

## Ultrasonographic, Cytological and Bacteriological Investigation of Endometritis in Arabian Mares

Mohamed M.S. Ibrahim, Mohamed M.M. Kandiel,  
Gamal A.M. Sosa and Mahmoud E.A. Abouel-Roos

Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, Egypt

**Abstract:** The objective of this study was to evaluate efficacy of diagnostic techniques of endometritis in Arabian mares. An ultrasonographic evaluation as well as cytological and bacteriological examinations of uterine swabs have been performed in Arabian mares (n=43) suffered from endometritis. Mares showed a highly significant ( $P<0.001$ ) impact of age on the occurrence of various grades of endometritis, with the occurrence of severe grade (E4) in mares elder than 18 years. The mean number of neutrophils in the cytological examination of endometrial swabs was  $3.79\pm 0.22$ ,  $4.90\pm 0.32$ ,  $6.50\pm 0.65$  and  $9.66\pm 0.33$  in E1, E2, E3 and E4, respectively ( $p<0.001$ ). There was a highly significant ( $P<0.0001$ ) difference in the echogenicity of the inflammatory fluid and endometrial wall and the diameter of uterine horn and lumen among different grades endometritis. The overall incidence of single and mixed bacterial infection in mares endometritis was 67.35% and 30.23 %, respectively. E.coli (33.3%) and Streptococcus (21.43) were the most isolated microorganisms from uterine bacterial culture. We could conclude that the ultrasonography is a rapid, reliable diagnostic technique for mare endometritis, when combined with endometrial cytology and bacteria isolation.

**Key words:** Arabian Mares • Bacteriological • Cytological • Endometritis • Ultrasound

### INTRODUCTION

Endometritis is a major cause of infertility, inflicts major losses in the equine breeding industry and therefore its diagnosis is very important in veterinary practice [1]. Several diagnostic tools have been developed to check the reproductive soundness in mares as rectal palpation, vaginal speculum examination, ultrasound examination, or more invasive methods as endometrial cytology, uterine culture and endometrial biopsy [2].

Transrectal ultrasonography is used routinely to assess the reproductive tract of the mare. The non-invasive nature of ultrasonography allows frequent serial examinations to evaluate normal morphological as well as pathological changes within the endometrial layer of the uterus and the presence of fluid inside uterine lumen [3].

Cytological examination of endometrial swab is often used in the evaluation of the reproductive tract in humans and domestic animals. Such method is believed to

increase the ability to detect endometritis [4]. Moreover, the endometrial cytology is a fast and reliable way to assess the uterus during quick-response situations [5].

Bacterial contamination of the mare's uterus is common and in certain circumstances is normal [6]. However, endometritis is most commonly associated with aerobic bacteria [5]. Nevertheless, isolation of bacteria does not necessarily prove the presence of endometritis nor does failure to isolate bacteria eliminate it [7].

The aim of the current study was to evaluate different diagnostic methods including ultrasonographic evaluation, endometrial cytology and bacteriological examination of uterine swab for the verification of uterine inflammation grades in Arabian mares.

