et al. (1999).

Significant reduction in TWBCs followed by significant increases started from 4th week pi of our study come in accordance with Karram et al. (1991) in *T. evansi* infected camels; Mwangi (1991); Williams et al. (1991) and Onah et al. (1996) in cattle and sheep experimentally infected with *T. evansi*; Mdachi et al. (2005) in horses experimentally infected with *T. evansi*. Leucopenia at the first weeks pi. was a result of decrease in the numbers of lymphocytes, thereafter there was leucocytosis as a result of lymphocytosis while the proportions and numbers of neutrophils decreased irrespective of the increase TWBCs. Similar results have been reported in the observations of Onah et al. (1996) and Goosens et al. (1998) in *T. congolense* infected sheep; Gutierrez et al. (2005A) in *T. evansi* infected dromedary camels and Mdachi et al. (2005) in *T. evansi* experimentally infected horses. It is likely that the massive lymphocytosis may have partly accounted for the inability of the persistently infected host to clear parasites from their system. Moreover, Onah et al. (1999) recorded that the infection of sheep with *T. evansi* alters the lymphocyte composition and numbers of T- and B-cells in sheep peripheral blood.
The results of the current study indicated significant alterations in several biochemical parameters; hyperproteinaemia and hyperglobulinaemia with hypoalbuminaemia. Similar findings were observed by Akinbamijo et al. (1992) in *T. evansi* infected WAD goats; Nessiem (1982) in *T. evansi* infected buffaloes; Hilali et al. (2006) in *T. evansi* infected buffalo calves; Taiwo et al. (2003) in *T. congolense* and *T. brucei* infected sheep and Dalal and Faten (2005) in *T. evansi* infected ram. This increment in total protein and globulins might be attributed to the increase of gamma globulin fraction and was linked to the initiation of the immune response which is stimulated by trypanosome antigen and antibody production following the massive increase in B-cell numbers as recorded by Van Dam et al. (1996) and Onah et al. (1999). The low levels of albumin have been also reported in *T. evansi* infection by Chaudary and Iqpal (2000). It is possible that trypanosomal uptake of albumin-bound fatty acids for their metabolism and growth and hemodilution might account for the decrease of plasma albumin as stated by Vicerman and Tetley (1979) and Holmes (1976).

There are reports proved that blood lipids play important roles in the pathogenesis of trypanosomosis (Tizard et al., 1978 and Robert, 1984). Previous studies have indicated that plasma cholesterol levels decrease during trypanosome infection in sheep (Katunga-Rwakishaya et al., 1997).

In the current study, there was hypocholesterolaemia and significant decrease in HDL within the infected ewes throughout the experimental period. It is interesting to note that the decline in concentration of cholesterol & HDL commences with the appearance of trypanosomes in the circulation. This decline might be associated with products released by trypanosomes as cholesterol & HDL undergo lysis or uptake by trypanosome as recorded by Katunga-Rwakishaya et al. (1999). There is evidence that lipids constitute 15–20% of trypanosome dry weight (Venkatesan and Ormerod, 1976) and trypanosomes obtain cholesterol from the host by uptake and degradation of density (Coppens et al., 1987 and Gillett and Owen, 1987) or high density lipoproteins (Traore-Leroux et al., 1987). It has also been demonstrated that trypanosomes require cholesterol for growth and multiplication (Black and Vanderweerd, 1989) and that is the main sterol in trypanosomes (Carrol and McCroire, 1986).

Thyroxin (T₄) is required for animal growth and maturation and is revealed to various phases of
reproduction inducing fertility, pregnancy and ovulation (Coles, 1980). Parasitic infections are known to induce euthyroid syndrome at the peak of Acute Phase Response (APR). In the present study, there were significant decrease in the levels of serum T3 and T4 in the infected ewes. These results come in harmony with those of Al-Qarawi et al. (2001) in T. evansi infected camels and Lomo et al. (1996) in T. congolense infected rabbits. In addition, Ogwu et al. (1992) reported progressive decrease in the levels of T3 and T4 of T. congolense infected Zebu heifers resulted from severe pathological changes in the thyroid gland. Mutayoba et al. (1988) stated that the decrease in plasma T4 in goats infected with trypanosomiasis was related to the severity of clinical symptoms of infection and this decline was attributed to the degenerative changes in the thyroid glands led to rapid impaired thyroid gland function and subsequently decrement in T3 and T4. Finally, we support the opinion of Kahl et al. (2002) who attributed this decrement to diminished sensitivity to thyroid stimulating hormone-stimulation and in turn suppression of synthesis of secretion of thyroid hormones.

On the other hand, serum progesterone levels revealed no change in the infected pregnant ewes.

Adequate reproductive performance is essential component in efficient animal production. There are several advantages of keeping small ruminants as compared to cattle in traditional livestock production system and in terms of reproductive performance.

Our study revealed abortion at the late stage of pregnancy and new natal death among the infected ewes. These results matched with Edeghere et al. (1992) in T. brucei brucei infected ewes; Akinbamijo et al. (1994) in T. vivax infected WAD ewes; Rowlands et al. (1995) in Trypanosomes-infected Zebu cows; Elhassan et al. (1995) in T. vivax infected ewes; Bealby et al. (1996) in goat herds infected with different species of trypanosomes in Zambia; Kashiwazaki et al. (1998) in T. evansi infected heifers; Garcia et al. (2000) in T. vivax infected ewes with absorption of alive faetus and dystochia and Bawa et al. (2000) and Gutierrez et al. (2005b) in outbreak in T. evansi infected camels.

The present results are in concomitant with reports stated that the reason of abortion would be that, a progression of stress on animals as pregnancy developed and the immunity was suppressed as a result of infection (Kashiwazaki et al., 1985 and Elhassan et al., 1995), reduction in dry matter intake (Akinbamijo et al., 1990),
reduced nitrogen and energy balance (Zwart et al., 1991), increased basal metabolic rate (Stephen, 1986), increased catabolism of tissue reserve (Akinbamijo et al., 1992) and possibly uptake of host nutrient by the parasites.

Pathological results observed in the uteri of this study were similar to those obtained by Patel et al. (1983), Bowry (1984) and Chandra et al. (2000) in rabbits. These pathological alterations could be considered as a good defense of the uterine tissue against *T. evansi* infection. This suggestion was supported by Bowry (1984) and Chandra et al. (2000) who recorded that the mononuclear cell infiltration in the stroma and engorged blood vessels appeared to be the part of the immunoproliferative response of the host against trypanosome infection. These histopathological findings play a great role in the occurrence of abortion in pregnant ewes and death of lambs during or after parturition. Uterine fibroblastic proliferation, inflammatory cells infiltration and glandular damage were responsible for infertility and low productivity of the animals as reported by Gonzalez et al. (1985) and Mahdy (1988) who believed that uterine damage alters protein synthesis. In addition, Manspeaker et al. (1983), Messier et al. (1984), Gonzalez et al. (1985) and Amer (1992) stated that periglandular fibroblastic proliferation plays an important role in the reduction of uterine milk and consequently early embryonic death. Moreover, necrosis in the cotyledons and / or caruncles was considered as a main cause of abortion or stillbirth.

**CONCLUSION**

The present study proved that *T. evansi* infection causes various reproductive disorders in pregnant ewes leading to reduction in reproductive capacity like abortion, stillbirth and neonatal death.

In our point of view, these disorders mainly due pathological, hematological and some biochemical alterations that are considered as contributing factors manifest themselves as poor reproductive performance. Further investigations should be done for detection of the pathogenesis of *T. evansi* infection in pregnant ewes that leads to abortion or death of lambs.

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