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PATHOLOGICAL CLASSIFICATION AND ETIOLOGICAL PREVALENCE OF ENDOMETRITIS IN EWES SUFFERING FROM INFERTILITY PROBLEMS

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ABSTRACT

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Ewes in Sohag Governorate, Egypt are suffering from many infertility problems such as abortion, repeat breeder, anoestrus, cystic ovary and endometritis. The purpose of this study is to investigate reproductive problems that may occur in sheep at Sohag governorate, Egypt using pathological, bacteriological and serological examinations. One hundred ewes slaughtered in abattoirs at Sohag Governorate, Egypt were included in this study. These animals were suffered from many infertility problems according to the owners complain, their age ranged from 3 to 6 years. Blood samples were collected from each animal before slaughtering for serological examination. After slaughtering, the genital tracts were collected and examined grossly. Bacteriological swabs were taken from the lumen of the uterus and processed for bacteriological examination. Uterine samples were processed for histopathological examination. The uterine lesions were classified into 30 cases lymphoplasmocytic endometritis, 5 cases lymphoplasmocytic metritis, 18 cases acute catarrhal endometritis, 29 cases chronic catarrhal endometritis, one case fibrinonecrotic endometritis, 4 cases suppurative endometritis and 8 cases adenomyosis. Serological examination of blood samples and bacteriological examination of swabs of 60 cases indicated that, there are various species of bacteria were present and confirmed serologically positive for *Brucella*, *Salmonella* or toxoplasma. This examination revealed that *Staph aureus* which isolated from 2 cases (3.33%), *Staph aureus* with *Streptococcus* species isolated from 1 (1.66%) case, *Streptococcus* species was isolated from 3(5%) cases, *Streptococcus* isolated from 1(1.66%) case associated with serologically positive *Brucella*, *Proteus* species was isolated from 1 (1.66%) cases, *E-coli* were isolated from 1(1.66%) case associated with *Salmonella* species, *Salmonella* species was isolated and detected by widal test in 9 cases (15%), *Salmonella* species detected in association with toxoplasma antibodies in 1 (1.66%), toxoplasma antibodies were serologically detected also in another 6 cases (10%) and *Brucella* antibodies were serologically detected by rose Bengal test in association with toxoplasma antibodies in one case (1.66%).

Keyword: Ewes, endometritis, metritis, toxoplasma, *Brucella*

INTRODUCTION

Sohag Governorate considered the second province in sheep production after Kina governorate. It has 416472 head of sheep and 423141 head of goat (Agriculture ministry and land reclamation, 2009). Ewe in Sohag Governorate are suffering from many infertility problems such as abortion, repeat breeder, anoestrus, cystic ovary and endometritis. Sheep have high survival rates under drought conditions compared to cattle. Moreover, They provide their owners with a vast range of products and services such as meat, milk, skin, hair, horns, bones, manure and urine for cash, security, gifts, religious rituals, and medicine, etc. (Alemu and Merkel, 2008). Due to their short reproductive cycles (short lambing/kidding

interval) and high incidence of multiple births. This allows farmers/producers a quick interval of selling part of their flock and generating cash income. (Alemu and Merkel, 2008).

Female reproductive pathology seems to be underestimated in ovine species and, in most cases; severe gross lesions are missed due to the lack of specific clinical signs. Moreover, pathologists may support flocks dealing with subtle reduced fertility owing to subclinical endometritis by means of endometrial biopsies and histological examination. Although this technique could be a valuable diagnostic and research tool for assessing postpartum uterine function or health, many defects may escape notice by owners, resulting in poor reproductive

performances of the flock. Appropriate careful clinical examination and examination of slaughterhouse uterine samples should be made for a sound breeding program.

The purpose of this study is to investigate reproductive problems that may occur in sheep at Sohag Governorate, using pathological, bacteriological and serological examinations.