### MATERIALS AND METHODS

**Animals:** The current study was conducted from June 2012 to June 2014 at El-Zahraa Stud for Arabian horse

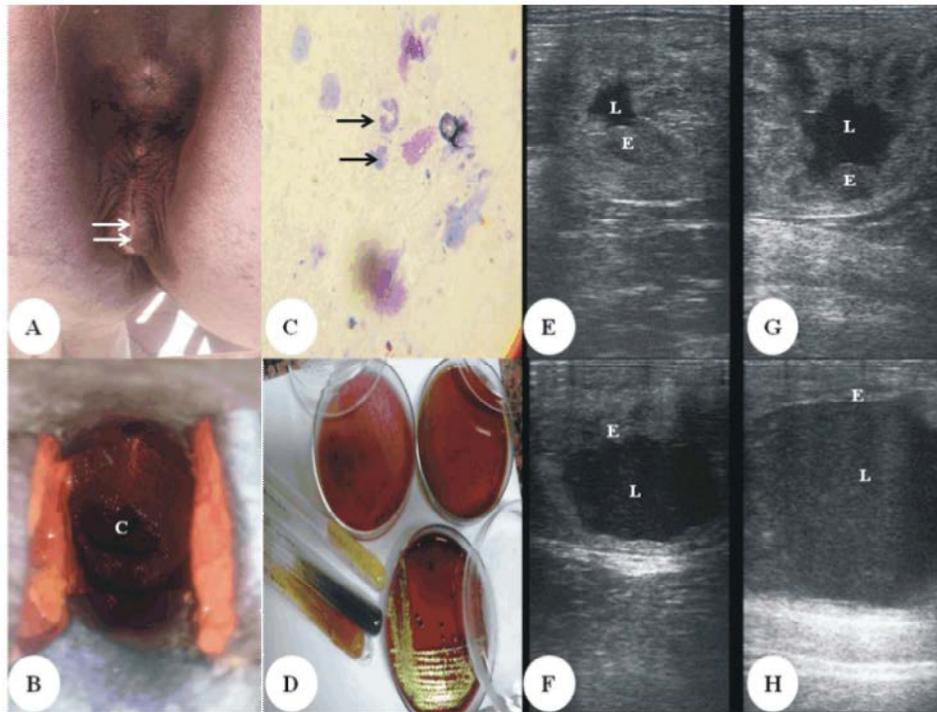


Fig. 1: Representative photographs of diagnostic techniques for evaluation of endometritis in Arabian mares. A) Physical examination showed leakage of abnormal vulvar discharge (Arrows). B) Vaginoscopy showed sever vaginitis and cervicitis (C). C) Endometrial cytology smears stained with Field stain and photographed at x400 magnification. D) E.coli colonies on Macconkey agar, XLD agar and EMB agar with biochemical tests; Citrate -ve, Urease -ve and TSI. E-H) Ultrasound images of endometritis of 1<sup>st</sup> (E), 2<sup>nd</sup> (F), 3<sup>rd</sup> (G) and 4<sup>th</sup> (H) degree. Note the presence of an intra-uterine accumulation of an echogenic fluid inside the uterine lumen (L) surrounded with a hypoechoic thickend endometrial lining (E)

breeding, E.A.O., Cairo, Egypt. Mares were subjected to the same conditions of management. They were fed as sweet clover (25 kg) and barley (6 kg) during winter (Green season) and hay (6 kg) and barley (6 kg) during summer (Dry season). A total number of 43 mares, with mean body weight (450 kg) and age (From 3 to 23 years), were selected according to their breeding history and thorough clinical and gynaecological examination.

## Methods

**Physical and Vaginal Speculum Examination:** General physical examination involved the inspection of the axis of the vertebral column as well as vulvar conformation (Figure 1A).

Vaginal speculum examination was achieved with a reusable well lubricated fiberglass speculum provided with light source. The entire vestibule, vagina and external cervical opening were evaluated according to Brinsko *et al.* [8]. The cervix and vagina were examined for their

appearance in relation to cyclic state (Moist, pink and relaxed in estrus; dry, pale and tight in diestrus) and for signs of injury, incompetence, discharge and /or vaginal urine pooling (Figure 1B).

**Ultrasonographic Examination:** The mare's reproductive tract was examined via transrectal ultrasonography (Real-time B-mode scanner, Esaote Aquila vet pro, Netherlands and equipped with 6.0/8.0 MHz transrectal linear array transducer) in accordance with Brinsko *et al.* [8] and Abou-El-Roos and El-Maghraby [9]. Mares were grouped with respect to preliminary ultrasonographic findings into: Group 1 (E1; n=26) had small amount of an intrauterine anechoic fluid. Group 2 (E2; n=10) had moderate intrauterine slightly echogenic fluid. Group 3 (E3; n=4) had moderate intrauterine echogenic fluid. Group 4 (E4; n=3) suffered from pyometra and were characterized by the presence of huge amounts of an intrauterine echogenic fluid. The mean gray value was measured as a

quantified indicator of the changes in the echogenicity of the endometrium and the intra-uterine fluid using the built in measuring system of the ultrasound device.

