

PATHOLOGICAL STUDIES ON SHEEP ENDOMETRITIS

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Received: 1. 1. 2007.

Accepted: 23. 1. 2007.

SUMMARY

The study was carried out on 106 non-gravid uteri of slaughtered ewes in Cairo slaughter house. Blood and tissue samples were collected and used for serological, bacteriological and histopathological examination. Endometritis was detected in 48.1% of examined cases, which were divided into: non suppurative endometritis (33 cases), acute suppurative endometritis (6 cases), chronic suppurative endometritis (7 cases) and granulomatous endometritis (5 cases). Two cases of granulomatous endometritis showed spherical yeast-like fungi invading the epithelial covering. *Toxoplasma gondii* and *Salmonella spp.* antibodies were detected in 31(29.2%) and 21 (19.8%) cases of endometritis respectively .

Keywords: ewes, endometritis, pathology, causes.

INTRODUCTION

In Egypt, sheep are more popular for human consumption and are widely spread animals because they are a cheap source of meat. It has been recorded that sheep population in Egypt is about 4.672 millions (FAO, 2004). Endometritis occurs as a result of infection by microorganisms. Most lesions are classified as acute and chronic catarrhal endometritis, and acute and chronic suppurative endometritis (Bahgat et al., 1993). Infections of the genital tract are affecting the fertility of animals by altering its uterine environment, resulting in impaired fertilization or unsuitability for development of conceptus causing reproductive problems as early embryonic death, abortion, stillbirth and/or newly born deaths (Boundurant 1991). The non gravid uterus is relatively resistant to infection although bacteria are always present in the vagina. Most uterine infections

begin in the endometrium and are associated with mating, pregnancy or post-partum uterine involution (McEntee, 1990). Approximately 25-40 % of embryos in cattle and sheep are lost around the time of implantation (Hafez and Jainudeen, 1992). The most important infectious agents in small ruminants are: *Brucella ovis*, *Brucella melitensis*, *Campylobacter fetus*, *Salmonella abortus ovis*, *Chlamydia psittaci*, *Toxoplasma gondii* and *Neospora caninum* (Jubb et al., 1993). The causative organisms of endometritis may reach the uterus from the vagina and cervix or from the blood stream.

MATERIAL AND METHODS

Animals: A total number of 106 non-gravid ewes slaughtered in Cairo slaughter house during the period from January to June 2002 were used in this study.

Sampling:

-Blood samples were collected from slaughtered animals into dry and clean tubes and kept at room temperature. Serum samples were harvested and stored at (-20°C) until used.

-Tissue samples of uteri were collected at time of slaughter.

1- Bacteriological examinations:

Parts from the uteri obtained from the slaughtered ewes were collected under aseptic conditions for cultivation onto suitable agar media. Swabs from these tissues were inoculated onto blood agar, nutrient agar, brain heart infusion

agar, MacConkey agar, SS agar, Brucella agar, Skirrow media (Skirrow, 1977) and Sabouroud dextrose agar media at 37°C for 24-48 hours. Isolation and identification of growing colonies were carried out according to (Bolton et al., 1992 and Nachamkin, 1999).

2- Serological examinations:

Collected serum samples were used for the detection of antibodies for the following organisms:

A- *Brucella spp.* using Rose Bengal Plate Agglutination test and Rivanol test (Alton et al. 1988).

B- *Salmonella spp.* using SAS febrile antigen purchased from SA Scientific, Inc. San Antonio, Texas, USA (Alton et al., 1975).

C- *Chlamydia psittaci* (*Chlamydochila abortus*) using complement fixation test (Appleyard et al., 1983).

D- *Toxoplasma gondii* using SAS toxoplasma latex kit purchased from SA Scientific, Inc. San Antonio, Texas, USA (Candolfi et al., 1987)

3- Pathological examinations:

A- Gross Examination:

Post mortem (PM) examination of uteri was carried out to detect any gross lesions.

B- Histopathological examination:

Tissue samples from the uteri of slaughtered ewes were collected and fixed in neutral buffered formalin 10%. The fixed specimens were washed, dehydrated and embedded in paraffin wax. The tissues were sectioned at 4-5 thickness and stained with haematoxylin and eosin.

(H & E) as routine work for histopathological examinations according to Bancroft et al. (1996). Some tissue sections were stained with special stains as: Periodic acid Schiff (PAS), Giemsa stain, Modified Ziehl-Neelsen stain, Von Kossa stain and Verhoeff-Van Gieson stain (Sheehan and Hrapchak, 1980) and Gridleys stain for detection of fungi according to (Bancroft et al., 1996).

