

Diagnostic Study On Infertility Of Buffalo-Cows With Uterine Infection

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ABSTRACT

The present study was carried out during the year 2004 on 50 female buffalo-cows collected from the abattoir of Mansoura City, Dakahlia governorate, Egypt. A preliminary manual exploration of the reproductive tract was carried out before ultrasonographic scanning for determining the location and rectal findings of the genitalia. The ultrasonographic image of the longitudinal section of the uterus revealed that the uterine wall runs parallel to the longitudinal axis of the body and appears as dorsal and ventral layers surrounding the central anechoic (black) lumen. During the follicular phase the uterine echotexture was heterogeneous, shades of hypo- and hyper-echogenicity, with increased thickness of the uterine wall (echoic = white). The lumen of the uterus was anechoic due to its fluid contents. The uterine echotexture during the luteal phase, was homogenous with decreased thickness of the uterine wall. The ultrasonographic examination of buffalo-cows showed a mild degree of endometritis evidenced by a mild thickening of the endometrium and segmental accumulation of anechoic fluid within the uterine lumen. Pyometra was characterized by great distention of uterine lumen with pus, which contained hypoechoic particles showing continuous motion (snow storm). The prevalence rate of endometritis (mild and severe) was 14% besides pyometra (4%). After slaughter the genital tract was removed shortly after evisceration for macroscopic and microscopic examinations. The histological alterations, observed in the investigated materials could be classified into mild (6%), moderate (4%) and severe chronic catarrhal endometritis (8%). The microscopic picture of the uterus was correlated with that of the ultrasonography, to diagnose endometritis as a cause of infertility.

It could be concluded that, the ultrasonography helps the diagnosis of infertility in addition to causes of buffalo-cows infertility. The use of the sonar, for the predication of infertility problem in buffalo-cows is recommended.

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INTRODUCTION

The bovine uterine infections are a major cause of economic loss in cattle industry. It has been established that postpartum uterine infection in cows, may interfere with their subsequent fertility even after apparent recovery. It may predispose to further anoestrus and repeat breeder syndrome (Hartigan, 1). The uterine disorders are important factors that impair the fertility of animals. The most common forms of the uterine disorders are endometritis, pyometra and intrauterine embryonic fetal death (Khan, 2). Implementation of the ultrasonography in the diagnosis of infertility problems, at early stages is helpful and more accurate (Spreche, 3, Farin, 4 and Griffin, 5). On transrectal ultrasound examination, the mild endometritis was characterized by a distended lumen,

presence of varying amounts of fluid which was partially echogenic and snowy in a short segment of the uterus. However, in many cases of these mild cases, the ultrasonography could not detect the lumen. The severe form of endometritis, showed distended uterine horns with exudates along their entire length. The inflammatory fluid contained echogenic patches which were in a continuous motion to cause snow storm like image (Fissore, 6, Kahn, 7 and Rajamehendram, 8). Fadel (9) described endometritis to be characterized by enlarged hyperechoic uterine wall, thick hyperechoic endometrium and accumulation of anechoic uterine secretions. Fissure (6) reported that the pyometral fluid, sonographically was characterized by presence of follicular reflections with varying echogenicity, according to the consistency of pyometral fluid. It appears more hypoechoic

than the uterine wall depending on its content of cellular elements. If the pyometral fluid was very thick with many cellular elements, the echogenicity, would increase nearly similar to that of the uterine wall and become separated from it by a fine anechoic surrounding line.

In order to acquire good practice in the manipulation of the ultrasonography for diagnosis of any problems of infertility, it is necessary to be acquainted with the normal findings of the genital system. For this reason, the histological investigation was involved in the present study for the accurate identification of the normal and pathological changes in the examined organs.

This study was planned to correlate the ultrasonographic results with rectal, histopathological and macroscopic examinations of the genitalia.

MATERIALS AND METHODS

The current work was carried out on 50 buffalo-cows during the year 2004 in the abattoir of Mansoura City, Dakahlia province, Egypt. Before transrectal scanning, the animal was properly restrained and the scanning unit was placed at a suitable distance from the animal (on the side opposite to the operator's arm used for rectal examination). The monitor of the scanner was placed at the level of the operator's eyes, and the ambient light was reduced as much as possible for good observation. The animal's rectum was evacuated from all feces prior to the introduction of gel lubricated transducer. A preliminary manual exploration of reproductive tract was carried out before ultrasonographic scanning for determining the location and rectal findings of the genitalia. The transducer scan head was covered with the coupling ultrasonic gel inserted in a cupped, lubricated hand through the rectum with scan head presses firmly against the rectal mucosa to prevent air interference. The transducer was moved along the dorsal surface of the reproductive tract to examine the uterus as described by (Pierson, 10 and Pierson, 11). The buffalo-cows were slaughtered and the reproductive tracts were removed shortly after evisceration. The

macroscopical picture of the reproductive organs was described. The ovarian structures were studied to detect the phase of the reproductive cycle. The stage of the estrus cycle, in each organ was determined by studying ovarian structures. Specimens of about 1x1 cm were collected taken from the uterine horns. Five microns thick paraffin sections were prepared according to Bancroft (12), stained and examined microscopically.

RESULTS

Ultrasonographic examination:

The ultrasonographic image of the longitudinal section of the uterus revealed that the uterine wall runs parallel to the longitudinal axis of the body and appears as dorsal and ventral layers echoic surrounding the central anechoic lumen (Fig. 1).

Two distinctly different forms were found during the follicular and luteal phases.

a) Follicular phase: The uterine echotexture was heterogenous, as it showed shades of hypo and hyper-echogenicity, with increased thickness of the uterine wall. The lumen of the uterus was found to be non echogenic (anechoic) due to its fluid contents (Fig. 2).

b) Luteal phase: The uterine echotexture was homogenous with decreased thickness of the uterine wall. The fluid contents decreased than in case of the follicular phase (Fig 3).