**Cytological Diagnosis:** It was performed through examination of smears prepared from endometrial swabs and stained with Field stain (BioLab Diagnostics; Part A: methylene blue for 15 sec. and Part B: Eosin for 5 sec.). Mares which had more than 1-2 neutrophils (PMN) per 5 high power microscope (400x) fields were classified as having an active inflammation in the uterine wall (Figure 1C).

**Bacteriological Examination:** Uterine swabs were collected from mares during early estrus, when the cervix was relaxed, moist and easing the swab's passage according to the method of Sertich [3]. Briefly, a double-guarded swab (Minitube® Company, Germany) was guided into the vagina, placed in the cervical lumen and further proceeded into the uterine lumen where the swab was exposed. The swab was left in the uterine lumen for 30-60 seconds to absorb uterine contents and was retracted back into the guard prior to removing the swab from the uterus. The swab samples were placed in transporting media (Nutrient broth) for microbiological examination according to the method described previously [4, 8].

**Statistical Analysis:** Data are presented as mean  $\pm$  standard error of mean (SEM) and analyzed using simple one-way analysis of variance (ANOVA) and post-hoc multiple comparisons with LSD using Statistical Analysis System (SPSS Ver. 16). P value was set at 0.05 or less for statistical significance.

## RESULTS

Mares under the conditions of this investigation showed a highly significant ( $P < 0.001$ ) impact of age on the occurrence of various grades of endometritis, with the occurrence of severe grade (E4) in mares elder than 18 years. On the other hand, milder forms (E1, E2 and E3) of the uterine inflammation did not reveal significant differences in its incidence among mares below 15 years old (Table 1).

**Physical and Vaginal Examination:** The incidence of swayback, an excessive downward curvature of the spinal vertebral column in the dorsal region, in the investigated mares was 13.95%. The mares with swayback and suffered

from different grades of endometritis was 3.80%, 10.00%, 25.00% and 100.00% in E1, E2, E3 and E4, respectively.

The incidence of abnormal perineal and vulvar conformation as a predisposing factor for endometritis was 16.00%. Its incident rate was 7.69%, 10.00%, 25.00% and 100.00% in mares with E1, E2, E3 and E4 grades of endometritis, respectively.

Vaginal speculum examination of the cervix (Figure 1B) revealed that the incidence pneumovagina, urovagina, vaginal varicose veins and incompletely closed cervix was 13.65%, 4.65%, 2.32 % and 4.65%, respectively in mares with uterine wall inflammation.

**Cytological Diagnosis of Endometritis in Arabian Mares:** Cytological examination of uterine swabs revealed that the incidence rate of varying grades of endometritis (E1~ 4) was 55.81, 23.25, 9.30 and 6.98%, respectively. The mean number of neutrophils in the cytological examination was significantly different in smears prepared from different grades (E1-E4);  $3.79 \pm 0.22$ ,  $4.90 \pm 0.32$ ,  $6.50 \pm 0.65$  and  $9.66 \pm 0.33$  per high-power field (HPF), respectively (Table 1).

**Ultrasonographic Examination of Mare Endometritis:** Ultrasound inspection of uterine inflammation showed the presence of an echogenic intra-uterine fluid in 41 out of 43 cases (95.34 %), which is considered as an indicator of clinical inflammatory condition of the uterine wall (Fig. 1E-H) but the other 2 cases (4.66%) represent subclinical uterine inflammation. Ultrasonography revealed that the rate of endometritis grade 1, 2, 3 and 4 was, 60.47, 23.25, 9.30 and 6.97%, respectively (Table 1).

Grading of the uterine inflammatory fluid echo-pattern by grayscale echogenicity revealed that the mean uterine fluid echogenicity  $1.38 \pm 0.15$ ,  $2.80 \pm 0.13$ ,  $3.80 \pm 0.20$  and  $4.67 \pm 0.33$  in cases of endometritis grade E1, E2, E3 and E4, respectively (Table 1). These results indicated a highly significant ( $P < 0.0001$ ) difference in the inflammatory fluid echo-pattern among different grades of endometritis in mares.