RESULTS

1-Microbiological examinations:

A-Bacteriological isolation:

Bacterial isolations from the 106 non-gravid uteri revealed the presence of *Staphylococcus aureus* (9 cases), *Streptococcus pyogenes* (14 cases), *Arcanobacterium pyogenes* (17 cases), *Salmonella spp.* (5cases), *Campylobacter fetus* (4 cases), mycotic organisms (17cases) and *Anthracooides* (18 cases). These isolates were detected either as single or mixed infections.

B- Serological examinations:

Serological examination revealed that 58 cases out of 106 reacted positively for either *Toxoplasma gondii* (31 cases), *Salmonella spp.* (21 cases), *Brucella spp.* (3 cases) and *Chlamydia psittaci* (3 cases) with percentage of 53.4%, 46.5%, 5.2%, and 5.2% respectively.

2- Pathological examinations:

A- Gross pathology:

Uterine gross pathological changes were observed in 51cases out of 106. According to ovarian activity, 40 cases were in luteal phase and 11 cases were in follicular phase. The other remaining 55 uteri were apparently normal and according to state of ovarian activity, 37 were in follicular phase and 18 cases in luteal phase.

B- Histopathological Findings:

The microscopic examination of the 51 uteri which showed gross pathological changes revealed that there were different types of endometritis which can be classified according to the type of exudates and inflammatory response into:

- 1- Non suppurative endometritis.
- 2- Acute suppurative endometritis.
- 3- Chronic Suppurative endometritis.
- 4- Granulomatous endometritis.

1-Non suppurative endometritis:

It was the most common form in the examined ewes in which 33 out of 51 cases of endometritis showed non suppurative endometritis representing (64.7%) (26 in luteal phase and in follicular phase). The microbiological examination of these cases revealed the isolation of 56 isolates from 33 cases as following

Campylobacter fetus (4 cases), *Arcanobacterium pyogenes* (9 cases) *Streptococcus pyogenes* (10 cases), *Salmonella spp.* (3 cases) and *Staphylococcus aureus* (7 cases), mycotic isolates (12 cases) and *Anthracoïds* (11 cases).

Pathological findings:

Macroscopically, the mucosa of the endometrium was congested, edematous and red in color. The uterine wall was thickened and flappy. Microscopically, the endometrial surface showed partial degeneration and desquamation of the lining epithelium cells. The endometrial stroma was highly and diffusely infiltrated with inflammatory cells mainly lymphocytes, plasma cells, macrophages and few neutrophils. The uterine glands were lined by columnar epithelium cells, some of which showed degenerative and necrotizing changes. Periglandular aggregations of mononuclear cells mainly lymphocytes, macrophages and few plasma cells were observed. The majority of blood vessels showed vasculitis, with severe thickening of their wall and narrowing of their lumina as a result of proliferation of sub intimal elastic connective tissue, in addition to mononuclear cell infiltration of the wall of blood vessels. There was marked edema in between the muscle fibers of the myometrium . In some cases vacuolar degeneration of myometrium and focal infiltration of mononuclear inflammatory cells were observed. The perimetrium showed proliferation of capillaries which were dilated and engorged

with blood. In addition to, edema and few neutrophils were observed.

2- Acute suppurative endometritis:

Six cases showed acute suppurative inflammation out of the 51 cases of endometritis, representing 11.7 6%. (4 in luteal phase and 2 in follicular phase).

Eight isolates were detected in these six cases as single or mixed infection: *Streptococcus pyogenes* (2 cases), *Arcanobacterium pyogenes* (3 cases) and *Anthracoïds* (3 cases).

Pathological findings:

Macroscopically, most of the examined uteri were fluctuating and appeared to be distended. The myometrium was edematous, swollen, thickened and easily to be ruptured. The uterine lumina contained accumulations of thick creamy, yellow or brownish purulent exudate (pus) covering the uterine mucosa associated with offensive odour (Fig.1). Microscopically, the endometrial mucosa was lined by pseudostratified columnar epithelium and showed partial to complete desquamation of the epithelial lining associated with leukocytic cell exocytosis mainly neutrophils on the epithelial surface. The endometrial stroma was intensively infiltrated with neutrophils. In one case, large focal abscess was observed in the endometrium. The uterine glands were degenerated and massively infiltrated with neutrophils. Intraluminal accumulation of numerous neutrophils and cellular debris were also seen (Fig.2). In some

cases, the uterine glands were completely degenerated and replaced with inflammatory cells. Periglandular infiltration by inflammatory cells mainly lymphocytes and neutrophils were noticed accompanied by dilatation and congestion of blood vessels. Frequently, large numbers of lymphocytes and plasma cells were also present in the endometrial stroma.