The ultrasonographic examination of cows with a mild endometritis revealed a mild thickening of the endometrium and segmented accumulation of anechoic fluid (black) within the uterine lumen (Fig 4). The severe endometritis was characterized by thickened endometrium and widespread accumulation of uterine fluid within the uterus (Fig, 5). Pyometra was characterized by great distention of the uterine lumen with pus which contained hypoechoic particles ultrasonographic image showing continuous motion (snow storm), Fig (6).

The mild endometritis was characterized by moderate periglandular leukocytic infiltration (Fig, 7 & 8). The uterine glands were lined by columnar epithelium, with the presence of

cellular debris in their lumens. Gland hyperplasia was seen in some cases.

The endometrium showed round cell infiltration, brown pigments and fibrous tissue proliferation (Figs. 9 & 10). Moreover, the coiled arterioles were congested and showed thickened walls (Figs. 11 & 12). The epithelial lining of the uterine glands showed apical vacuolation with the presence of interstitial edema (Fig. 13). The endometrium showed diffuse leukocytic infiltration (Figs. 14 & 15). The uterine glands showed several changes which include mucinous degeneration, hyperplasia of the epithelial lining, desquamation of the glandular epithelium, cystic dilatation of the uterine glands and vacuolation of the epithelium in the

upper part with congested blood vessels in the deep layer.

The severe endometritis (Figs. 16 & 17) showed ulcerative endometrium with submucosal neutrophilic infiltration and few lymphocytes. On the other hand, hyperplasia of the surface epithelium was noticed. Diffuse interstitial leukocytic infiltration with lymphocytes was encountered, particularly periglandular. Moreover, focal lymphocytic aggregations were observed. Leukocytic infiltration was present intraglandular with interstitial edema. Blood vessels were congested leading to a diffuse bleeding.

Table (1) Prevalence of different lesions in buffalo-cows. Rectal examination of the 50 uteri

Degree of severity of endometritis	No.	Prevalence %
Mild	-	-
Moderate	2	4
Severe	4	8
Pyometra	-	-
Total	6	12

Table (2) Prevalence of different lesions in 50 buffalo-cows uteri by ultrasonography.

Degree of severity of endometritis	No.	Prevalence %
Mild	3	6
Moderate	-	-
Severe	4	8
Pyometra	2	4
Total	9	18

Table (3) Prevalence of different degrees of endometritis by macroscopic examination of 50 buffalo-cows uteri

Degree of severity of endometritis	No.	Prevalence %
Mild	1	2
Moderate	2	4
Severe	2	4
Pyometra	2	4
Total	7	14

Table (4) Prevalence of different degrees of chronic catarrhal endometritis by microscopic examination of 50 buffalo-cows uteri

Degree of severity of endometritis	No.	Prevalence %
Mild	3	6
Moderate	2	4
Severe	4	8
Pyometra	-	-
Total	9	18

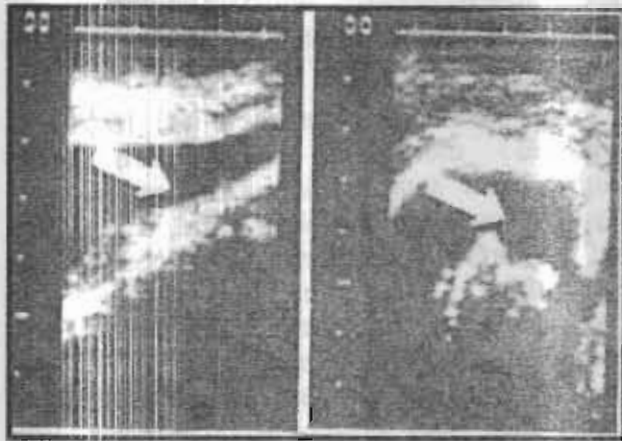


Fig (1): A longitudinal section through a bovine uterus to show anechoic lumen (arrow) and echoic wall.

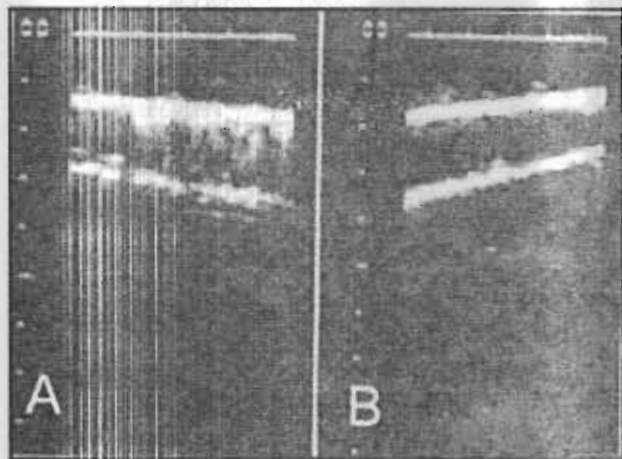


Fig (2): A longitudinal section through a bovine uterus, during the follicular phase.(A & B) to show heterogenous echotexture of a thickened uterine wall with echoic lumen.

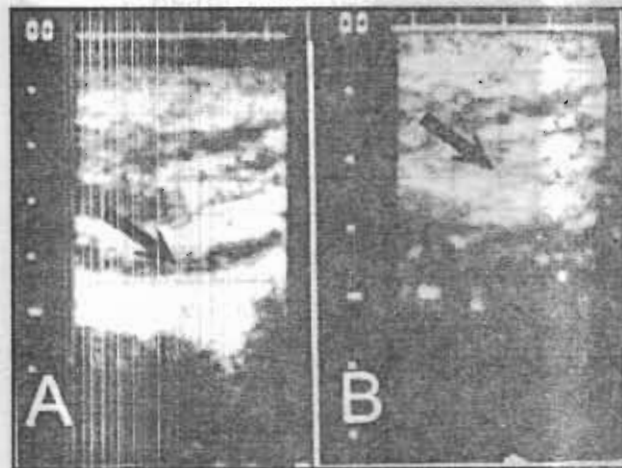


Fig (3): A longitudinal section through a bovine uterus during the luteal phase to show a homogenous echotexture of a thin uterine wall with less anechoic fluid-content.

