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HISTOPATHOLOGICAL AND MICROBIOLOGICAL STUDIES ON UTERI OF BUFFALO-COWS

(With 2 Tables and 12 Figures)

By

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دراسة هستوباثولوجية وميكروبيولوجية على بعض الإصابات
في رحم الجاموس

أحمد محمد صابر عزب ، محمد عبيد إسماعيل ، محمود عبدالحليم حمودة ،
عز الدين أحمد صقر

تم تجميع الرحم من ٣٤٠ جاموسة بالغة غير عشار من مجزري الحوامدية والمنيب بمحافظة
الحيزة خلال فترة ٢٤ شهر. وقد تم إجراء الفحص الظاهري والفحص الهستوباثولوجي
بالمجهر الضوئي واخذ عينات للعزل البكتريولوجي لجميع عينات الرحم بينما تم اختيار عدد
٨ عينات رحم بها التهابات رحمية للفحص بالمجهر الإلكتروني النافذ. وقد تم تسجيل
التغيرات الباثولوجية في رحم ٣٤٠/٤٥ حيوان وشملت: التهاب بطانة الرحم في ٢٩ حالة
(٨,٥٣%)، التهاب كل طبقات الرحم في ٥ حالات (١,٤٧%)، تكيس وزيادة عددية في غدد
بطانة الرحم في ٥ حالات (١,٤٧%)، ورم ليفي حميد في ٦ حالات (١,٧٦%)، تكيسات
بالجدار الخارجي للرحم في ١١ حالة (٣,٢٤%)، تواجد غدد بطانة الرحم في طبقة عضلات
الرحم (اينومايوزيس) في ٢ حالة (٠,٥٨%)، عقيدات بالجدار الخارجي للرحم في ٢٦ حالة
(٧,٦٥%)، والتصاقات رحمية في ٣ حالات (٠,٨٨%). وأظهر فحص التراكيب الدقيقة
بواسطة المجهر الإلكتروني النافذ لعينات الرحم التي تم تشخيص التهابات رحمية بها وجود
خلايا بلعمية متعددة الأنوية تميزت باحتوائها على عدة فراغات في السيتوبلازم ظهر في
بعضها أجسام ليكترونية عسوية كثيفة في حالات الالتهاب الحاد لبطانة الرحم. أما في
حالات الالتهاب المزمن لبطانة الرحم أو كل طبقات الرحم فقد تميزت بوجود خلايا
الفيبروبلاست والفيروسايت وعدد قليل من الخلايا وحيدة النواة وألياف الكولاجين في مقاطع
عرضية وطولية حول غدد بطانة الرحم بالإضافة لوجود خلايا متحللة تحتوي على عدة
فراغات ليكترونية غير كثيفة في السيتوبلازم وبقايا ليكترونية كثيفة داخل تجاويف الغدد
الرحمية في حالة الالتهاب الحاد لكل طبقات الرحم. وقد أظهر الفحص البكتريولوجي وجود
عدد ١٩ حيوان إيجابي للعزل البكتري من ٣٤ حيوان تم تشخيص التهاب رحمي بها
وشملت العترات المعزولة: الميكروب العنقودي الذهبي، ميكروب السودوموناس ايروجينوزا،
الميكروب القولوني العصوي، ميكروب الكليسيلا أوكسي توكا، ميكروب البروتيا

فولجارس، ميكروب الإنتيروباكترايبروجينيز، وميكروب السيتروباكتر فريونداي ودايفيرسيس. وبالتحليل الإحصائي وجد أن التغيرات المرضية في الرحم في فصلي الربيع والصيف (٣٠,٣٣% و ٢٦,٦٦% على التوالي) كانت بمعدل أعلى من التي وجدت في فصلي الشتاء والخريف (٢٥,٠٠% و ١٧,٢٤% على التوالي). وقد وجد أن هناك زيادة معنوية ($P > 0.05$). بين حدوث التغيرات المرضية في الرحم بين فصلي الربيع والخريف بينما لم توجد زيادة معنوية بين فصول الربيع والصيف والشتاء أو الخريف والصيف والشتاء.