3- Chronic suppurative endometritis:

Seven uteri were found to show chronic suppurative endometritis, in a percentage of 13.72% of the affected cases (six in luteal phase and one in follicular phase). The microbiological examination of these cases revealed the presence of eight isolates either single or mixed infection: *Arcanobacterium pyogenes* (2 cases) *Salmonella spp.* (2 cases) and *Anthracoïds* (4 cases).

Pathological findings:

Macroscopically, the uteri of three cases showed small, hard and atrophied caruncles, which were covered with thick dark brownish exudate. The mucous membrane was necrotic. The other four cases showed accumulation of creamy yellowish green exudate with flappy uterine wall which could easily be ruptured.

The histopathological examination revealed that the covering epithelium was focally degenerated and invaded with numerous neutrophils and some mononuclear inflammatory cells. The lamina

propria was intensively infiltrated with neutrophils, macrophages and plasma cells. There were marked periglandular infiltration with polymorphonuclear cells and fibroblastic proliferation. The latter collected the glands into groups forming nests. There were accumulations of yellowish brown granules of irregular size and shape in endometrial stroma which stained positive with Prussian blue stain. In some cases, the glands were degenerated, necrotic and infiltrated with inflammatory cells. Some glands contained aggregations of neutrophils in their lumina. Large numbers of lymphocytes and plasma cells were aggregated around the blood vessels and the uterine glands.

Considerable numbers of large phagocytic cells with eccentric nucleus and granulated cytoplasm could be seen infiltrating the endometrial stroma in some cases. The cytoplasm granules stained positive with PAS. Some of the blood vessels showed severe narrowing of its lumina with hyalinization of tunica media and invasion of its wall with mononuclear inflammatory cells. Perivascular accumulations of round inflammatory cells which may extend to the wall, in addition to free granules of yellowish brown pigments were seen. Haemosiderin laden macrophages were often present in the stroma and stained blue with Prussian blue stain. Presence of some uterine glands within the myometrium (adenomyosis) was seen in two cases.

4- Granulomatous endometritis:

Five cases showed granulomatous endometritis representing 9.8% of the examined uteri (4 in luteal phase and one in follicular phase). The results of the microbiology showed that *Staphylococcus aureus* was isolated from one case, *Arcanobacterium pyogenes* was isolated from three cases and mycotic infection was found in five cases, as single or mixed infection.

Pathological Findings:

Macroscopically, the uterine mucosa was congested and oedematous while in two cases the mucosa was rough, dry, dull and covered with dark brown exudate. Microscopically the cases infected with *Arcanobacterium pyogenes* revealed partial desquamation of the covering epithelium of the endometrium and presence of granuloma in the wall of the uterus consisting of aggregations of macrophages and lymphocytes with central caseous necrosis associated with calcification in 2 cases, which stained positively brownish black with "Von Kossa" stain and surrounded by fibrous connective tissue capsule (Fig. 3). Periglandular fibroblastic proliferations and infiltration with mononuclear cells were noticed. The uterine glands showed degenerative and necrotizing changes of the lining epithelium. Concerning the cases of granulomatous endometritis in which the causative agents were undoubted fungi, two types of isolates were detected in a mixed infection with the mycotic agents. *Staphylococcus aureus* was isolated from one case and *Streptococcus pyogenes* was isolated from two cases. The microscopic examination showed that the epithelial covering of the endometrium and

the subepithelial stroma were invaded by the causative organism. It appears as spherical yeast-like cells of different sizes, about 20 μ -40 μ in diameter with double contoured refractile wall (Fig.4). It stains positive with PAS reaction and Gridley's stain (Fig.5). Different developmental stages of the yeast-like organisms were seen either free in the uterine lumen or invading the epithelium lining or being present in the subepithelial layer. Each yeast-like cell contains eosinophilic material which has different shapes, either homogenous or granular or remnant in the form of eosinophilic filaments. Some times it is either laminated or like-cart wheel. The organisms form a cystic space occupied with refractile unstained bodies (Spherule containing endospores). Some yeast-like cells appears as empty cysts and the capsule stains with eosin and sometimes the inner third is unstained and outer two thirds stained deeply with eosin. Some degenerated yeast-like cells were invaded with neutrophils. The endometrium showed granuloma with a central core of neutrophils and caseous necrosis surrounded by macrophages, epithelioid cells and lymphocytes. Some granulomas showed central calcification. The uterine glands were either small and atrophied or large cystically dilated with polymorphs inflammatory cells within its lumen. Some times these cystically dilated uterine glands showed hyperplasia of its epithelial lining with projection into the lumen in the form of villi. Large abscess was found in the glandular portion of the endometrium surrounded with connective tissue capsule. All the blood vessels and capillaries were dilated and engorged with blood.

