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Diagnosis of Endometritis in Arabian Mares Using Ultrasonography and Microbial Isolation with Lipid Peroxidation and Antioxidant Assessment

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Abstract

THE present study aimed to diagnose mare endometritis using ultrasonography, microbial isolation and identification, in addition to blood biochemical characteristics. The study was carried out on a total of 22 Arabian mares divided into 2 groups: normal uterus (n=5) and endometritis (n=17) groups. Depending on age, mares were classified into 2 groups (≤ 10 years and > 10 years). Mares were subjected to ultrasonographic examination to determine uterine wall thickness, edema, and echogenicity. Endometrial samples (swabs) were collected and cultured for microbial isolation and identification. Blood samples were collected, and serum was separated and analyzed to determine the levels of total antioxidant capacity (TAC), nitric oxide (NO), malondialdehyde (MDA), and interleukin-6 (IL-6). The results revealed an increased incidence of endometritis ($P < 0.05$) in older mares (> 10 years, 85.7%) compared to young ones (≤ 10 years, 37.5%). Endometritis increased the uterine wall thickness, uterine wall echogenicity, and intrauterine fluid ($P < 0.000$) as well as TAC ($P < 0.05$). In microbial isolation, the most frequently isolated bacteria were α-hemolytic Streptococcus and E. coli (23.8%). Two fungi, including Aspergillus flavus (2.44 %) and *Candida albicans* (9.76 %), were isolated from some cases. It could be concluded that endometritis in mares is affected by the age of the mare, and is associated with increased uterine wall thickness, uterine wall echogenicity, intrauterine fluid, as well as serum levels of TAC. The most common microbial causes of endometritis are α-hemolytic streptococcus and Escherichia coli (E. coli) bacteria, in addition to Candida species and Aspergillus fungi.

Keywords: Arabian Mares, Endometritis, Ultrasonography, Microbial isolation, Biochemical parameters.

Introduction

In broodmares, endometritis is a major cause of failure to conceive and embryonic loss [1]. Among 1149 equine practitioners surveyed, endometritis was ranked the third most common medical problem for adult horses [2]. There are several clinical signs of endometritis, including intrauterine fluid, excessive or unusual patterns of endometrial edema, vaginitis, vaginal discharge, abnormal estrous cycles, and cervicitis [3,4]. When ultrasound is used during estrus, susceptible mares are defined as those with a depth of intraluminal fluid greater than 2 cm [5]. It can be challenging to diagnose subclinical conditions with hidden clinical signs, such as the absence of intrauterine fluid [6]. In mares, 25%–60% of uterine infections are caused by bacteria [7]. Most commonly, anaerobic streptococci, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae have been isolated from the uterus [8]. Clinical manifestations can differ based on the chronicity of endometritis and the bacterial species involved. For instance, Escherichia coli has a strong adherence to the endometrial epithelium, which can hinder its detection by making it more difficult to recover during endometrial sampling for microbiological [9]. Uterine fungal infections are less common than bacterial infections

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and are caused by fungi such as Candida spp. and Aspergillus spp. [10].

The potential use of indirect diagnostic markers that eliminate the need for uterine sampling has been investigated in mares with endometritis [11,12]. This includes assessing oxidative stress (OS) parameters in blood serum, such as malondialdehyde (MDA), total antioxidant capacity (TAC), and nitric oxide metabolites (NOMs) [11]. TAC is commonly used to assess the antioxidant status of biological samples and to evaluate the antioxidant response to free radicals generated in various diseases **[**13]. NO is an important smooth muscle relaxation mediator in the different organs, including the uterus [14]. It is formed from L-arginine by various isoforms of nitric oxide synthase (NOS). The Inducible NOS (iNOS) is usually expressed at sites of inflammation and produces large quantities of NO [15]. MDA is a lipid peroxidation marker formed as a relatively stable degradation product of oxidized lipids and can be used as an oxidative stress indicator [16].). Interleukin-6 (IL-6) is a pleiotropic cytokine produced by various types of immune and non-immune cells as well as skeletal and smooth muscle cells [17]. IL-6 plays a vital role in acquired immunity by stimulating antibody production and T-cell development. Additionally, it promotes differentiation or proliferation of several non-immune cells [18].

The Levels of nitric oxide metabolites (NOx) and malondialdehyde (MDA) were significantly elevated ($p < 0.05$) in mares that tested positive for endometritis via cytology compared to cytologically negative mares. In contrast, total antioxidant capacity (TAC) exhibited an opposite trend, showing lower values in the endometritis-positive group [19]. The serum concentrations of IL-6 were remarkably increased in mares with endometritis compared with healthy mares with endometritis compared with healthy mares [20]. Serum concentrations of IL-6 showed a significant increase in subclinical and clinical endometritis in cows [21].