SUMMARY

Female genital organs of 340 adult breeding non-pregnant buffaloes were collected from slaughter houses. The uterine lesions were recorded in 45/340 animals and included: endometritis in 29 cases (8.53%), metritis in 5 cases (1.47%), endometrial hyperplasia in 5 cases (1.47%), fibroma in 6 cases (1.76%), serosal cysts in 11 cases (3.24%), adenomyosis in 2 cases (0.58%), perimetrial nodules in 26 cases (7.65%) and uterine adhesions in 3 cases (0.88%). Ultrastructure examination of uterine specimens with inflammatory lesions by transmission electron microscopy revealed presence of degenerated polymorphonuclear cells with many vacuoles in their cytoplasm that sometimes contained electron dense rod-like structures and macrophages in case of acute endometritis. Fibroblasts, fibrocytes, few mononuclear cells, and collagen fibers in longitudinal and cross sections around the endometrial glands were prominent in case of chronic endometritis or metritis in addition to presence of degenerated cells with intracytoplasmic electron translucent areas and electron dense debris in lumina of the endometrial glands in acute metritis. The bacterial isolation was positive only in cases of endometritis and metritis in 19/34 animals (55.88%) and included: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Citrobacter freundii* and *Citrobaacter diversus*. The frequency of uterine pathological findings in spring and summer (30.33% and 26.66%, respectively) were higher than in winter and autumn (25.00% and 17.24%, respectively) and there was significance ($P < 0.05$) between incidences of uterine lesions in spring and autumn.

Key words: *Uterus, buffalo-cows, histopathology, microbiology*

INTRODUCTION

The buffaloes have an outstanding potentiality as a source of good quality lean meat for human consumption (Farrag, 1978).

Examination of genital tracts of non-pregnant buffaloes at the abattoir suggests that the reproductive problems among buffaloes may be more than those of cows (El Khouly, 1985). Microbial agents play an important role in the infertility problem in buffaloes that lead to great economic losses (Aisha Ali and Hala Ibrahim, 2001).

Samad *et al.* (1984) found that the endometritis represents 87.3-93.5% of the recorded reproductive disorders (23.7-47%) in infertile buffaloes, while Shalaby (1997) stated that endometritis is the main cause of conception failure in buffaloes in Egypt. Wahba and Kholeaf (1996), reported endometritis, metritis, and pyometra in various incidences in infertile buffaloes. Endometrial atrophy, adenomyosis, serosal cysts on the uterine surface, endometrial glandular cystic hyperplasia, uterine adhesions, mucometra, hydrometra, were also observed in buffaloes (Khan *et al.* 1992, Ahmed and Khan, 1993).

Dwivedi and Singh (1975) and Deeb *et al.* (1979) observed fibromyoma in Indian buffaloes (0.17%) and in buffaloes in Egypt (0.28%), respectively, while Deeb *et al.* (1976) reported adenocarcinoma in buffaloes (1.2%). McEntee (1990) mentioned that the uterine neoplasms in domestic mammals were comparatively rare with the exception of leiomyoma (in cows and bitches) and uterine carcinoma. Shalash (1991) observed the uterine tumors in buffaloes and cows as 0.71% and 0.93%, respectively, while Garcia-Iglesia *et al.* (1995) found 3 cases of adenocarcinomas and 3 cases of leiomyoma in female bovine genitalia. This study was carried out to explore the pathological alterations in uteri which might be responsible for infertility in buffaloes in Egypt.

MATERIALS and METHODS

A- Animals

The uteri of 340 adult breeding non-pregnant buffaloes (3-6 years) of unknown previous history of fertility were collected from El Hawamdya and El Moneeb slaughter houses at Giza province from June 2002 to May 2004.

B- Histopathological and electron microscopy study

The obtained uteri were incised along the mid-dorsum and necropsy specimens from horns and body were taken and fixed in 10% formol saline solution, processed, sectioned, stained with Harri's haematoxylin and eosin (Wilson and Gamble, 2002) and Van Gieson (Jones, 2002) stains, and examined by light microscopy.