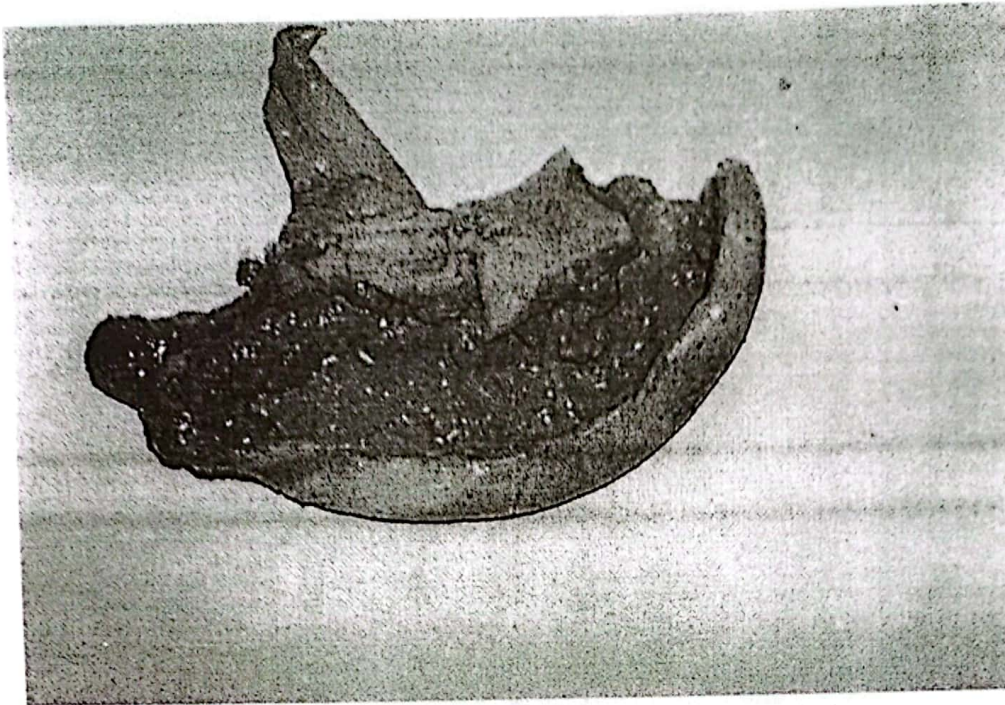


Fig. (1): Uterus of ewe showing accumulation of thick creamy yellowish to brownish purulent exudates covering the uterine mucosa.

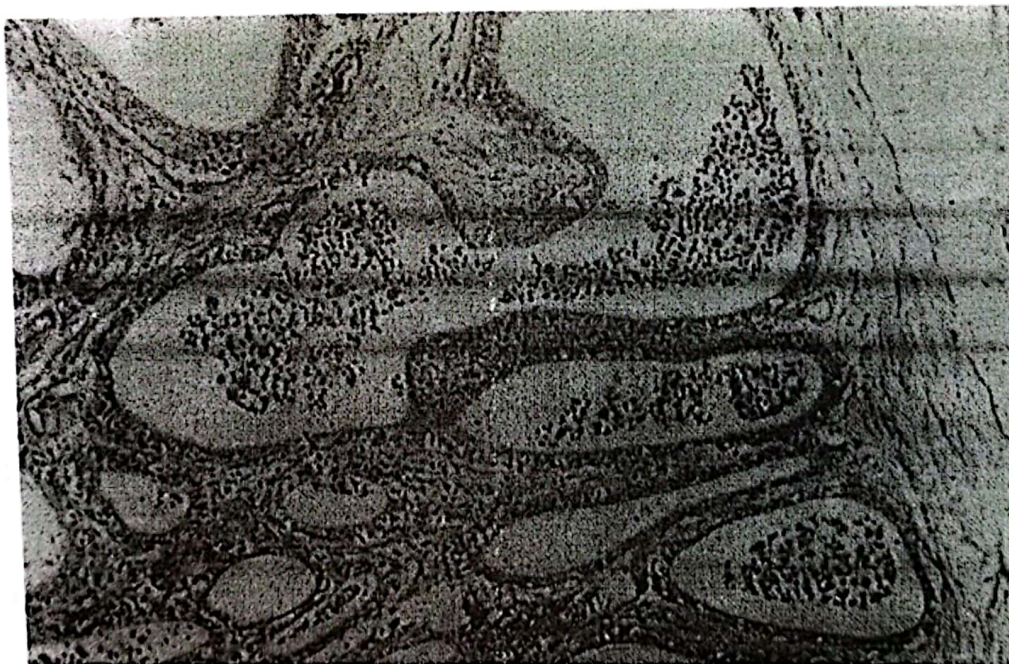


Fig. (2): Suppurative endometritis with cystic dilatation of uterine glands and accumulation of neutrophils in its lumen. [H&E stain, X100].