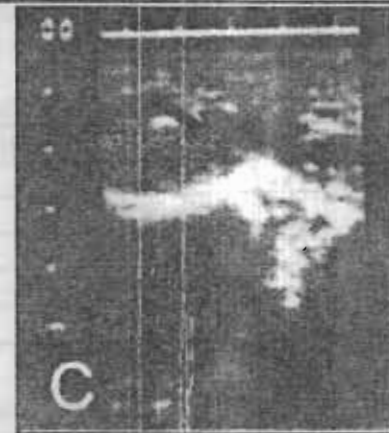
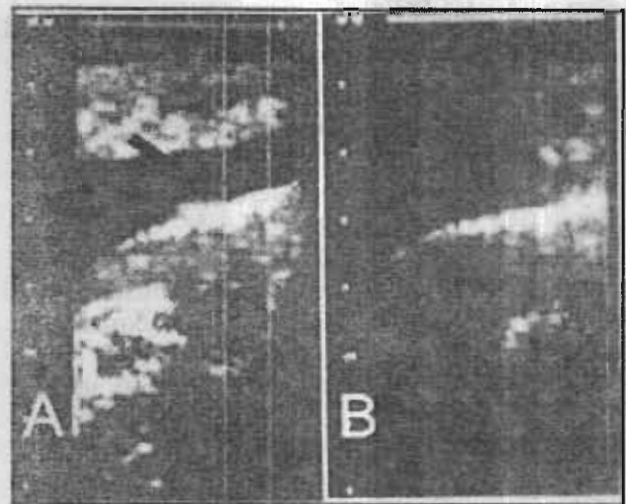


Fig (4): A bovine uterus with mild endometritis showing moderately thickened endometrium with a segmented accumulation of anechoic fluid in the uterine lumen.

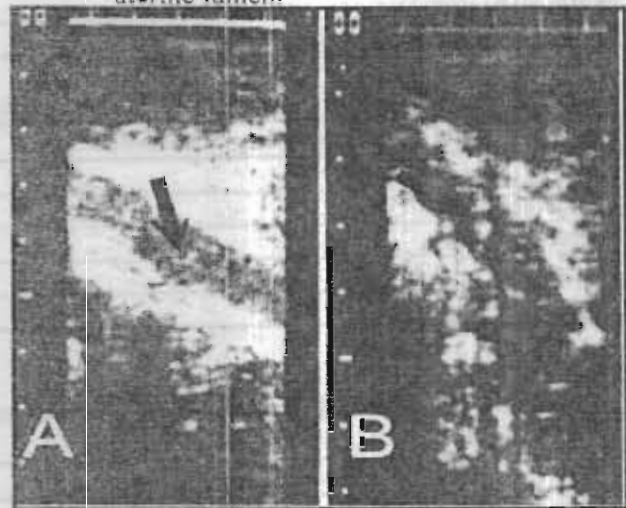


Fig (5): A bovine uterus with a severe endometritis (A & B) showing a thickened endometrium and a widespread accumulation of intrauterine fluid.

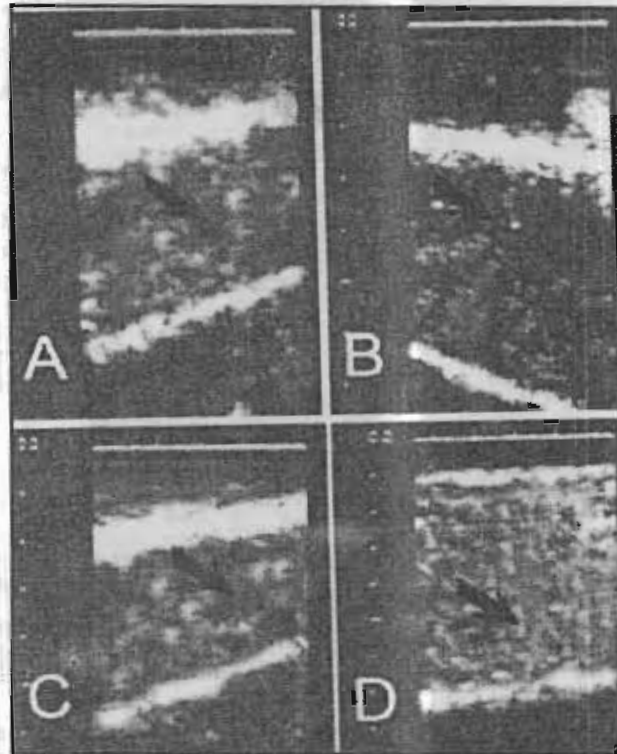


Fig (6): A case of pyometra (A, B, C & D) showing a highly distended uterine lumen with pus displaying hypoechoic particles (snow- storm).

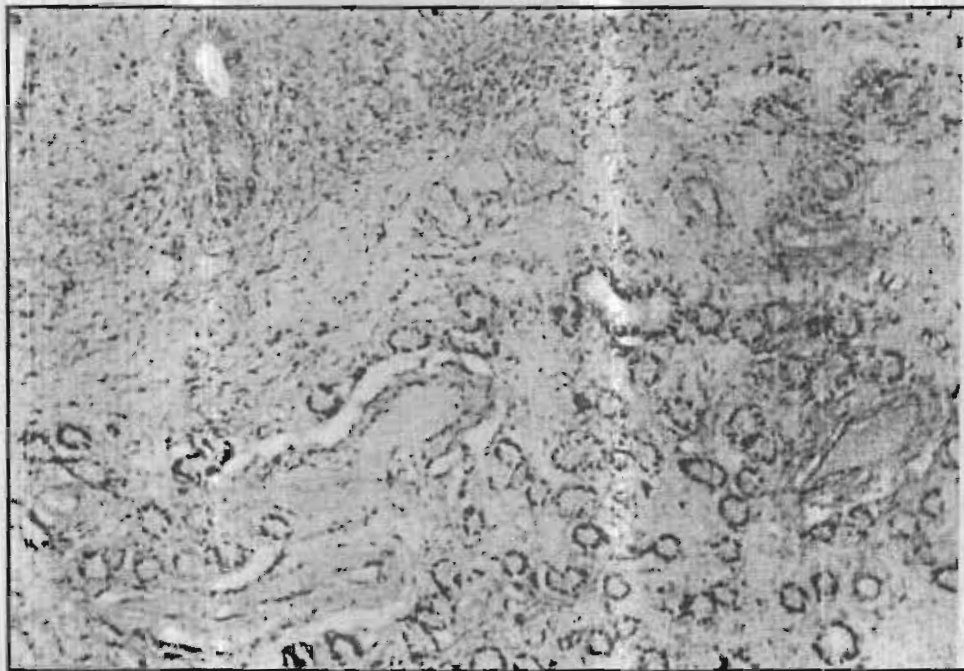


Fig. (7): Mild chronic catarrhal endometritis in a buffalo-cow showing mild periglandular leukocytic infiltration besides edema in the deeper portion, H&E x 100.