MATERIALS and METHODS

A total 100 genital tracts collected from ewes slaughtered at abattoirs in Sohag governorate during 2011. Female sheep aged 1-6 years was used for this study. These animals were suffered from infertility problems according to the owners complain. Specimens from uterus of examined animals were taken after the gross pathological examination. The samples were fixed in neutral buffer formalin. Bacteriological swabs were taken from uterus as well as blood sample collected for serological examination

Preparation of tissue specimens for Pathological examination

The tissue specimens were fixed in neutral buffer formalin, dehydrated in ascending grades of ethyl alcohol, cleared and embedded in paraffin blocks. Sections of 5-7 μ m were made and stained with H and E stain (Bancroft and Stevens 1982) and examined by light microscope.

Preparation of samples for Bacteriological examination

A- Direct microscopic examination:

Direct smears were made with a platinum loop on clean sterile slides. Screening study of morphology and staining character of the prevalent microorganisms, were carried out using stained films.

B- Isolation and identification of micro-organisms:

For detection of *S. aureus* & *Streptococcus*: Culture of swab samples using Nutrient broth, Nutrient agar, Mannitol salt agar, Baird Paker agar, Blood agar plates, The plates were incubated for 24 hours at 37°C. Gram stain, catalase, coagulase and Voges-Proskaver (VP) tests were conducted on suspected colonies.

For detection of *Proteus*, *E. coli* & *Salmonella*: Culture of swab samples using Nutrient agar, Semisolid soft agar, Blood agar plates, S.S agar, Eosin methylene blue agar & MacConkey's agar were used for isolation of coliforms and other members of the family *Enterobacteriaceae*. The plates were incubated at 37°C for 24h. Five randomly selected colonies from MacConkey's agar and 10 per cent sheep blood agar plates were picked up and subculture on eosin methylene blue (EMB) agar plates to observe the characteristic metallic sheen of *E. coli*. *Proteus* Sp show swarming phenomena on nutrient agar and show motility on semisolid soft

agar. The culture was characterized by biochemical tests i.e., API 20E test kit, Indole Test, Methyl Red, Voges-Proskauer Test, Citrate Utilization Test, Carbohydrate Fermentation Test, Nitrate reduction test and Catalase Test for identification of isolates (Holt *et al.*, 1994)

C- Serological examination of serum samples

Rose Bengal test routinely done to all collected serum samples for detection of brucella antibodies Serum plate agglutination test (TAYTEC TOXO LATEX KIT, Canada) for detection of toxoplasma antibodies, Slide rapid agglutination test (ACCUCARE™ WIDAL SLIDE TEST, India) for detection of salmonella antibodies.

RESULTS

Pathological examination

In this study, 100 female genital tract from ewes slaughtered at abattoirs in sohag Governorate were examined grossly and histopathologically. The pathological examination revealed many pathological lesions which could be summarized in table (1).

According to the histopathological examination, the uterine lesions were classified into: 1- lymphoplasmocytic endometritis (Fig. 1) recorded in 30 cases, 2- lymphoplasmocytic metritis (Fig. 2) in 5 cases, 3- acute catarrhal endometritis (Fig. 3) in 18 cases, 4- chronic catarrhal endometritis (Fig. 4) in 29 cases, 5- fibrinonecrotic endometritis (Fig. 5) in 1 case, 6- suppurative endometritis (Fig. 6) in 4 cases, and 7- adenomyosis (Fig.7) in 8 cases.

Bacteriological examination

Lesions associated with microorganisms isolated from 60 cases were summarized in table (1). Serological examination of blood samples and bacteriological examination of swabs of 60 cases indicating that, there are various species of bacteria were present at the uterus as well as serologically positive cases for either *Brucella*, *Salmonella* or *toxoplasma*. This examination revealed that: *Staph aureus* which isolated from 2 cases (3.33%), *Staph aureus* with *Streptococcus* species isolated from 1 (1.66%) case, *Streptococcus* species was isolated from 3(5%) cases, *Streptococcus* isolated from 1(1.66%) case associated with serologically positive *Brucella*, On the other hand, *Proteus* species was isolated from 1 (1.66%) cases, *E-coli* were isolated from 1(1.66%) case associated with *Salmonella* species, *Salmonella* species was isolated and detected by widal test in 9 cases (15%), *Salmonella* species detected in association with *toxoplasma* antibodies in 1 (1.66%).