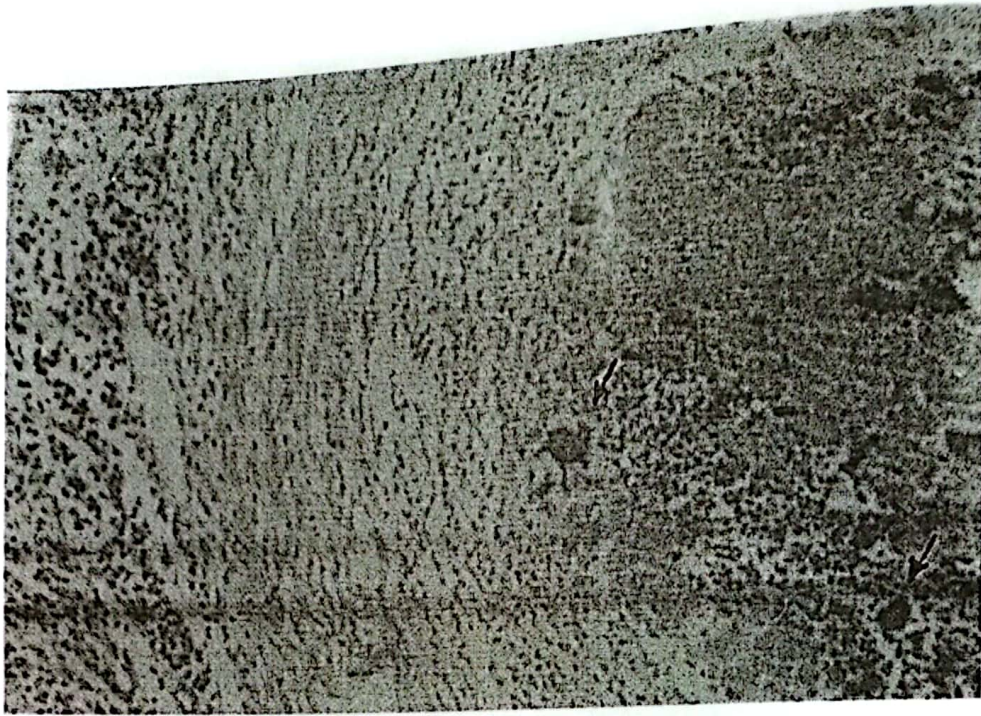


Fig. (3):Notice the presence giant cells (arrow) in the granulomatous lesion in uterine wall. [H&E stain, X 200]