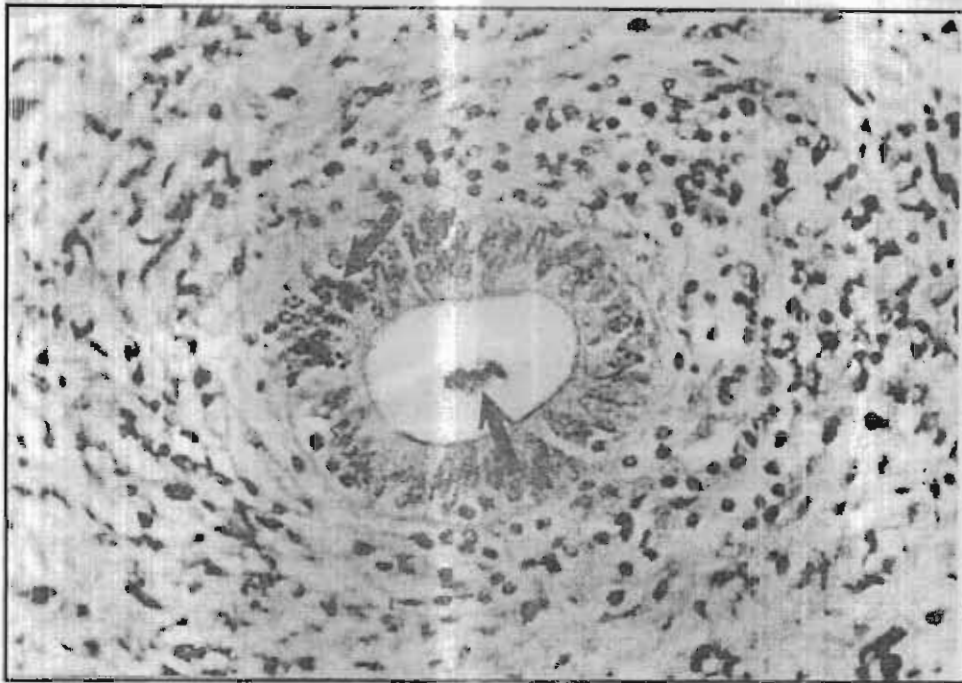


Fig. (8): A high power for Fig (7) to show periglandular few round cell aggregation besides necrotic debris in its lumen (mild endometritis), H&E x 400.

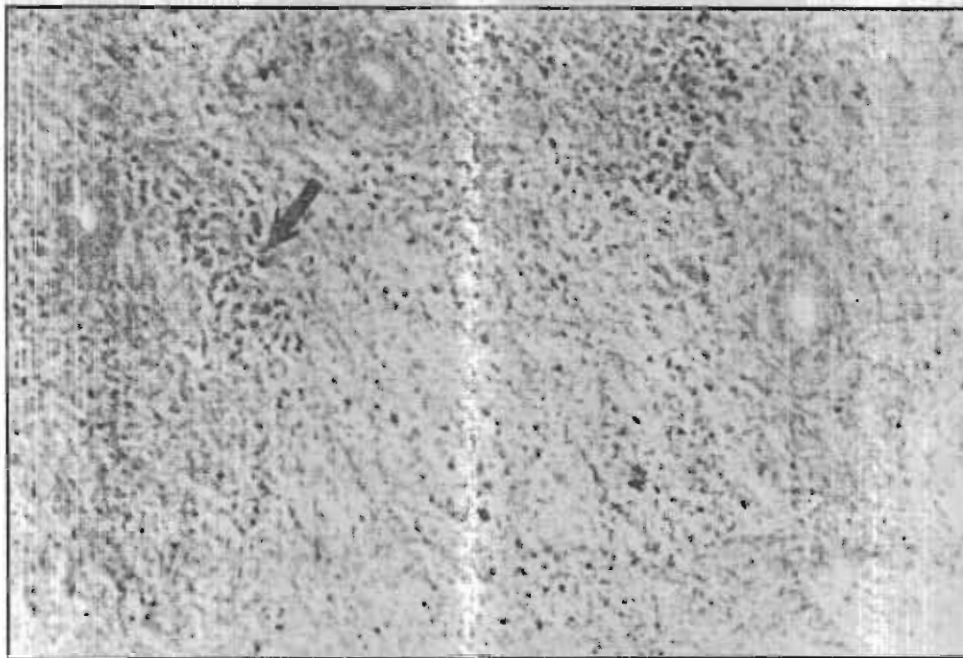


Fig. (9): Mild chronic catarrhal endometritis in a buffalo-cow showing dark brown pigments (arrow) besides round cell infiltration, H&E x 100.