Eight uterine specimens with endometritis (5 cases) and metritis (3 cases) from which bacterial isolates were frequently associated were selected to be examined by transmission electron microscopy. The specimens fixed in 5% cold cacodylate buffer glutaraldehyde (4⁰C, 0.1 M, pH 7.2) for 2 hours, postfixed in 1% osmium tetroxide for 2 hours, dehydrated in ascending grades of ethyl alcohol, placed in propylene oxide and in propylene oxide-epon mixture (1:1), and finally embedded in epon. Semithin and ultrathin sections were obtained and stained with 0.25% toluidine blue and 2% uranyl acetate and 0.5% lead citrate stains, respectively, and examined in a Jeol electron microscope (Jeol-EM-100 S) at VACSERA-Electron Microscopy Unit (Woods and Stirling, 2002).

C- Microbiological study

The bacteriological study was carried out for isolation and identification of bacteria and fungi according to Koneman *et al.* (1983), Carter and Cole (1990), Alton *et al.* (1975), and Ruhnke and Rosendal (1989).

D- Statistical analysis

The results were compared using analysis of variance test (ANOVA) for seasonal variations of the incidence of lesions (Snedecor and Cochran, 1989).

RESULTS

(A) Histopathological findings

The examination of 340 buffalo-cows showed that the incidence of lesions in spring and summer (30.33% and 26.66%, respectively) were higher than in winter and autumn (25.00% and 17.24%, respectively) as shown in Table 1. There was significance ($P < 0.05$) between incidences of uterine lesions in spring and autumn, while there was no significance between spring, summer and winter or autumn, summer and winter.

1- Endometritis

Endometritis was recorded in 29 cases (8.53%) with positive bacteriological isolation in 16 of them (Table 2). The uterine lesions were as follows:

1-a) Acute endometritis

It was observed in 3 cases (0.88%). Macroscopically, the uterine mucosa was hyperemic and edematous. On cut section, the uteri had offensive odor and the uterine mucosa was hyperemic and edematous with swollen red caruncles that showed necrotic spots and erosions. Microscopically, the acute reaction varied in its severity but the

polymorphonuclear leucocytes were the predominated cells. The endometrial epithelium was completely necrosed and the endometrial stroma contained multiple abscesses (Fig. 1).

1-b) Chronic endometritis

Chronic endometritis was recorded in 26 cases (7.65%) with positive bacteriological isolation in 13 of them (Table 2). Macroscopically, the perimetrium had adhesions with the surrounding peritoneum with nodules or cysts and most uteri had thick wall. Microscopically, in most cases of chronic endometritis, the endometrial epithelium appeared infiltrated with a large number of lymphocytes alongside fibrosis in stratum compactum and stratum spongiosum (Fig. 2). This was accompanied by atrophy and cystic dilatation of endometrial glands. In some cases, moderate (2-3 layers) to severe (4-5 layers) periglandular fibroblastic proliferation (Fig. 3) in addition to sclerosis was also noticed.

2- Metritis

Metritis was recorded in 5 cases (1.47%). (Table 2).

2-a) Acute metritis

Acute metritis was found in one case (0.29%). Microscopically, the surface epithelium of endometrium showed vacuolar degeneration with neutrophilic infiltration and erosion in most areas (Fig. 4). The myometrium had mild neutrophilic and mononuclear cells infiltration and the perimetrium showed inconsistence thickening with edema and neutrophilic infiltration.

2-b) Chronic metritis

Chronic metritis was observed in 4 cases (1.18%). Macroscopically, the uterine wall was thick and the outer surface was rough with grayish nodules. Microscopically, endometrial epithelium showed lymphocytic exocytosis and appeared eroded in some areas. Some glands were completely destructed and replaced with lymphocytes (Fig. 5) or showed marked periglandular fibroblastic proliferation. The myometrium showed moderate mononuclear cell infiltration, while the perimetrium appeared thickened with fibroblastic proliferation.

3- Endometrial hyperplasia

Hyperplasia with cystic dilatation of endometrial glands was observed in 5 cases (1.47%) in association with chronic endometritis. Microscopically, the lining epithelium was low cuboidal to flat with wide distended empty lumina or contained homogeneous eosinophilic material and/or desquamated cells.

4- Fibroma

Fibroma was found in 6 cases (1.76%). Microscopically, fibroma consisted of bundles of collagen fibers running in different directions in focal areas in the stroma. The bundles of collagen fibers whirl around blood vessels (Fig. 6).