The mean uterine horn diameter was clearly ( $P < 0.001$ ) different in mares suffered from endometritis. The diameter was  $6.35 \pm 0.21$  cm,  $7.28 \pm 0.17$  cm,  $7.75 \pm 0.48$  cm and  $7.50 \pm 0.29$  cm in grade E1, E2, E3 and E4, respectively (Table 1).

The mean diameter of the uterine lumen was considerably ( $P < 0.001$ ) different between various groups. It was  $1.27 \pm 0.15$  cm,  $2.66 \pm 0.28$  cm,  $4.43 \pm 0.63$  cm and  $4.67 \pm 0.67$  cm in mares showed endometritis grade E1, E2, E3 and E4, respectively (Table 1).

Table 1: Cytological and ultrasonographic findings in Arabian mares with endometritis

Item	Grades of endometritis					
	E1	E2	E3	E4		
Number of animals graded by ultrasound	26 (60.47%)	10(23.25%)	4(9.30%)	3 (6.98%)		
Age (years)	10.35±0.64 <sup>b</sup>	11.90±0.96 <sup>b</sup>	15.33±3.18 <sup>ab</sup>	18.67±1.20 <sup>a</sup>		
Cytological findings	Incidence of neutrophils in UCE	n	24	10	4	3
		Incidence	55.81%	23.25%	9.30%	6.98%
	Number of cells/HPF	Mean	3.79±0.22 <sup>c</sup>	4.90±0.32 <sup>b</sup>	6.50±0.65 <sup>b</sup>	9.66±0.33 <sup>a</sup>
		Range	(2-6)	(3-6)	(5-8)	(9-10)
Ultrasonographic findings	Presence of uterine fluid		+ve	+ve	+ve	+ve
			(Except for 2 cases)			
	Uterine fluid echogenicity	Mean	1.38±0.15 <sup>c</sup>	2.80±0.13 <sup>b</sup>	3.80±0.20 <sup>a</sup>	4.67±0.33 <sup>a</sup>
		Range	(0.00 – 2.00)	(2.00 – 3.00)	(3.00 – 4.00)	(4.00 – 5.00)
	Uterine horn diameter (cm)	Mean	6.35±0.21 <sup>b</sup>	7.28±0.17 <sup>ab</sup>	7.75±0.48 <sup>a</sup>	7.50±0.29 <sup>ab</sup>
		Range	(4.00 – 9.00)	(6.50 – 8.00)	(7.00 – 9.00)	(7.00 – 8.00)
	Uterine lumen diameter (cm)	Mean	1.27±0.15 <sup>c</sup>	2.66±0.28 <sup>b</sup>	4.43±0.63 <sup>a</sup>	4.67±0.67 <sup>a</sup>
		Range	(0.40 – 3.00)	(1.00 – 4.00)	(3.00 – 6.00)	(4.00-6.00)
	Endometrial thickness(mm)	Mean	2.14±0.13	2.24±0.13	2.40±0.25	2.50±0.29
		Range	(1.00 – 4.00)	(1.50 – 3.00)	(2.00 – 3.00)	(2.00 – 3.00)
Endometrial wall echogenicity	Mean	3.00±0.14 <sup>b</sup>	3.80±0.13 <sup>a</sup>	4.75±0.25 <sup>a</sup>	4.67±0.33 <sup>a</sup>	
	Range	(2.00 – 5.00)	(3.00 – 4.00)	(4.00 – 5.00)	(4.00 – 5.00)	

UTC: uterine cytological examination. HPF: high-power field. Values with different superscripts within the same row were significantly different at P<0.05.