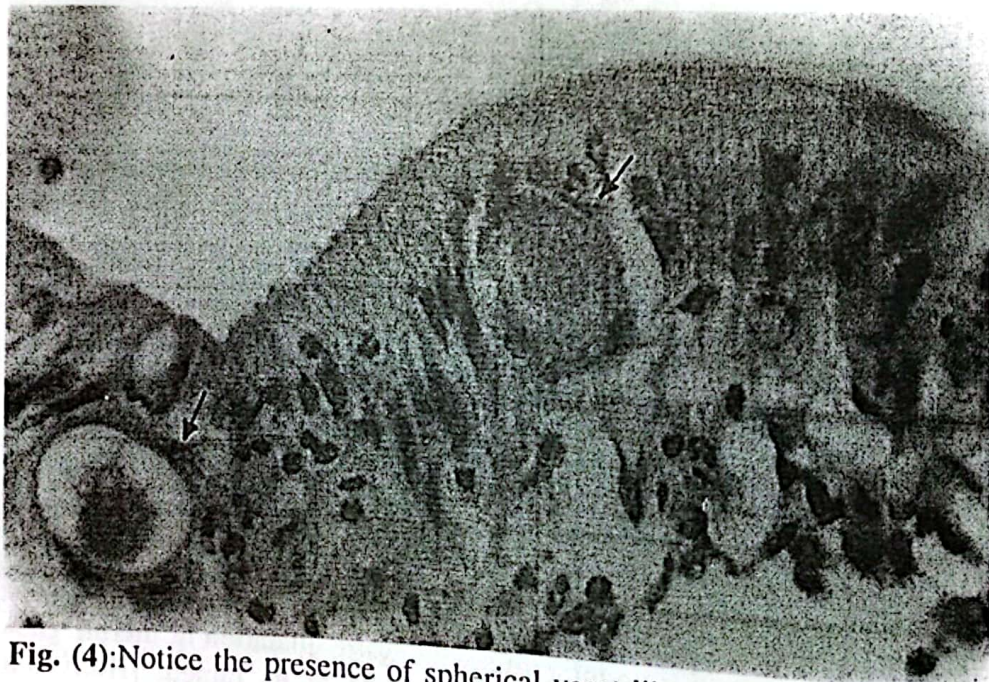


Fig. (4):Notice the presence of spherical yeast-like cells of different size with double contoured wall (arrow) in the epithelial cells lining the endometrium and in the sub epithelial stroma. [H&E stain, X400].

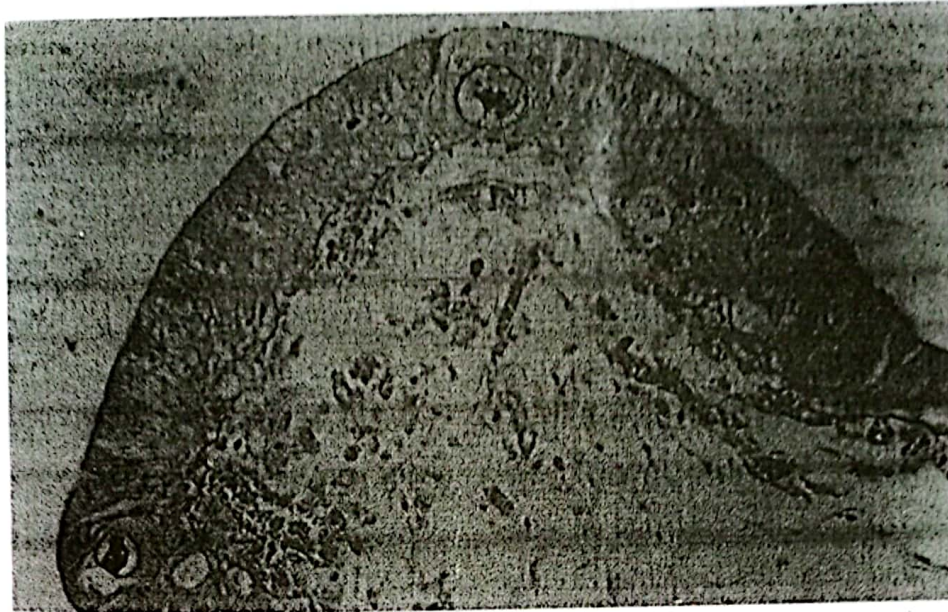


Fig. (5): Endometrium of ewe showing presence of spherical yeast-like structure invading the epithelium covering. [Gridley's stain, X 200].

DISCUSSION

The present study was carried out to study the histopathological changes of endometritis induced by various microorganisms in ewes. Concerning the serological examination of slaughtered ewes, the present study revealed that 78 samples out of 106 reacted positive to *Brucella*, *Salmonella*, and *Toxoplasma gondii*. *Salmonella spp.* antibodies were detected in serum samples of 21 cases (19.8%) out of the 106 examined non pregnant ewes using vidal test. Serological results of this investigation indicated that 31 (29.24%) serum samples out of 106 examined ewes had been exposed to *Toxoplasma gondii* antibodies. Trees et al. (1989) suggested that latex agglutination test (LAT) and indirect fluorescent antibody test (IFAT) showed rapid response

with antibody first appearing at two to three weeks after infection. They added that a negative LAT result seven days after abortion would eliminate toxoplasmosis as a cause of abortion. In this instance, Menzies and Miller (1997) concluded that titers of toxoplasma antibodies may persist from infection without occurrence of abortion, but elevated titers are indicative of recent infection. While ewes may exhibit high titers to toxoplasmosis at the time of parturition and yet produce normal uninfected lambs (Perry et al., 1979). Bacteriologically, neither *Brucella melitensis* nor *Brucella ovis* were isolated from any of the cases of this study. These results give us an idea that we can't depends for accurate diagnosis of brucellosis on serology alone where false positive reaction with various other bacterial infection may occur. These results were i