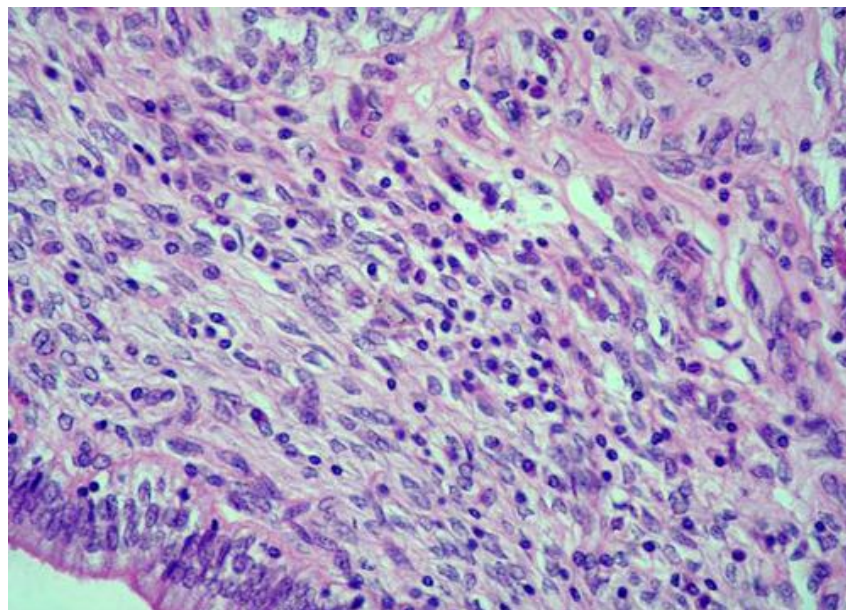
Serologically examination of serum revealed *Toxoplasma* antibodies were detected also in another 6 cases (10%) & *Brucella* antibodies were detected by Rose Bengal test in association with *Toxoplasma* antibodies at 1 case (1.66%).

Table 1: Lesions associated with microorganisms isolated from 60 cases.

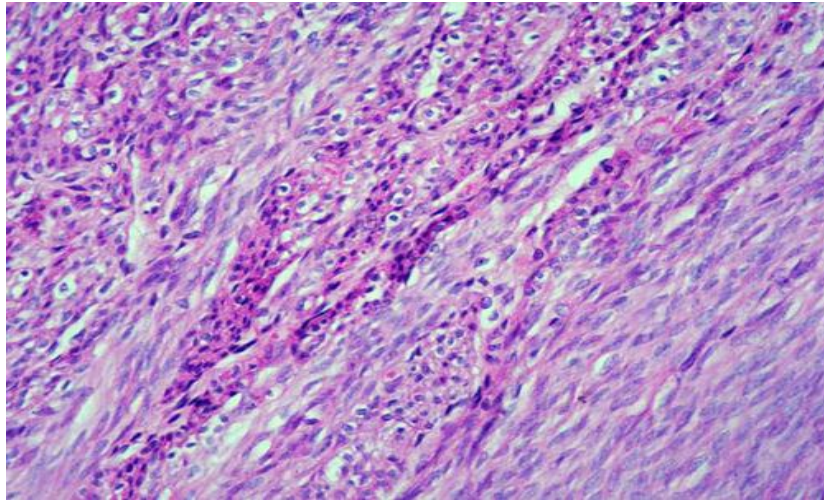
Isolated and serologically +ve Microorganisms	No	%	Lesions
<i>Staph aureus</i> *	2	33.3%	Fibrinonecrotic endometritis.
<i>Staph aureus</i> *; <i>streptococcus</i> *.	1	1.66%	Acute catarrhal endometritis.
<i>Streptococcus</i> *.	3	5%	-Acute suppurative endometritis. -Chronic catarrhal endometritis. -Cystic endometrial hyperplasia.
<i>Streptococcus</i> *; <i>Brucella</i> **.	1	1.66%	Lymphoplasmocytic endometritis.
<i>Proteus spp</i> *.	1	1.66%	Lymphoplasmocytic endometritis.
<i>E-coli</i> *; <i>Salmonella</i> * & **.	1	1.66%	Chronic catarrhal endometritis
<i>Salmonella</i> *, **.	9	15%	-Lymphoplasmocytic endometritis. -Acute catarrhal cervicitis
<i>Salmonella</i> *, **; <i>Toxoplasma</i> **.	1	1.66%	-Chronic catarrhal endometritis. -Adenomyosis
<i>Toxoplasma</i> **.	6	10%	-Lymphoplasmocytic endometritis. -Chronic catarrhal endometritis. -Adenomyosis. -Acute catarrhal cervicitis.
<i>Toxoplasma</i> **; <i>Brucella</i> **.	1	1.66%	Lymphoplasmocytic endometritis
Total	26	43.33%	