Table 2: Distribution of bacterial infection in mares with endometritis detected by means of direct smear

Incidence (%)	Single bacterial infection		Mixed bacterial infection	Absence of bacterial infection
	Gram +ve	Gram -ve		
23.25	44.10	30.23	2.33	
(n=10)	(n=19)	(n=13)	(n=1)	

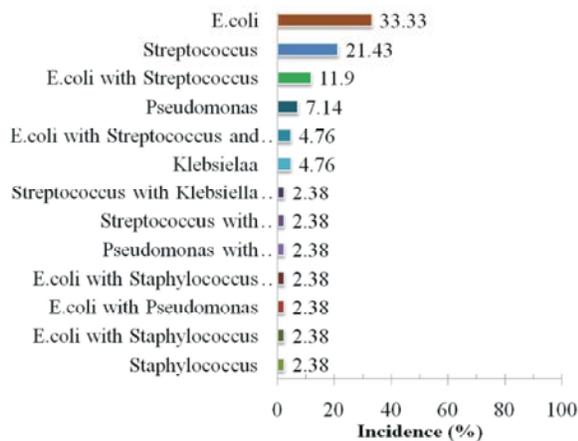


Fig. 2: The distribution of various bacterial colonies in mares with endometritis

The mean uterine endometrial thickness did not show statistical significance between mares with endometritis, though numerical differences were clearly stated. It was 2.14±0.13 cm, 2.24±0.13 cm, 2.40±0.25 cm, 2.50±0.29 cm, in E1, E2, E3 and E4 endometritis, respectively (Table 1).

The endometrial wall echogenicity was tested by grayscale echogenicity and displayed in table 1. It exhibited a highly significant (P<0.001) variation between different grades of endometritis. The echo-pattern of endometrial wall in grade E1, E2 and E3 was not significantly different, but were higher (P<0.05) in echogenicity than mild grade (E1) of endometritis (Table 1).

### Bacteriological Diagnosis of Endometritis in Arabian Mares

**Direct Smear:** Distribution of bacterial infection in mares with endometritis detected by means of direct smear was presented in Table 2. The incidence of bacterial infection detected by means of direct smear was 100% among the investigated cases. Endometrial infections with either Gram positive or Gram negative bacteria were 23.25 % and 44.10 %, respectively. On the other hand, the mixed bacterial infection was detected in 30.23 % of the examined cases. The freeing of direct smear form bacterial infection was detected in 2.33% of the cases.

**Bacterial Colony Isolation:** Results of bacterial incidence in the microbiological culture from uterine swabs is shown in Figure 2. The most isolated microorganisms from bacterial culture were in mares uterine inflammation were observed for *E.coli* (33.33%) and streptococcus (21.43%). On the other hand, mixed colonies were isolated from infected uterine swab at lower incidence, with the highest rate was for *E.coli* plus Streptococcus (11.90%) followed by *E.coli* with Streptococcus and Staphylococcus (4.76%). A representative *E.coli* colonies on MacConkey agar, xylose lysine deoxycholate agar (XLD agar) and eosin methylene blue (EMB) agar with biochemical tests (Citrate -ve, Urease -ve and TSI) was shown in Fig. 1D.

## DISCUSSION

Having a healthy mare is the first step in raising a healthy foal. If the mare is not in good health, her reproductive system is unlikely to perform optimally. The ability to maintain an environment in the uterine lumen that is compatible with embryonic and fetal life is a major factor governing reproductive efficiency in mares.

From the present study, it has been found that an advancement of age was associated with increasing the severity of endometritis. Aging was a significant predisposing factor for endometritis ( $P < 0.001$ ) as the older mares suffered from the severe grade of endometritis. The decline in fertility with age has been found to be associated with ovarian and uterine factors and these increased the susceptibility to post-coital endometritis [10] chronic progressive degenerative changes in the endometrium [11].