agreement with findings of Dokhan, (2000). *Campylobacter foetus* was isolated from endometrium of 4 cases (7.84%) suffered from non suppurative endometritis. In this respect Peters and Ball (1995) mentioned that in the non-pregnant cow the vibronic disease can cause endometritis but this condition also caused by non-specific bacterial invaders, e.g. *Corynebacterium pyogenes* and *E. coli*. Dokhan (2000) recorded that *Campylobacter foetus* was isolated from 2 cases (1.33%) suffered from chronic catarrhal endometritis and one case in acute non suppurative endometritis. *Salmonella spp.* microorganism was isolated from 5 cases out of 51 cases of endometritis of non-pregnant ewes (9.8%), These findings are supported by the observations of Nada (1988) and El-Hallawany (2000). In this respect, El-Hallawany (2000) suggested that infected ewes with *Salmonella* revealed mild to moderate degenerative changes of endometrium associated with edema and congestion, in addition to inflammatory cell infiltration particularly neutrophils, lymphocytes and macrophages. Nada (1988) reported that the occurrence of *Salmonellae* was more frequent among sheep (2.98%) than among goats (2.06%). Other microorganisms isolated from all cases of this study were detailed as *Arcanobacterium pyogenes*: 17 cases out of 51 cases (33.3%) associated with endometritis. On the other hand, *Staphylococcus* species were isolated from nine cases of endometritis, *Streptococcus spp.* were also isolated from 14 uteri out of 51 cases with endometritis (27.45%). These findings

were in parallel with those recorded by Sokkar et al. (1980), Bahgat et al. (1993) and Dokhan (2000) who reported that *Staphylococci*, *Corynebacterium* and *E. coli* were the most frequently isolated organisms from inflammatory condition of the uteri. They were more pathogenic than the less frequently isolated bacteria. In the present study, the different lesions in the uteri of ewe were seen in 51 out of the 106 examined ewe with percentage of 48.1%. An incidence which was higher than that recorded by Al- Dahash et al. (1993) (20.15%) and Dokhan (2000) (29.09%). The variations between these results and our results in this study might be due to the different methods of diagnosis.

The histopathological examination showed that 33 cases (64.7%) suffered from acute non suppurative endometritis, 6 cases (11.76%) were acute suppurative endometritis, 7 cases (13.72%) suffered from chronic suppurative endometritis, 7 cases (9.8%) suffered from granular endometritis. The classification of these types of endometritis based on the type of exudate, inflammatory response and duration of inflammation. These findings were in agreement with those mentioned by Adams (1975) and Sokkar et al. (1980). In this respect, Dokhan (2000) reported that the uterus is more susceptible to infection during the progestational phase of the estrus cycle because progesterone reduces the migration of blood neutrophils and reduces phagocytosis and killing of bacteria by uterine neutrophils. He also

ed that infiltration of leukocytes occurs in response to chemotactic substances released by bacteria and leukotriene B₄ from endometrium. On the other hand, the glandular changes might be attributed to the effect of hormones of the secretory active ovarian cysts (Jubb et al., 1993).

Pyometra was found in 4 cases which were characterized by accumulation of pus in the lumen of the uterus associated with the presence of persistent corpus luteum. These results were similar to the observations described in ewes by (El-Wishy et al., 1974 and Dokhan 2000). In this respect, Acland (2001) recorded that pyometra occurred due to either mechanical obstruction or functional obstruction of the cervix under the influence of progesterone produced by retained corpus luteum. The persistence of the corpus luteum could be due to degeneration and destruction of endometrial epithelium as the source of prostaglandins which in turn was responsible for lyses of corpus luteum.

In the current study, the histopathological examination of some cases of endometritis revealed granulomatous lesion (granulomatous endometritis) in five cases. *Staphylococcus aureus*, *Arcanobacterium* and mycotic isolates were demonstrated in these cases. These results were confirmed by those of Dokhan (2000) who recorded granulomatous endometritis in ewes. It is not easy to notice strong relation between specific pathological changes of the uterus and specific infectious

agents in natural cases. This is due to the difficulty to control so many factors such as purity of infecting organisms, the hormonal state of the uterus and the immune status of animals (Dokhan , 2000).

In the present work, two cases of granulomatous endometritis showed the evidence of mycotic isolates. The histopathological finding in these cases was characterized by the presence of spherical yeast like cells with double contoured refractile wall which stained positive with PAS reaction and Gridley's stain .Further investigations are needed to identified the type of this yeast cells. Endometritis due to mycotic infection was recorded in ewes by El-Naggar et al. (1997) Dokhan (2000) and El-Hallawany (2000).