The present study aims to diagnose endometritis in Arabian mares using ultrasonography, microbial isolation and identification, in addition to the blood biochemical characteristics (TAC, NO, MDA, and IL-6).

Material and Methods

The present study was carried out on a total of 22 Arabian mares, aged between 3 and 20 years, divided into two groups: a control group with a normal, healthy uterus (n = 5) and an infertile group with 2-3 unsuccessful cycles of no conception (n = 17). All mares appeared healthy with good body condition scores. Mares were kept indoors, and sufficient balanced food and water were given to each mare separately. All mares were examined, and sample collection was performed during the estrus phase. Depending on the age, mares were classified into 2 groups (≤ 10 years and > 10 years).

Ultrasound scanning

Mares underwent a transrectal ultrasound examination using a digital ultrasound (Sonoscape E1 with 5-7.5MHz linear transducer, China). According to [22], the mare was completely secured, feces were evacuated from the rectum, and a gel-lubricated transducer was inserted over the cervix and body of the uterus until the bifurcation of the uterus, the tip of one uterine horn, and the ovary. The ultrasound examination determines the echogenicity of the uterine wall and the presence of intrauterine edema (Fig. 1). Mares were considered positive for endometrial inflammation when they presented excessive edema that was not compatible with the stage of the cycle [23]. The presence of intra-uterine fluid (IUF) >2 cm during estrus has been suggested to indicate mare susceptibility to endometritis [5]. In the present study, uterine ultrasound parameters, including intra-uterine fluid (IUF), uterine wall thickness (UWT), and uterine wall echogenicity (uterine wall mean gray value, UWMGV), were accomplished using NIH Image J (National Institutes of Health, Bethesda, Maryland, USA), according to [24].

Swab Collection and Culture

Swab sample collection was performed according to [25]. Briefly, the mares were restrained in stock with their tails bandaged, and the perineal area was cleaned several times with water and povidone-iodine (Betadine®) and dried with paper towels. A lubricated double-guarded uterine swab was inserted through the vagina and cervix. The swab was placed in contact with the uterine wall, rolled clockwise on the endometrium for 15 seconds, and kept there for another 15 seconds. After that, the swab was retracted into the sheath, removed from the mare, and placed in transport media (brain heart infusion media).

According to [26], swaps were incubated at 37°C for 18-24 hours, then cultured on 3 different types of bacterial isolation media, including EDward, Mannitol salt agar, and EMB (eosin methylene blue), and fungal isolation medium, Sabouraud dextrose agar (SDA) containing 0.05mg/ml Chloramphenicol and HiCrome Candida Differential Agar (HIMEDIUM, India) (Fig. 2). Bacterial growth on the plates was assessed and identified over 24-48 h incubation at 37 °C. The plates without visible growth were re-incubated under the same conditions and re-examined after an additional 24 h [27]. The hemolytic behavior and morphological appearance of colonies were examined microscopically in a Gram-stained film, and the suspicious colonies were then transferred to semisolid agar for further identification [28]. The emergence of two or more colonies of any microbial species was considered indicative of an

existing uterine infection. Plates of Sabouraud dextrose agar were incubated at 27°C, and the fungal growth was assessed and identified after 24 hours of incubation. The plates without fungal growth were reexamined four days later at 27°C [28].

Blood sampling

Blood samples (10 ml) were collected from the jugular vein using an 18-gauge needle in 10 ml sterile plain vacutainer® tubes. According to [12], samples were centrifuged at 2000 rpm for 20 min, and serum samples were separated and stored at -80°C until being analyzed.

Biochemical analysis

Serum from different animals was analyzed spectrophotometrically. The levels of NO (NO kits, TA 25 33, Egypt) and TAC (TAC kits, TA 25 13, Egypt) were assessed according to [11].The MDA (MDA kits, MD 25 29, Egypt) and IL-6 (R&D systems-Quantikine ®Equine IL-6 ELISA Kit) levels were measured according to [12] and [29], respectively.

Statistical analysis

The incidence of endometritis in different ages was calculated as a percentage of mares with endometritis from the total number of mares within the same age range. The statistical difference was determined using the Chi-square test.

The results of uterine thickness, uterine edema, and uterine echogenicity, as well as TAC, MDA, NO, and IL-6 levels, are presented as Mean ± SEM, using SPSS software (IBM® SPSS® Statistics Version 25). The statistical difference between the groups was set at $P \leq 0.05$ and determined using an independent-sample t-test.

Results

The effect of age on the incidence of endometritis

As shown in Table 1, Fig. 3, the incidence of endometritis was higher ($P< 0.05$) in the old mares (> 10 years) compared to mares of young age (≤ 10 years) (85.7 %, 12/14, 37.5 %, 3/8, respectively).