5- Serosal cysts

Serosal cysts were found in 11 cases (3.24%). Macroscopically, the cysts were observed as multiple cysts or single cyst. They had thin wall and contained a clear serous fluid. Microscopically, the cyst appeared lined with flattened to low columnar epithelial cells surrounded by a thin fibrous tissue layer.

6- Adenomyosis

Adenomyosis was noticed in 2 cases (0.58%). Microscopically, the tunica muscularis showed few scattered endometrial glands at one site within the myometrium (Fig. 7).

7- Perimetrial nodules

The nodules were observed in 10 cases (2.94%). Macroscopically, the nodules appeared as single or multiple grayish elevated nodules of different sizes (Fig. 8). Microscopically, the nodules were characterized by presence of mononuclear cells infiltration and fibrous tissue proliferation.

8- Uterine adhesions

Adhesions of uterus with the surrounding tissues were found in 3 cases (0.88%) and grossly appeared as fibrous tissue strands attaching the perimetrium to peritoneum or to uterine tubes or to the broad ligament.

(B) Ultrastructure findings

Ultrastructure examination of endometrium in case of acute endometritis revealed presence of many degenerated polymorphonuclear cells and few macrophages. The cytoplasm of the degenerated polymorphonuclear cells contained many vacuoles that sometimes harbouring electron dense rod-like structure (Fig. 9) and degenerated mitochondria (marked vacuolation of mitochondrial matrix (Fig. 10).

In case of chronic endometritis and chronic metritis, the most prominent ultrastructural findings were periglandular collagen fibers in cross and longitudinal sections, fibroblasts, fibrocytes, and few mononuclear cells (Fig. 11).

In case of acute metritis, degenerated cells with intracytoplasmic electron translucent areas and electron dense debris in lumina of the endometrial glands were observed (Fig. 12).

(C) Microbiological findings

The bacterial isolation was positive only in cases of endometritis and metritis in 19/34 animals (55.88%). The bacterial isolates associated with endometritis and metritis included: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Citrobacter freundii* and *Citrobaacter diversus*. All uterine specimens were negative for brucella, mycoplasma, and fungi (Table 2).

Table 1: Seasonal incidence of affections in the uteri of female buffaloes:

Organ	Spring	Summer	Autumn	Winter	No. of cases with lesions	
					/Total cases with lesions	/Total animals
Uterus	27/89 (30.33±4.06%) ^a	28/105 (26.66±4.01%) ^{ab}	10/58 (17.24±2.26%) ^b	22/88 (25±2.39%) ^{ab}	87/154 (56.49%)	87/340 (25.58±2.14%)

N. B.:

-Data between parentheses represent the % ±MSE).

-Values with different alphabetical superscripts (a, ab, b) in the same row are significantly different at least at P< 0.05.

Table 2: Bacterial isolates in relation to endometritis and metritis:

	Endometritis (16 case)		Metritis (3 case)	
	Acute (3 case)	Chronic (13 case)	Acute (1 case)	Chronic (2 case)
Single	(2/3) <i>Klebsiella oxytoca</i> .	(11/13) <i>Citrobacter diversus</i> (4 cases). <i>Klebsiella oxytoca</i> (3 cases). <i>Proteus vulgaris</i> (2 cases). <i>Staph. aureus</i> (1 case). <i>Enterobacter aerogenes</i> (1 case).	(1/1) <i>Citrobacter diversus</i> .	(2/2) <i>Citrobacter diversus</i> . <i>Klebsiella oxytoca</i> .
Isolated organisms	<i>Enterobacter aerogenes</i> .			
Mixed	(1/3) <i>Klebsiella oxytoca</i> & <i>Pseudomonas aeruginosa</i> .	(2/13) <i>Citrobacter diversus</i> & <i>Klebsiella oxytoca</i> . <i>Citrobacter diversus</i> & <i>E. coli</i> .	(0/1)	(0/2)