Non-specific etiological agents, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Arcanobacterium pyogenes* and *Salmonella spp.* were incriminated in cases of endometritis in ewes examined in the present investigation which might be single or mixed infection. These were previously isolated by many other investigators and found to play an important role in uterine infection of ewes: Moorthy and Singh (1982), Bahgat et al. (1993 and Dokhan (2000). Infection of almost any tissue by members of the Gram-negative enteric group of bacteria can lead to loss of pregnancy especially in corpus luteum-dependent pregnancies. It is known that the release of endotoxin from such bacteria can induce a systemic prost.

glandin (PGF 2α) response, which results in luteolysis and subsequent pregnancy failure (Boundurant, 1991).

It is evident that endometritis influence fertility in two ways, firstly it reduced fertility and secondary, it can result in sterility due to irreversible pathological changes of genital tracts. Our results revealed that endometritis percentage was 48.1% which divided into: non suppurative endometritis (33 cases), acute suppurative endometritis (6 cases), chronic suppurative endometritis (7 cases) and granulomatous endometritis (5 cases). The diagnosis of infectious diseases causing abortion in sheep depends not only upon case history, clinical signs, bacteriological isolation, serological examination and histopathological changes, but more studies are essential to detect the relation between the infective agent and its specific pathological picture in the dams' uterus. These findings direct our attention for the importance to have a potential diagnostic laboratory centers for scientific dealing with this problem.

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دراسة باثولوجية عن التهاب الرحم فى الأغنام

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أستهدفت هذه الدراسة البحث وتوضيح التغيرات الباثولوجية فى الأرحام والأجنة المجهضة ووفيات حديثة الولادة وعلاقتها بالمسببات المرضية.

وقد تم إجراء هذه الدراسة على عدد مائة وستة (١٠٦) رحم بدون أجنة أخذت عينات دم أثناء الذبح فى المجزر وذلك لإجراء الإختبارات الإكلينيكية والمعملية (السيروولوجى). تم تجميع عينات نسيجية من الأرحام تم فحص هذه الأنسجة والأعضاء لمعرفة التغيرات التشريحية، وتم حفظها بالتجميد (-٢٠°) وفى محلول فورمالين ١٠٪ وذلك لإجراء العزل والتصنيف البكتيريولوجى لفحص التغيرات الهستوباثولوجية).

وقد توصلت الدراسة إلى النتائج التالى:

النتائج السيروولوجية:

٧٨ عينة من عدد ١٠٦ كانت إيجابية لأى من ميكروب البروسيللا - السالمونيلا - التومسويلزما أو الكزنيديا ٢ حالات من ١٠٦ حالة فى المجزر كانت إيجابية لميكروب البروسيللا بإستخدام إختبار روز بنجال. ميكروب السالمونيلا تم الكشف عن أجسامه المضادة فى ٢١ عينة سيرم من عدد ١٠٦ نعاج ليست عشارة. سجلت عدد ٣١ حالة من ١٠٦ نعاج غير عشارة إيجابية لميكروب التوكسويلزما.

العزل البكتيريولوجي:

- تم عزل ميكروب السالمونيلا من عدد خمس حالات إلتهاب بطانه الرحم (٥١ حالة).
- تم عزل ميكروب الفيبيريوفيتس من عدد أربع حالات إلتهاب لبطانة الرحم.
- كذلك سجل عزل بكتيرى لميكروبات الكورينى و الستاف والستربت من جميع الحالات بنسب متفاوتة.

النتائج الباثولوجية:

الصفة التشريحية: تميزت الصفة التشريحية للأرحام ذات الإلتهاب فى الغشاء المبطن للرحم حدوث إحتقان وإرتشاحات مائية وفى بعض الحالات الأخرى كانت مغطاة بطبقة سميكة من الإفرازات الصديدية ذات اللون الأصفر البنى.

التغيرات الهستوباثولوجية: أظهر الفحص الميكروسكوبى وجود تغيرات باثولوجية مميزة فى أنسجة الأرحام. - لوحظ وجود تغيرات التهابية فى بطانة الرحم والتي تم تقسيمها إلى: إلتهاب غير صديدى (٣٣ حالة)، إلتهاب صديدى حاد (٦ حالات) ، إلتهاب صديدى مزمن (٧ حالات)، إلتهاب جرانوليومى (حبيبي) (٥ حالات). فى حالات إلتهاب بطانة الرحم الجرانوليومى ، سجل وجود أجسام كروية للخمائر ذات جدار مزدوج متواجدة فى الخلايا الطلائية المبطنة لجدار الرحم.