The most critical component in preventing endometritis is to accelerate the physical clearance of uterine contents after foaling or mating. In the current findings, swayback and abnormal vulvar conformation (Incidence rate 13.95% and 16.00%, respectively) were correlated with the endometritis grades and the accumulated intrauterine fluid. The former condition was associated with pendulous uterus being at a level lower than the cervical axis. A pendulous uterus or incompetent cervix or degenerative changes that decreased uterine contractility, vascular elastosis or lymphangectasia predispose to the occurrence of endometritis in mares [12]. Newcombe [13] and Derbala [14] stated that the loss of the structural support of the caudal portion of the reproductive tract and stretching of the broad ligaments from repeated pregnancies result in the tilting of the uterus caudally and its drop ventrally in the abdomen, hinder the evacuation of uterine fluid and leads to

persistent uterine infection. In the meantime, former studies displayed a high predisposition of poor perineal and/or abnormal vulvar conformation towards endometritis in mares due to the defect in the barrier function causing air, feces and urine to enter the reproductive tract [7].

Vaginal speculum examination, a routine component of a mare reproductive evaluation, is of value in the detection of mucoid or purulent exudates, urine accumulation or inflammation of the reproductive tract [15]. In the present study, the commonest vaginal abnormalities in mares had uterine inflammation were pneumovagina (13.65%), urovagina (4.65%) and vaginal varicose veins (2.32). Newcombe [13] showed that the pneumovagina can lead to aspiration of air and debris into the genital tract causing pneumometra and continuing insult coupled with the inability to overcome infections results in chronic endometritis. Griggers *et al.* [16] stated that the vesicovaginal reflux (Urine pooling), a relatively common cause of subfertility/infertility, is seen predominantly in older multiparous broodmares. In urine pooling, the urinary osmolarity and pH markedly reduce spermatozoa motility and may prevent the spermatozoa from reaching the oviduct results in vaginitis, cervicitis and endometritis [17]. Pycock and Ricketts [18] reported that older maiden or multiparous mares have an abnormally tight cervix due to fibrosis or cervical lacerations. Once the tight cervix fails to relax properly during estrus, fluid is unable to drain, accumulates in the uterine lumen and increases the susceptibility to post-breeding endometritis.

Results presented in this study showed that the cytological examination of mares diagnosed to have varying grades of endometritis were positive in all mares except two mares belongs to the first grade that were bacteriologically positive. The number of neutrophils/hpf was concomitant with the grades of endometritis. These results were in complete agreement with Overbeck *et al.* [4] showed that the isolation of pathogens was not associated with positive cytological findings. Besides, Causey [19] reported that, if the examination was negative for neutrophils and the isolation was positive for organisms on culture, this indicated a contaminated swab and should be regarded as a negative culture. Riddle *et al.* [5] and Brinsko *et al.* [8] showed that the number of neutrophils increased with severity of endometritis as the normal cytology sample should contain less than 1 polymorphonuclear leukocytes (PMN)/hpf, while 1-2 PMN/hpf, 3-4 PMN/hpf and 5-6 PMN/hpf indicates mild, moderate and severe inflammation, respectively.

An accumulation of fluid inside the uterine lumen is very typical of mares suffering from endometritis. The amount of fluid varies according to the time of examination, stage of the cycle as well as individuality. In our study, the examination of mares using ultrasonography revealed that the highest incidence of endometritis was faced in E1 (60.47%), while the lowest was in E4 (6.97%). This might be attributed to the system of management, animal care after foaling, early diagnosis and treatment of uterine infection [20] which decreased the incidence of severe degree of endometritis and consequently veterinarian consultation and cases recordings. Fluid accumulations found during estrus may be physiologic, but may also be an early indication of endometritis in many cases [21]. The presence of an intrauterine fluid during diestrus is indicative of inflammation and is associated with subfertility related to early embryonic death and shortened luteal phase.

In the presented study the uterine fluid echogenicity increased with the grades of endometritis. This relation agree with former research by McKinnon *et al.* [22] and Pycock [23] graded the intra-luminal uterine fluid (Graded I to IV) according to the degree of echogenicity i.e. the more echoic the fluid, the more likely the fluid is contaminated with debris including white blood cells.

The amount of intrauterine fluid in the presented study was correlated with the grades of endometritis and this was in accordance with Brinsko *et al.* [24] who reported that the presence of > 2 cm depth of fluid during estrus was a predictor of susceptibility, in contrast of the small volume (< 2 cm) of intrauterine fluid during estrus didn't affect pregnancy rate. Additionally, Brinsko *et al.* [8] stated that the existence of an anechoic fluid of large volume (i.e. 1-3 cm or greater) during estrus or during the immediate post-ovulation period should be considered abnormal and could possibly affect fertility.