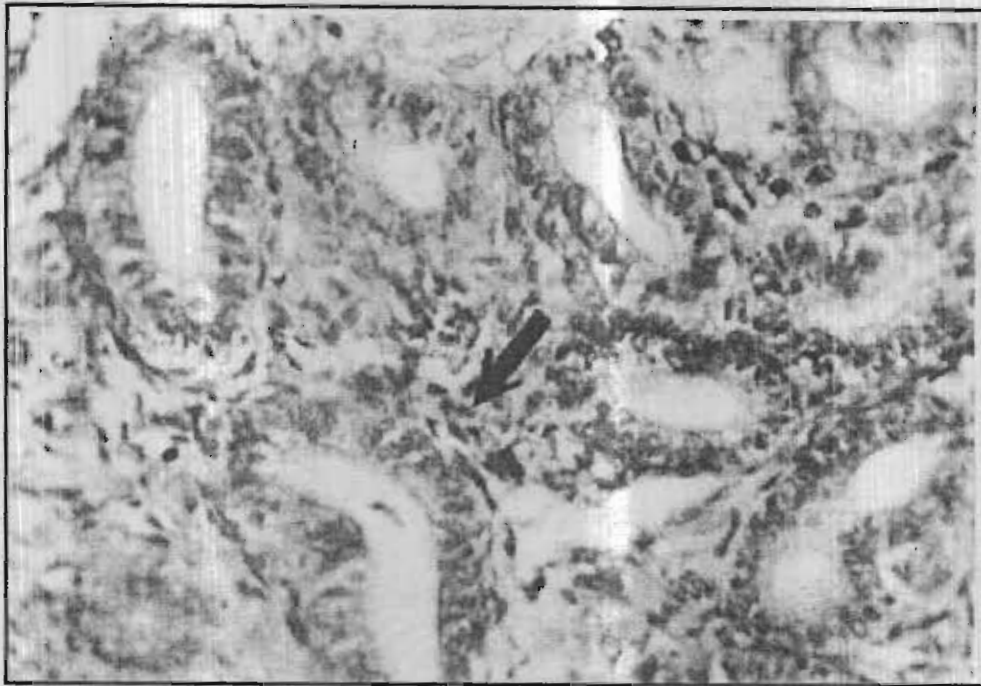


Fig. (10): Mild chronic catarrhal endometritis in a buffalo-cow showing interstitial leukocytic infiltration and fibroblastic proliferation (arrow) which increased in number during the luteal phase, H&E x 100.

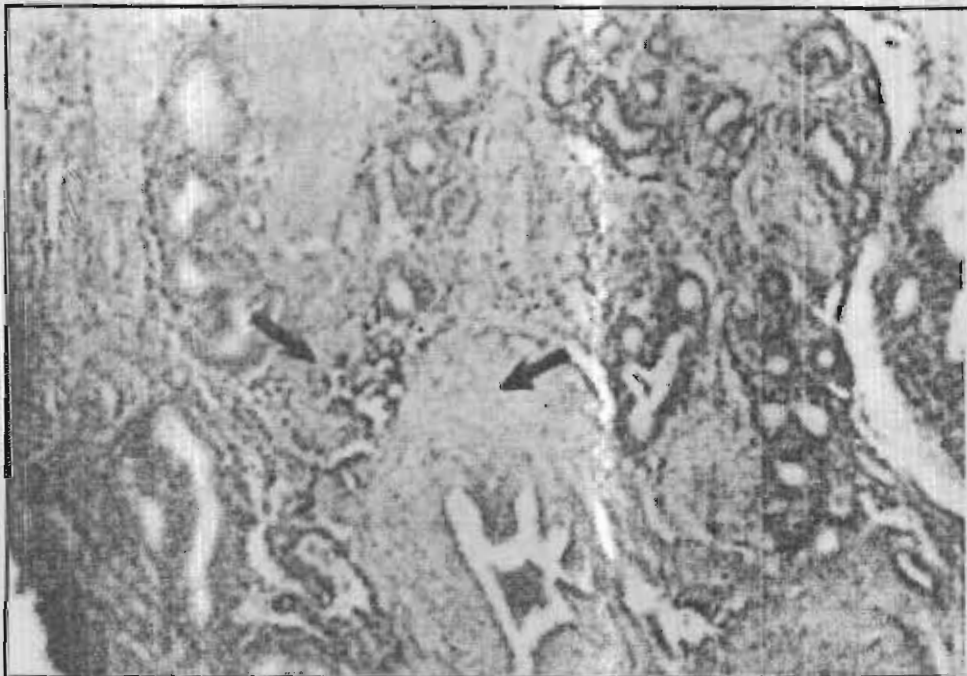


Fig. (11): Mild chronic catarrhal endometritis in a buffalo-cow showing dark brown pigments (arrow) besides thickened walls of blood vessel (arrow) and proliferated glandular epithelium, H&E., x 100.

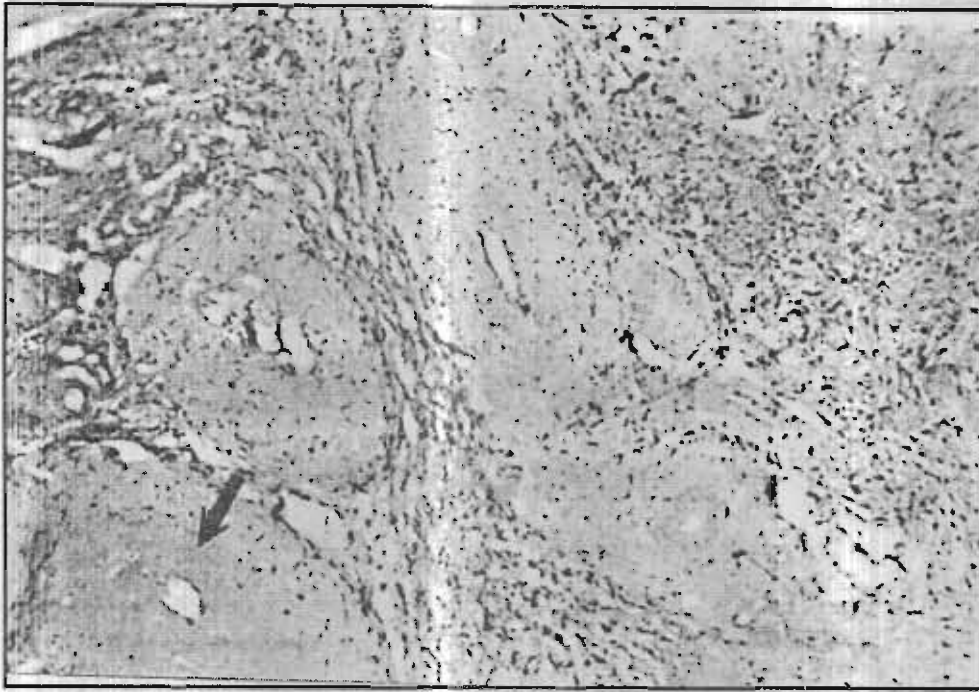


Fig. (12): Mild chronic catarrhal endometritis in a buffalo-cow showing thickened walls of the blood vessels (arrow), H&E., x 100.