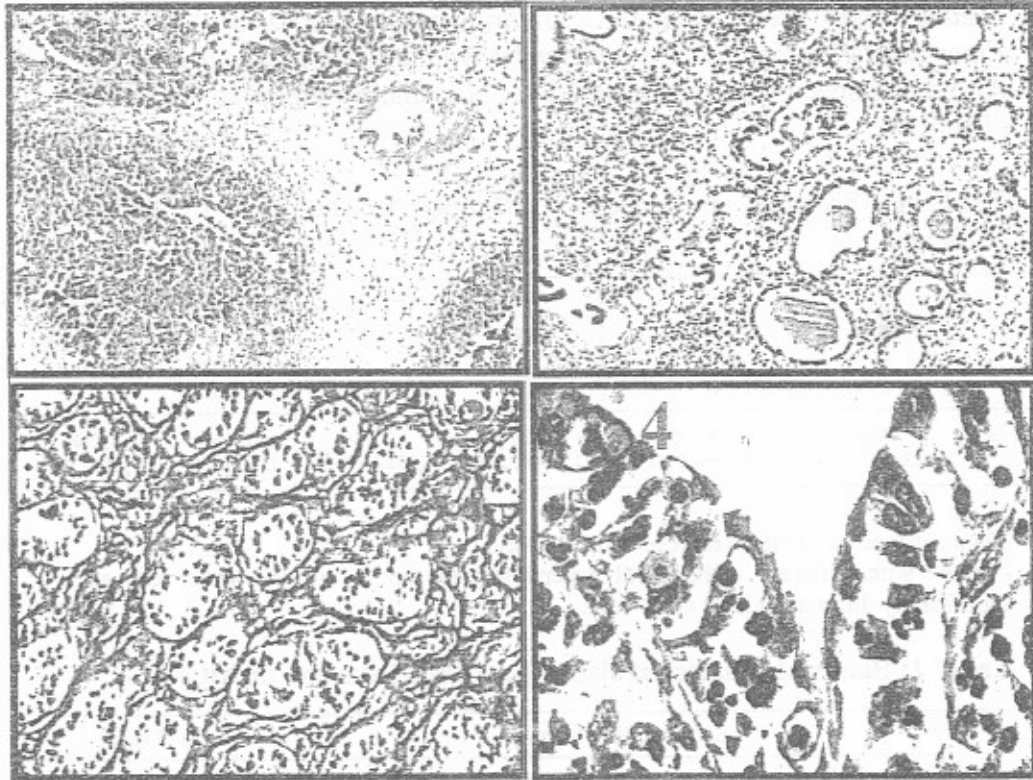


Fig. 1: Uterus from a case of acute endometritis showing multiple abscesses formation within the endometrial stroma. The endometrial glands are completely destroyed and replaced by degenerated neutrophils. (H&E, X40).

Fig. 2: Uterus from a case of chronic endometritis showing marked lymphocytic infiltration in between the dilated endometrial glands with periglandular fibrosis. (H&E, X40).

Fig. 3: Uterus from a case of chronic endometritis showing fibrosis around the endometrial glands (2-3 layers). (Van Gieson stain, X100).

Fig. 4: Uterus from a case of acute metritis showing vacuolar degeneration and neutrophilic infiltration (exocytosis) of the lining epithelium of the endometrial mucosa. (H&E, X400).