In the current study, the endometrial thickness didn't reveal a great significance difference with various grades of endometritis. This could be attributed to the difference of the day of examination in relation to estrus, in association with the changes in the circulating estrogen and progesterone concentrations [25]. Moreover, in mares suffered from endometritis, the degree of uterine thickening may be masked in instances of an excessive fluid accumulation and distension, but it is appreciated when the uterus was drained [21]. In our study, we checked the mares for endometritis irrespective of the time in relation to heat onset. Nonetheless, Brinsko *et al.* [8] and Abdel-Raziek [20] stated that a slight thickening of the uterine wall may be detected in some mares with acute endometritis.

In this study, the endometrial wall echogenicity increased with the severity of endometritis. This could be attributed to the existence of pathological fluid found in the uterus, filled the space between the uterine folds lead to blurry the layers adjacent to the folds especially in diestrus. Such fluid consisted of cellular particles of varying echogenicity lead to an increase in the endometrium and/or uterine wall echogenicity [9]. Causey [19] stated that endometritis caused the endometrial wall damaged, scarred, ulcerated, degenerated and dilatation of the capillary spaces between the folds. All of these increased the echogenicity of the endometrial wall with endometritis.

Uterine infections have long been recognized as one of the major causes of reduced fertility in mares [26]. In the present study, upon using bacteriological isolation of causative agents in cases of endometritis, we found that many micro-organisms were accused in inducing the inflammation, which were *E.coli* (33.33%), *Streptococcus zooepidemicus* (21.43), *Pseudomonas aeruginosa* (7.14%), *Klebsiela pneumonia* (4.76), *Staphylococcus aureus* (2.38), *Actinobacter* and *Corynebacterium*. According to Abdel-Raziek [20] *E.coli* (37.76%), *Streptococcus zooepidemicus* (15.31), *Staphylococcus aureus* (15.31%), *Klebsiela* (9.18%), *Pseudomonas aeruginosa* (4.08%), *Proteus* (4.08%), *Salmonella* (3.06%) and *Corynebacterium* (1.02%) as the most isolated micro-organisms from mares with endometritis. Fadel *et al.* [27] noted that *Pseudomonas aeruginosa*, *Streptococcus zooepidemicus*, *E.coli* and *Citrobacter Freundi* were the most isolated micro-organisms from mares with endometritis. The difference in the isolation rate as well as the types of bacteria, perhaps due to the difference in the season of the year, which impacts on the surveillance of micro-organisms as well as the body defense mechanisms in Arabian mares. Besides, its influence on the clearance of the organisms from the reproductive tract.

Our data indicated that *E.coli* was the micro-organism most frequently associated with infertility problems in the mare and that *Streptococcus zooepidemicus* came the second most frequent. These findings are in complete agreement with Abdel-Raziek [20] and Albihn *et al.* [28]. Do-Yeon Kwon *et al.* [29] reported that the most common isolate in mare endometritis was *E.coli* (38.5%) followed by *Klebsiela pneumonia* (7.7%), *Streptococcus zooepidemicus* (6.6%). On the other hand, Derbala [14] reported that the most frequently isolate micro-organisms from culture swab were *Staphylococcus aureus* firstly, *E. coli* secondly followed by *Streptococcus zooepidemicus*. We cannot give a causal explanation for this dominance of *E.coli* in the present study. This

difference in bacterial isolates might be attributed to environmental factors e.g. season, temperature and hygiene [14].

### CONCLUSIONS

Ultrasonography is a rapid, reliable field diagnostic technique for many reproductive problems in mares with special emphasis to the uterine inflammatory condition. When it is combined with other investigative methods, e.g. endometrial cytology, isolation of the causative bacteria and antibiotic sensitivity tests will eminently increased its accuracy of detection and consequently the manipulation decisions in Arabian mares.

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