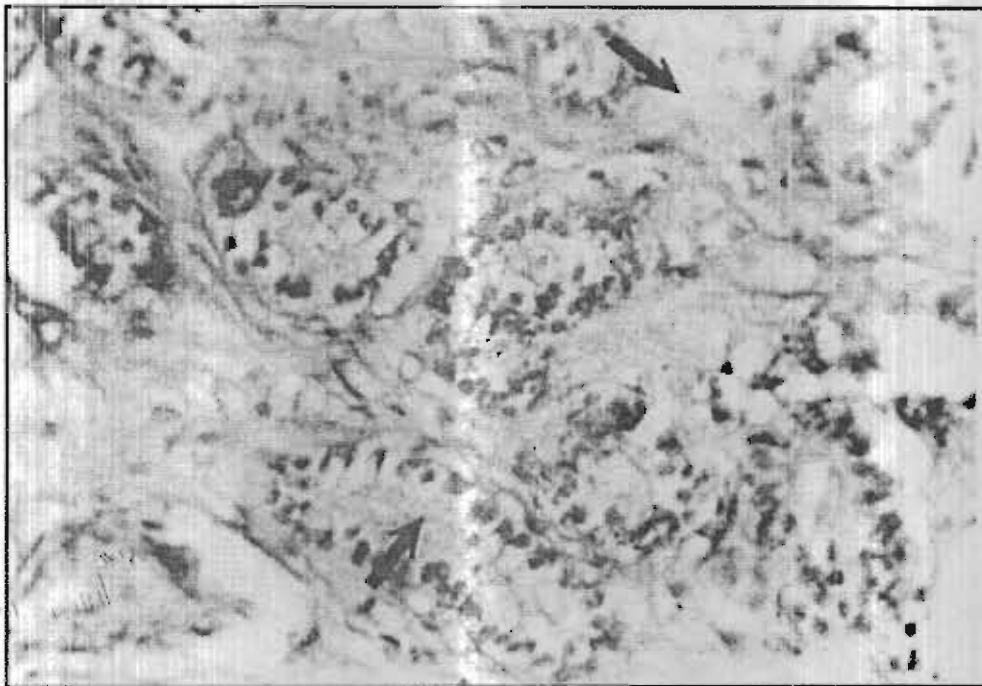


Fig. (13): Moderate chronic catarrhal endometritis in a buffalo-cow showing apical vacuolation of the glandular epithelium besides interstitial edema. H&E., x 400.

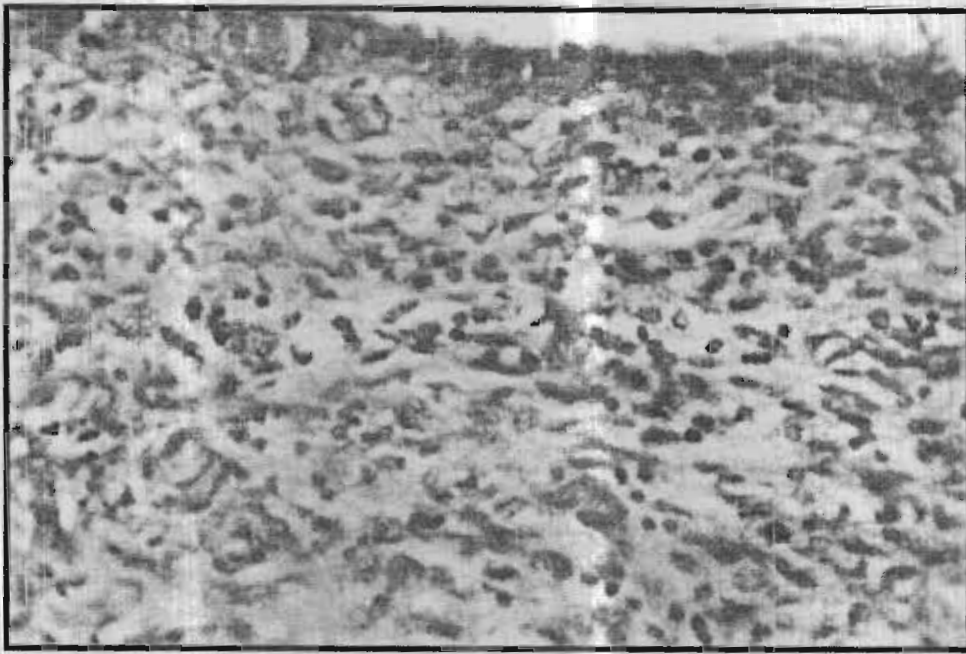


Fig. (14): Moderate chronic catarrhal endometritis in a buffalo-cow showing subepithelial round cell infiltration. H&E., x 400.