*: Bacteriological positive cases.

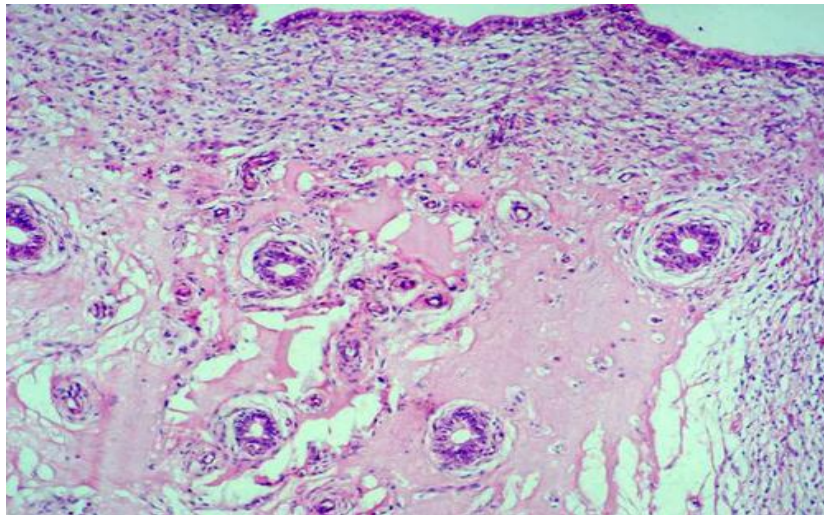
** : Serological positive cases.



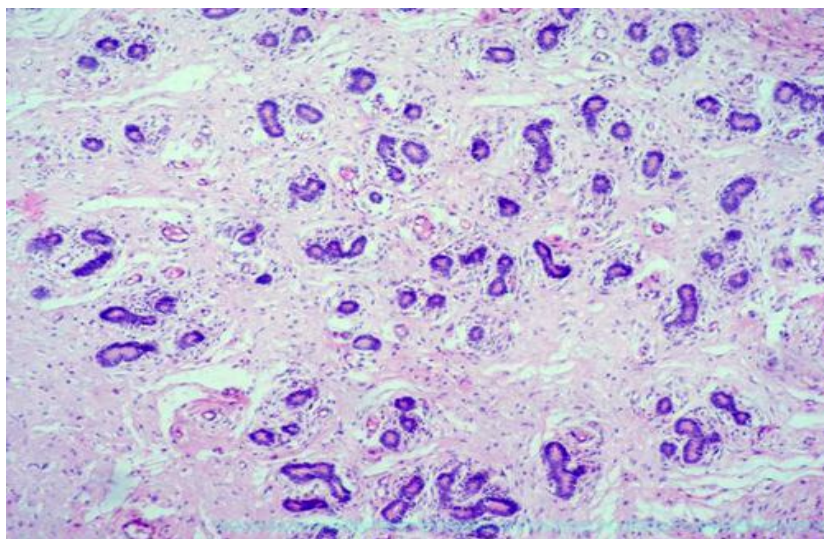
(Fig.1): Uterus of ewe showing lymphoplasmocytic endometritis(arrow). Lymphocytes and plasma cells were seen infiltrating subepithelial layer. H&E. X 40.