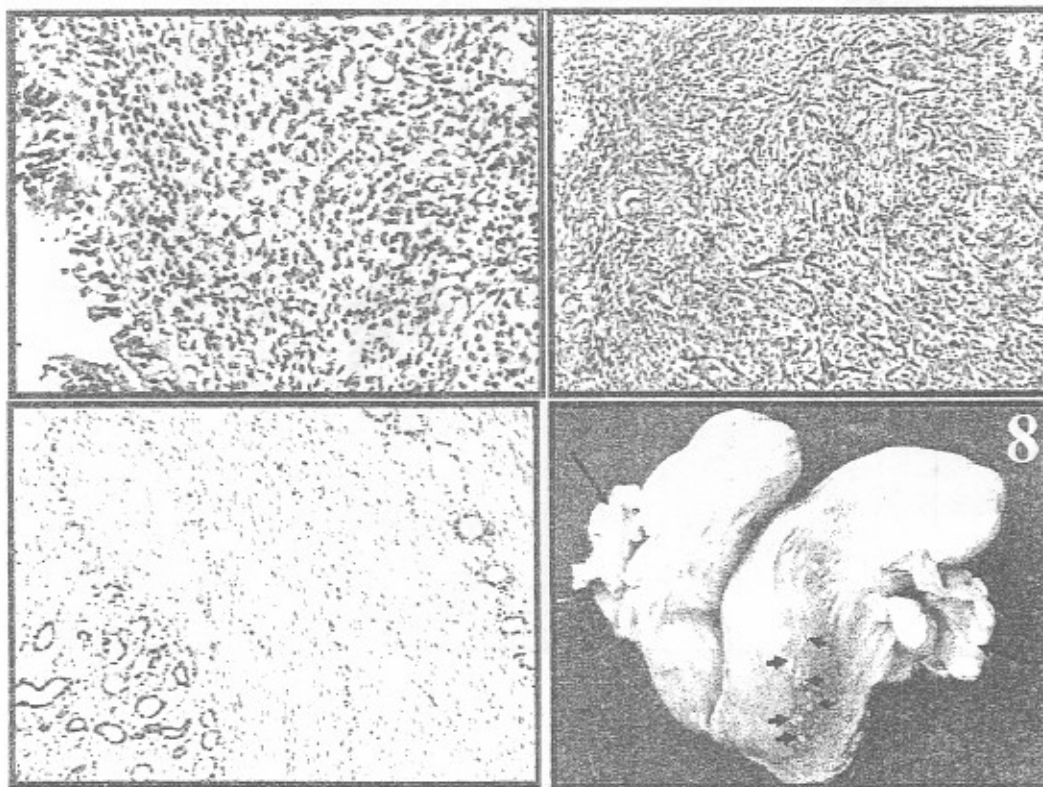
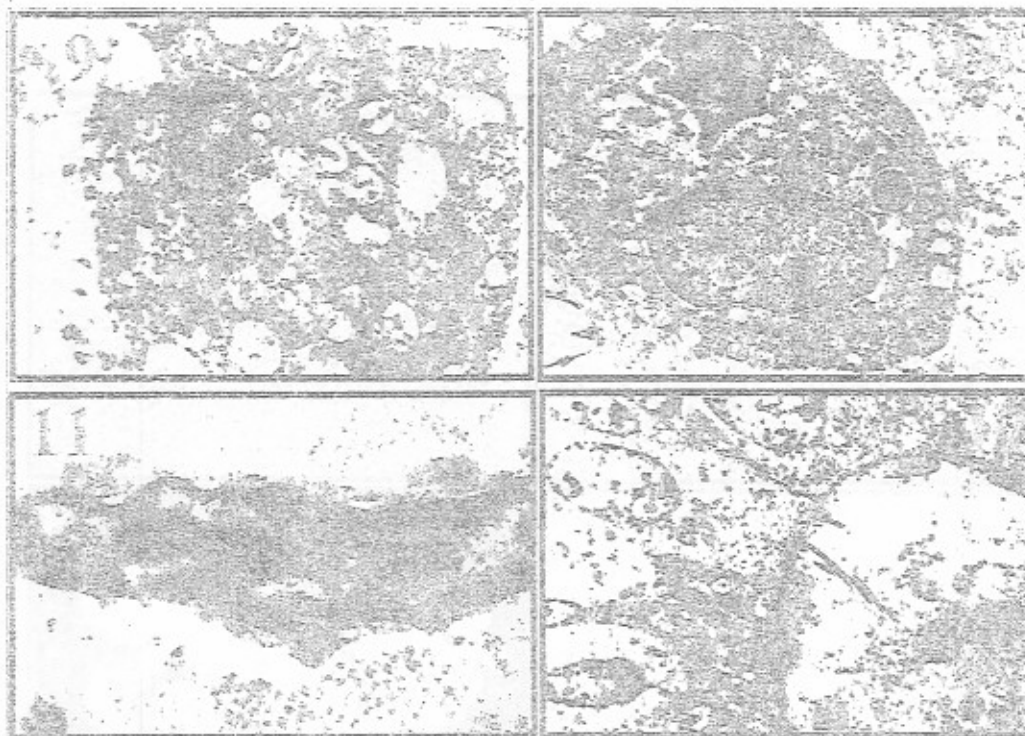


Fig. 5: Uterus from a case of chronic metritis showing destruction of most endometrial glands that replaced with lymphocytes. (H&E, X100).

Fig. 6: Uterus with fibroma showing circumscribed aggregate of fibroblastic proliferation and collagen fibers running in different directions within the endometrial stroma. (H&E, X40).

Fig. 7: Uterus has adenomyosis showing scattered endometrial glands and endometrial stroma among the muscle fibers of myometrium. (H&E, X40).