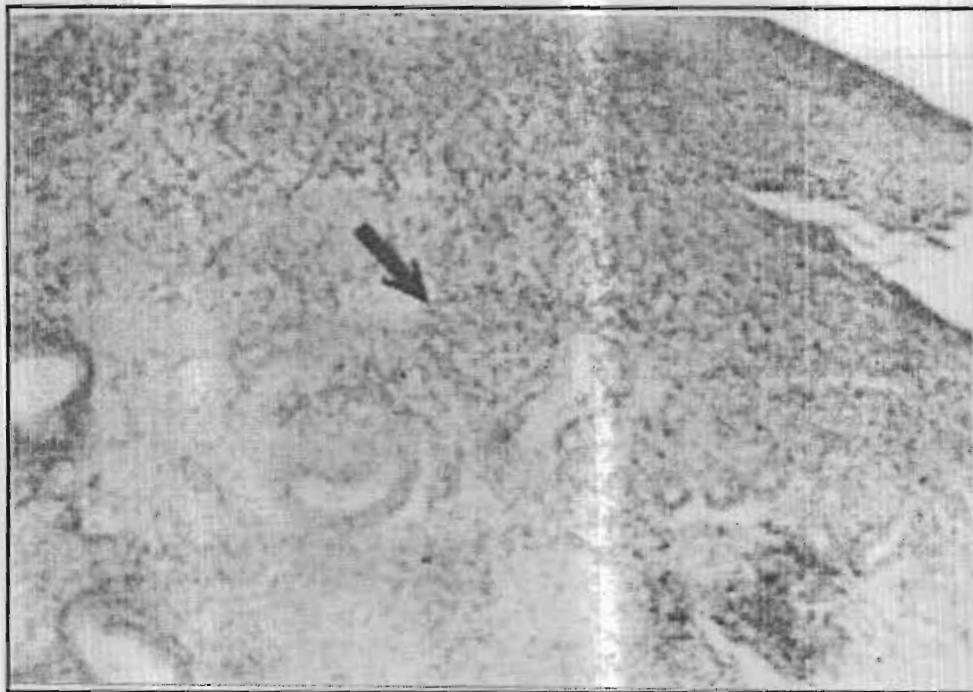


Fig. (15): Moderate chronic catarrhal endometritis in a buffalo-cow showing lymphocytic aggregations (arrow) in the lamina propria. H&E., x 400.

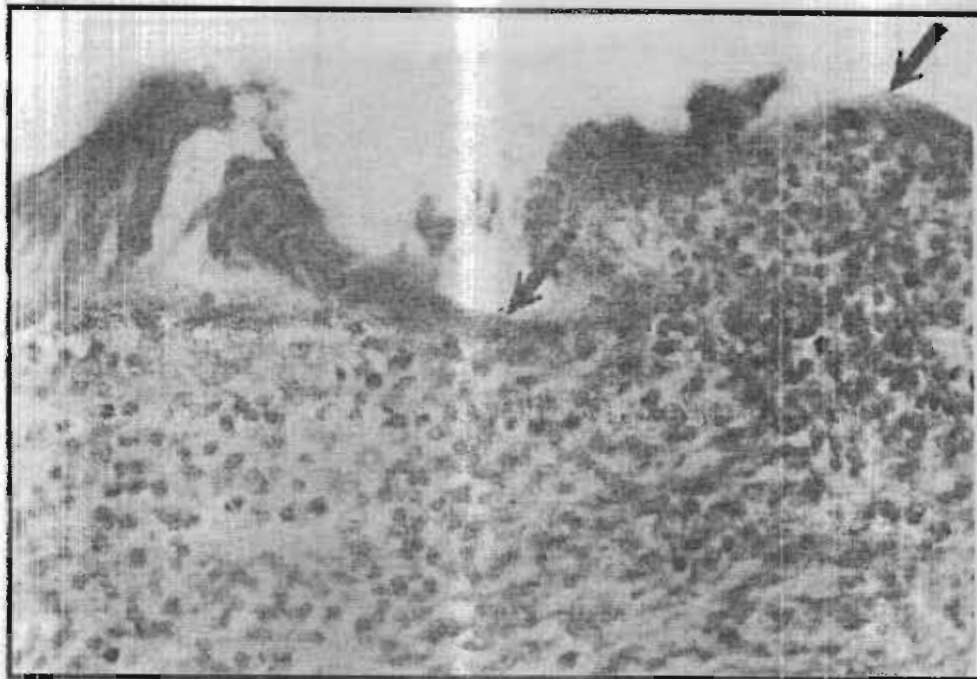


Fig. (16): Severe chronic catarrhal endometritis in a buffalo-cow shows desquamated surface epithelium leading to ulceration (arrows) and round cell infiltration. H&E., x 400.



Fig. (17): Severe chronic catarrhal endometritis in a buffalo-cow showing focal lymphocytic aggregations (arrow) besides dilatation of some endometrial glands. H& E., x 100.

DISCUSSION

Infertility isn't a disease, but it is a common sign for the action and/or interaction of multiple factors including hereditary abnormalities, management, environmental or pathological affections (*Erb, 13*). Early diagnosis of the possible causes of infertility is intended so that the reproduction and reproductive potential of the animal can be maintained. The implementation of ultrasonography in the diagnosis of infertility problems at early stages is helpful and more accurate (*Sprecher, 3, Farin, 4 and Griffin, 5*). Mastering the manipulation of the ultrasonography for diagnosis of infertility, requires the acquaintance with normal and abnormal findings in the genital system. For this reason, the histopathological investigations were involved in the present study. The cyclic changes, in the uterine echotxture included an increase in the heterognicity of the uterus with a mixed hypo- and hyperechogenicity during the follicular phase and homogeneity during the luteal phase. This observation is in agreement with the results obtained by (*Pierson, 10, Banafos, 14, Boyd, 15, Fadel, 9 and Kandiel, 16*). The change in the ultrasound image might be attributed to the turgidity of the uterus during estrus under the influence of estrogen and the flaccidity of the uterus during diestrus under the influence of progesterone (*Bartol, 17 and Roberts, 18*), besides the intrauterine estrous fluid accumulation (*Fissore, 6*) or edematous expansion of the endometrium (*Pierson, 10*).

The prevalence of endometritis (mild and severe) was 14%. This finding is in agreement with the results obtained by (*Khasatia, 19 and Son-Changho, 20*) who observed incidence rate of 15.68%. This result is lower than 20.60% observed by (*Fadel, 9*) and 18.33% mentioned by (*Kandiel, 16*). On the other hand, this result is higher than 7.4% observed by *Kang (21)*. On ultrasonography, mild endometritis appeared as a mild thickening of the endometrium and segmental accumulation of anechoic fluid within the uterine lumen. The severe endometritis was characterized by a thickening of the endometrium and a widespread accumulation of uterine fluid within the uterus. These findings are in agreement with those observed by (*Fissure,*

6, Kahn, 2, Rajahendran, 8, Fadel, 9 and Khandiel, 16).