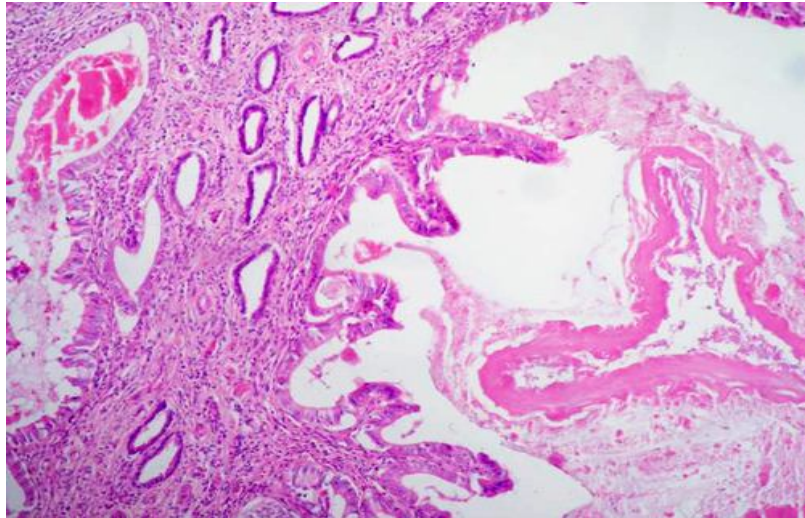
(Fig. 2): Uterus of ewe showing lymphoplasmocytic metritis. Severe infiltration by plasma cells and lymphocytes between and in all layers of myometrium (arrow). H&E. X 40.



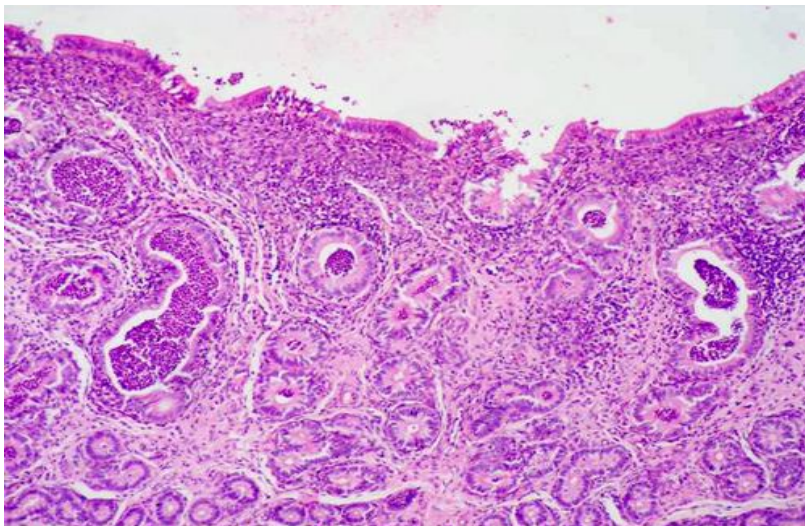
(Fig. 3): Uterus of ewe showing acute catarrhal endometritis. Catarrhal exudates with inflammatory cells were noticed at sub epithelial layer (arrow). H & E stain. X 10.