Fig. 8: Outer surface of uterus has multiple grayish elevated nodules of different sizes (short arrows). Marked partial dilatation of both fallopian tubes (long arrows) is seen.



- Fig. 9:** Electron micrograph of a degenerated neutrophil from a case of acute endometritis showing multiple vacuoles within the cytoplasm and some of them contained an electron dense rod-like structures (arrows) along with shrunken condensed nucleus. (Uranyl acetate and lead citrate stain, X11 200).
- Fig. 10:** Electron micrograph of a polymorphonuclear cell from a case of acute endometritis with multiple vacuoles within the cytoplasm. Mitochondria appeared with translucent space (marked degeneration). (Uranyl acetate and lead citrate stain, X8 400).
- Fig. 11:** Electron micrograph from a case of chronic endometritis showing collagen fibers in cross sections around the endometrial glands (periglandular fibrosis). (Uranyl acetate and lead citrate stain, X8 400).
- Fig. 12:** Electron micrograph from a case of acute metritis showing degeneration in the lining epithelium of endometrial gland with intracytoplasmic translucent areas and many electron dense debris within the lumen. (Uranyl acetate and lead citrate stain, X4200).

DISCUSSION

The reproductive abnormalities are attributed mainly to either hereditary or environmental factors and considered as one of the major factors causing infertility. The incidence of pathological findings in uterus was significant ($P < 0.05$) between spring and autumn, while there was no significance between spring, summer and winter or autumn, summer and winter. Uterine inflammatory lesions were the most common. Amer (1992) recorded similar findings in buffaloes and he stated that the inflammatory uterine lesions could be considered the main problem affecting the fertility of female buffaloes.

Some cases (15/34, 44.11%) with uterine inflammatory lesions were negative for bacterial isolation. Laila Ali *et al.* (1987) account such negativity for the high immunity during the estrogenic phase that leads to bacterial clearance or due to previous treatment with antibacterial drugs.

Inflammatory cells that noticed in most cases of chronic endometritis were lymphocytes and/or few plasma cells. Gonzalez *et al.* (1985) mentioned that presence of such inflammatory cells may be associated with local production of sperm antibodies which interfere with conception. Also, Mahdy (1988) stated that the inflammatory cells cause infertility and failure of implantation.

Moderate to severe periglandular fibroblastic proliferation was observed in all cases of chronic endometritis. Gonzalez *et al.* (1985) and Mahdy (1988) reported that the periglandular fibroblastic proliferation plays an important role in the reduction of uterine milk, early embryonic death and consequently repeat breeders. Additionally, Kenney (1978) stated that fibrosis comprises the function of involved glands and collagen may interfere with the integrity of the epithelium resulting in its atrophy.

Ultrastructural findings in case of acute and chronic endometritis were similar to those observed by Fetaih *et al.* (1992) in acute and chronic endometritis in she-camels and Sahar (2000) in chronic endometritis in equines.

Metritis was found in an incidence higher than that obtained by Zaki *et al.* (1963) and lower than that obtained by Kumar and Singh (1985b) in buffaloes.

Endometrial hyperplasia was observed in association with chronic endometritis. It was similar microscopically to that described

in cattle by Kennedy and Miller (1993) who stated that hyperplasia superimposed by cystic hyperplastic endometritis.

Fibroma was observed in association with chronic endometritis in a higher incidence than that recorded in cows and buffaloes by Shalash (1991) and Amer (1992).

Presence of adenomyosis in a low incidence agrees with the findings of Rao and Rajya (1976) in buffaloes and Deeb *et al.* (1976) in cows. In contrary, Kumar and Singh (1985a) recorded adenomyosis in a higher incidence in buffaloes (20%). Kennedy and Miller (1993) stated that the adenomyosis may arise as a malformation or due to hyperplastic overgrowth of the endometrium.

It was noteworthy that the perimetrial nodules and uterine adhesions were mostly recorded in chronic cases and could be attributed to continuous irritation with the surrounding tissues.

In conclusion, the present study covered a wide range of affections in uteri of female buffaloes. Not only the clinical examination of the animals but the endometrial biopsy coupled with bacteriological culture is very important in diagnosis and prognosis of endometritis.

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