The prevalence of pyometra was 4%. A finding which is higher than 2.3% and 1.9% detected by (*Son-Changho, 20 and Fadel, 9*), respectively. On the other hand, this finding is lower than 12%, 5.9% and 18.33% reported by (*Etherington, 22, Kang, 21 and Kandiel, 16*) respectively. By ultrasonography, pyometra appeared as a great distention of the uterine lumen with pus which contained hypoechoic particle as appeared in the live ultrasonographic image, showing continuous motion (snow storm). This finding is in agreement with that reported by (*Fissure, 6, Fadel, 9 and Kandiel, 16*).

The histological picture of the uterus was employed for the diagnosis of endometritis to correlate its picture with that of the ultrasonography. The histological examination of the uterus gave a true picture for the entire uterus (*Brus, 23*). The histological alterations, observed in the investigated materials, could be classified into mild, moderate and severe chronic catarrhal endometritis. The inflammatory condition of the endometrium has to be evaluated with special care in comparison with the leukocytic cycle occurring during the different phases of estrus where the polymorphnuclear leukocytes prevail during the follicular phase of the estrus cycle and the lymphocytes during the luteal phase. In normal estrus, the presence of neutrophils is due to the estrogenic effect and is required for the high resistance against the uterine infection (*Griffin, 24 and Priedklans, 25*). One of the pathological alterations, observed in case of endometritis, was diffuse and/or focal infiltration with inflammatory cells specially lymphocytes and plasma cells. These findings are in agreement with (*Brus, 26*) who stated that endometritis could be diagnosed with more or less certainty if lymphocytes and plasma cells could be observed. Also, the occurrence of these cells was less favorable for pregnancy than the occurrence of granulocytes. Although (*Skjerven, 27*) found small numbers of lymphocytes in the normal endometrium, yet he stated that more general occurrence of these cells, could probably be considered pathological and less favourable from the prognostic point of view.

The present work showed vacuolar degeneration, proliferation, desquamation, and erosion of the uterine epithelial lining. Moreover hyperplasia, focal and diffuse cellular infiltration were noticed. The uterine glands showed proliferation and desquamation of their epithelial lining, with subsequent leukocytic infiltration, and debris. These findings are in agreement with those obtained by (El-Nagger, 28, El-Hariri, 29, El-Azab, 30 and Gonzalez, 31).

Diffuse or focal leukocytic infiltration in the endometrial stroma and around the uterine glands was present, and constituted the significant lesion of endometritis. These findings are in consistency with the findings of (Brus, 23 & 26, Moss, 32 and DeBois, 33) who mentioned that the formation of inflammatory cell foci around the endometrial glands, was considered pathognomonic for endometritis. Congestion of blood vessels, edema and erythrocytic extravasation, lymphocytic infiltration and fibrosis of the endometrial stroma were indicative for chronicity and these findings are in agreement with (Hartigan, 34, El-Nagger, 28, Dzhurova, 35, Gonzalez, 31 and Kubar and Jalakas, 36). Atrophy of the endometrial glands seemed to be of inflammatory origin as indicated by the periglandular fibrosis which resulted in pressure atrophy, or the fibrosis around the duct resulted in their obstruction and subsequent dilation of the glands as a result of flattening of its epithelial lining. Consequently, the endometrium was unfavourable for implantation at it lost its secretory activity (Cupps, 37).

In this study, it was concluded that ultrasonography was additive method for diagnosis and identifying the causes of buffalo-cows infertility, so, we advice the use of sonar for predication of infertility further in buffalo.

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الملخص العربي

دراسة تشخيصية عن نقص الخصوبة في الجاموس في حالات إصابة الرحم

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أجريت هذه الدراسة خلال عام ٢٠٠٤ على خمسين أنثى جاموسى من خلال مجزر المنصورة - محافظة الدقهلية مصر وكان الغرض من هذه الدراسة هو مقارنة طرق الفحص المختلفة والتي تشمل الفحص بالموجات فوق الصوتية والفحص الإكلينيكي للجاموس من خلال المستقيم قبل الذبح وفحص الجهاز التناسلي وأخذ عينات من جدار الرحم لإعداد شرائح ميكروسكوبية وصبغتها بصبغة (H&E) بعد الذبح. وقد تبين من نتائج الفحص بالموجات فوق الصوتية أن الرحم يظهر غير متجانس مع ظهور تجويف أسود أثناء الشبق في حين يظهر متجانس مع وجود تجويف أسود رقيق لوجود جسم أصفر نشط. هذا وقد ظهرت صورة الرحم في حالة الالتهاب الرحمي بها زيادة في سمك جدار الرحم مع وجود سوائل في أجزاء من قرني الرحم كما في حالة الالتهاب الخفيف أو في كل تجويف الرحم كما في الالتهاب الشديد. وفي حالات أدى الالتهاب الصديدي المزمن للغشاء المبطن للرحم وما صاحبه من تجمع صديدي بتجويف الرحم الى زيادة في تجويف الرحم لامتلائه بالصديد والذي ظهر على هيئة سحب. وبالفحص بالموجات فوق الصوتية لوحظ وجود تغيرات مرضية لعدد ٧ (١٤%) الالتهابات الرحمية البسيطة والوسط والشديدة بالإضافة إلى ٢ (٤%) الالتهاب الرحمي الصديدي المزمن. وبالفحص الميكروسكوبي للشرائح المعدة من جدار الرحم لوحظ وجود تغيرات مرضية لعدد ٩ (١٨%) وقد تم تقسيم الالتهابات الرحمية إلى بسيط ووسط وشديد وقد وجد من بين هذه الحالات (٦%) بسيط و (٤%) وسط ، (٨%) شديد الالتهاب.

ولذلك ينصح باستخدام السونار لتحديد مستقبل الإخصاب في إناث الجاموس.