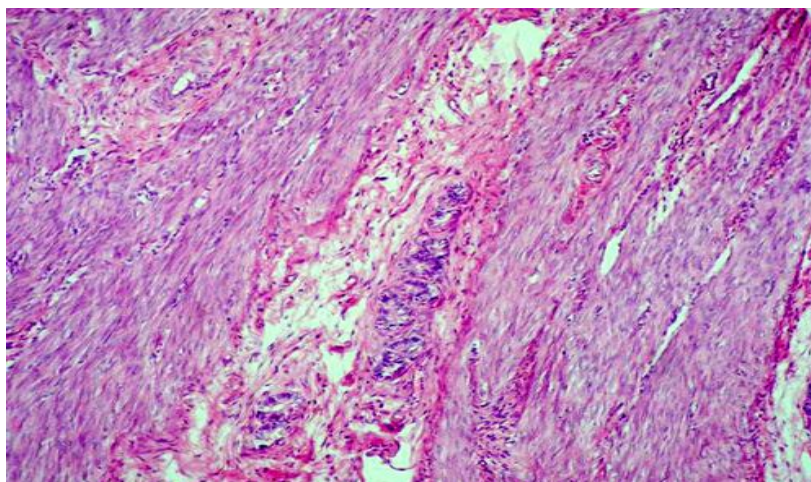
(Fig. 4): Uterus of ewe showing chronic catarrhal endometritis. Endometrial glands were atrophied (arrow) and separated from each others with connective tissue fibers. H & E stain. X 10.



(Fig. 5): Uterus of ewe showing fibrinonecrotic endometritis. Clots of fibrin at lumen mixed with inflammatory cells (neutrophils), hyperemia of blood vessels (V) and lymphocytic cell reaction at sub epithelial layer. H & E stain. X 10.



(Fig. 6): Uterus of ewe showing suppurative endometritis. Sloughed and necrotic epithelium of endometrium (arrow) with heavy infiltration of neutrophil cells (Head arrow) . H & E stain. X 10.



(Fig. 7): Uterus of ewe showing adenomyosis. Nests of endometrial glands with surrounding stroma had grown from endometrium in between muscles layers. H & E stain. X 10.

DISCUSSION

In this study we deal with the gross and microscopic lesions encountered in 100 genital tract collected at slaughterhouse specimens from Sohag abattoirs.

Since the healthy endometrial condition is necessary for the fertility of the animal so, any alteration in the normal histological structure may impair fertility or cause permanent sterility. This consideration was reported by El-Naggar and El-Sherry (1974).

From our results 95 cases out of 100 slaughtered ewes showed pathological changes in the uterus. These pathological changes included lymphoplasmocytic endometritis in 30 case, lymphoplasmocytic metritis in 5 cases, acute catarrhal endometritis in 18 cases, chronic catarrhal endometritis in 29 case, fibrinonecrotic endometritis in 1 case and suppurative endometritis in 4 cases, while adenomyosis was observed in 8 cases. Sokkar *et al.* (1980) reported that, out of 100 uteri of ewes collected from a slaughterhouse, 44 had endometritis. These cases were classified as acute endometritis (18%), acute lymphocytic endometritis (59%), chronic non-suppurative endometritis (16%) and suppurative endometritis (6.8%).

Acute lymphoplasmocytic endometritis was recorded in 30 cases. this result agree with (Barlund *et al.*, 2008; Gilbert *et al.*, 2005 and Kasimanickam *et al.*, 2004), they stated that subclinical endometritis prevalence ranges from 11% to 53%.

In these cases we isolate *Proteus* species from 1 case, *Salmonella* from 2 cases. *Streptococcus* was recorded in 1 case in association with *Brucella* antibodies in the serum. *Toxoplasma* antibodies was detected in serum of 1 case, also it was detected at another case in association with antibodies of *Brucella spp.* These types of metritis and/or endometritis was recorded at the postpartum period (Arthur and Pearsonl 1982). In postpartum period, the uterus seems to be able to prevent bacteria from achieving infection unless suppression to uterine defense mechanism occurs (Seals *et al.*, 2002). The microfloras have no pathological effect without supporting from other predisposing or stress factors (Azawi *et al.*, 2008). Previous studied recorded that 56.5% of genital tracts contains microflora including, *Arcanobacterium pyogens*, *Escherichia coli* and *Streptococci Spp* commonly isolated from uteri of ewes (Aziz *et al.*, 2000 and Al-Hamedawi *et al.*, 2002). Also members of the genus *Staphylococcus sp.* have been reported, in ewes (Sawyer, 1977).

Acute catarrhal endometritis which described in our results, in 18 cases, characterized by roughness in mucosal surface with petechiae and some accumulation of thin or viscid fluid in the uterine

lumen. Microscopically, there were hydropic degeneration at epithelial lining, with sever infiltration of endometrium by plasma cells and some lymphocytes. At 4 cases of them the acute inflammation extended to include all layers, in which the myometrium infiltrated by plasma cells and lymphocytes. The preimetrium showing congestion, edema and lymphocytic cell infiltration. Similar findings of endometritis were described by (Runnells *et al.*, 1965; Bollo *et al.*, 1990 and Jubb *et al.*, 1993).

In these cases we isolate *staphylococcus aureus* associated with *Streptococcus* species were isolated from 1 case and the antibodies of *toxoplasma* detected in 1 case Antibodies of *Salmonella* species were detected in another case of acute catarrhal endometritis.

Chronic catarrhal endometritis which described in our results in 29 cases, characterized by desquamation in the epithelium lining the endometrium, hyperplasia or metaplasia in some areas, other areas showed epithelial necrosis and desquamation. Lymphocytic cell infiltration of the epithelial layer, or subepithelial layer or surrounding the endometrial glands were also recoded. At 2 cases of chronic catarrhal endometritis, the endometrium was markedly expanded by many irregular cystic and hyperplastic glands, some of these glands contained scattered neutrophils, sloughed epithelial cells, cellular debris, and fibrin. The glands were lined by either normal or hyperplastic epithelium. The endometrial vessels showed perivascular fibroblastic proliferation and infiltration of lymphocytes and plasma cells. In 4 cases of them, the chronic catarrhal inflammation extended to include all layers, in which the infiltrated inflammatory cells were seen between the degenerated myometrium layer and at perimetrium. Nearly similar picture of endometrits were previously described by El-Naggar and El-Sherry (1974) and Jubb *et al.* (1993). *Streptococcus* species was isolated from 1 case, *E-coli* associated with *Salmonella* species was isolated from 1 case, antibodies of *Toxoplasma* were detected in serum of 1 case, and with antibodies of *Salmonella* from another q case.

Our results indicated that fibrinonecrotic endometritis was recorded in one case in which the utrine lumen contained a large amount of clotted exudates admixed with blood. The fibrinous exudates appeared as a red network of fibrin clots at the surface entangling acute inflammatory cells, mostly lymphocytes and neutrophiles. The epithelial layer of endometrium was necrosed and sloughed. At sub epithelial layer lymphocytic cells aggregated and blood vessels were congested. This observation was recorded also by Runnells *et al.* (1965). *Staphylococcus aureus* was isolated from this case.

Our results revealed that, there were 4 cases out of 100 female genital tracts showed suppurative endometritis which characterized by degenerative changes in some areas of endometrial epithelium, other areas showed epithelial hyperplasia and other showed epithelia degeneration and necrosis. At upper third of endometrium there are sever infiltration by neutrophils. Endometrial glands showing cystic hyperplasia with preiglandular fibrosis, the lumen of these glands filled with suppurative exudates infiltrated with neutrophils. Similar results were previously described by Runnells *et al.* (1965), Bollo *et al.* (1990) and Dawood (2010).

In these 4 cases, the endometrium was markedly expanded by many irregular cystic and hyperplastic glands, some of these glands contained widely scattered neutrophils, sloughed epithelial cells, cellular debris, and fibrin. The glands were lined by either normal, hyperplastic epithelium. Some endometrial epithelial cells were swollen with clear to foamy cytoplasm. The blood vessels showed medial hyperplasia. Similar lesions were previously described by (Feldman and Nelson, 1996). We isolate *Streptococcus species* from 1 case of suppurative endometritis.

Bacterial organisms are considered to be the predominant cause of endometritis. Bacteriological examination of our investigated ewes revealed many isolates of bacterial associated with the different types of endometrial lesions as *Staphylococcus aureus*, *streptococcus*, E-coli, *Salmonella*, *toxoplasma* and *Brucella species*. Endomeritis due to bacterial infection was described by several authors as Sokkar *et al.* (1980), who reported that, *Corynebacterium pyogenes*, *Escherichia coli* and *Streptococci* are the organisms commonly isolated from the uteri of ewes and *E. coli* and *streptococci* appear to be the organisms commonly associated with endometritis. Also Al-Hamedawi *et al.* (2002) and Aziz *et al.* (2000) reported that 56.5% of genital tracts contains microflora including, *Arcanobacterium pyogens*, *Escherichia coli* and *Streptococci Spp* which are commonly isolated from uteri of ewes. Also Sawyer (1977) isolate genus *Staphylococcus sp* from cases of endometritis in ewes.

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التصنيف الباثولوجي ومدى انتشار المسبب لمرض التهاب بطانة الرحم في إناث الأغنام التي تعاني من مشاكل العقم

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تعاني إناث الأغنام في محافظة سوهاج، بمصر من العديد من المشاكل مثل العقم والإجهاض، وتكرار الشياح وتكيس المبيض والتهاب بطانة الرحم. وكان الغرض من هذه الدراسة للتحقيق من هذه المشاكل وذلك باستخدام الاختبارات الباثولوجية ، البكتريولوجية والسيروولوجية. في هذه الدراسة تم فحص عدد ١٠٠ رأس من إناث الأغنام في مجازر محافظة سوهاج، بمصر، تراوحت أعمارهم ٣-٦ سنوات وقد عانت هذه الحيوانات من العديد من مشاكل العقم حسب شكاوي أصحاب هذه الحيوانات. تم جمع عينات الدم من كل حيوان قبل الذبح للفحص السيروولوجي. بعد الذبح ، تم جمع الأعضاء التناسلية لهذه الحيوانات وتم فحصها. أخذت مسحات بكتريولوجية من تجويف الرحم والمهبل وتجهيزها للفحص البكتريولوجي. تم تجهيز عينات الأنسجة من الرحم لفحص الأنسجة. بناء على التغيرات الهستوباثولوجية التي شوهدت في أرحام الحيوانات قسمت إلى: ٣٠ حالة التهاب خلوي ليمفوبلازمي ببطانة الرحم ، ٥ حالات التهاب خلوي ليمفوبلازمي بالرحم ، ١٨ حالة التهاب بطانة الرحم المخاطي الحاد، ٢٩ حالة التهاب بطانة الرحم المخاطي المزمن، حالة واحدة التهابا تليفي متفرحا ، ٤ حالات التهاب بطانة الرحم التقيحي و ٨ حالات تمدد ثنايا الطبقة المبطن للرحم إلى داخل طبقه العضلات. فحصت ٦٠ حالة من عينات الدم والمسحات المهبلية فحصا سيروولوجيا وبكتيريا، ووجد انه يوجد أنواعا عديدة من البكتريا تصيب الجهاز التناسلي لتلك الحيوانات كالاتي: الميكروب الكوري العنقودي تم عزله من حالتين بنسبه (٣.٣٣%)، الميكروب الكوري العنقودي تم عزله مرتبطا بالميكروب الكوري السبحي من حالة واحدة بنسبه (١.٦٦%)، الميكروب الكوري السبحي تم عزله منفصلا من ٣ حالات أخرى بنسبه (٥%)، الميكروب الكوري السبحي تم عزله من حالة واحدة وجد أنها موجبه للبروسيل بنسبه (١.٦٦%)، ميكروب البروتيس تم عزله من حالة واحدة بنسبه (١.٦٦%)، الميكروب المعوي وميكروب السالمونيلا عزلا معا من حالة واحدة بنسبه (١.٦٦%)، تم عزل ميكروب السالمونيلا وكذلك كانت موجبه للسالمونيلا بواسطة اختبار الفيدال من ٩ حالات بنسبه (١٥%)، حالة واحدة بنسبه (١.٦٦%) كانت موجبه سيروولوجيا للسالمونيلا والتوكسوبلازما ٦ حالات بنسبه (١٠%) كانت موجبه سيروولوجيا للتوكسوبلازما وحاله واحدة بنسبه (١.٦٦%) كانت موجبه سيروولوجيا للتوكسوبلازما والبروسيل.