Effect of endometritis on uterine wall thickness (UWT), Uterine wall echogenicity (UWMGV), and intrauterine fluid (IUF)

Mares with endometritis showed a significant ($P < 0.000$) increase in the uterine wall thickness, Uterine wall echogenicity, as well as intrauterine fluid (Table 2) compared to the mares with the normal uterus.

Effect of endometritis on serum levels of antioxidants, Lipid peroxidation, and IL-6

As presented in Table 3, endometritis increased ($P < 0.05$) the serum level of TAC compared to that in normal mares.

The isolated microorganisms are shown in Table 4. The majority of bacterial endometritis was caused by *E. coli* (24.4%), α-hemolytic *Streptococcus spp*. (22 %), and β-hemolytic *Streptococcus spp.* (17 %). Two fungi, including *Aspergillus flavus* (2.44 %) and *Candida albicans* (9.76 %), were isolated from some cases

Discussion

Endometritis is one of the main causes of pregnancy failure and embryonic loss in mares [1]. The diagnosis of endometritis and effective treatment can improve the mare's fertility and increase the chances of pregnancy [30]. A thorough reproductive history, ultrasonography, and uterine sampling are usually necessary for diagnosing endometritis [31].

Age-related reduced fertility is an important factor observed in clinical assistance in mares [32]. Reproductive performance has been reported to be lower in older mares than in younger ones [33]. Moreover, age was reported to be one of the main factors contributing to subfertility in well-managed mares [32]. The present study revealed an increased incidence of endometritis with increased age, which is coordinated with that reported by [6,34].The subfertility in older mares has been attributed to the physiological and anatomical changes that occur with increasing age, including reduced uterine tone and uterine clearance, altered conformation of the reproductive tract, and degenerative changes in the endometrium, which lead to an increased susceptibility to endometritis [22,35].

In the present study, the presence of intrauterine fluid (IUF) with a size of ≥2 cm was considered an indicator of endometritis depending on the findings reported by [9].The present results revealed increased uterine wall thickness, uterine wall echogenicity, and intrauterine fluid, which is coordinated with those reported by [36] in mare, [37] in queen, and [38] in bitch.

The diagnosis of mare endometritis by using indirect markers in blood serum, such as malondialdehyde (MDA), total antioxidant capacity (TAC), and nitric oxide metabolites (NOMs). was recently applied [11, 12] In this study, the TAC increased in serum samples from mares with endometritis compared to clinically normal ones. However, [11], recorded a significant decrease in serum TAC in mares with endometritis. Moreover, [39] reported the decreased level of TAC in uterine lavage samples from mares with endometritis. The discrepancy in the results, especially with [39], may be attributed to the measurement of TAC in serum instead of uterine lavage. In this study, the higher persistent TAC levels in serum could be a host response to the highly propagating oxidative stress or a compensatory mechanism for depleted antioxidant components [40].

Higher levels of NO have been recorded in mares with endometritis compared to healthy mares [11].However, in the present study, serum nitric oxide (NO) levels did not differ between mares with endometritis and those with a normal reproductive tract. It may be attributed to the rapid consumption of NO by superoxide anions, which are released by activated neutrophils and form peroxynitrite [41].

In the present study, the level of lipid peroxidation marker (MDA) did not differ between mares with endometritis and those with a normal reproductive tract. On the other hand, MDA levels in plasma [42] and serum [12] were reported to be significantly increased in mares with endometritis. It has been stated that certain inflammatory reactions are not strongly associated with oxidative stress, and the effective antioxidant mechanisms balance the production of free radicals, resulting in stable MDA levels [43].

IL-6 has been reported to promote the proliferation of several non-immune cells in addition to stimulating antibody production and T-cell development [18]. In the present study, serum IL-6 values did not differ between mares with endometritis and those with a normal reproductive tract. On the other hand, [44] recorded an increased level of serum IL-6 in mares with endometritis compared to the controls. In addition, increased levels of IL-6 were found in uterine lavage samples from mares with endometritis compared to clinically healthy mares [20,39]. This discrepancy of the results may be attributed to the fact that IL-6 remains unchanged in some inflammatory states, as its release is transient, peaks early, and declines even if inflammation persists [45]. In addition, the inflammatory response is mainly driven by other cytokines [46].

Although endometritis can be triggered by various factors, such as pneumovagina, urine accumulation, or retention of semen in the uterine lumen, bacterial infection, especially by aerobic microorganisms, remains the most common and important etiological factor [7].In this study, the most frequently isolated bacterium among our 42 isolates was α-hemolytic streptococcus and Escherichia coli (E. coli), followed by β-hemolytic streptococcus and Staphylococcus aureus. These results are coordinated with those reported by [47]. However, β-hemolytic streptococci and E. coli were reported as the most common isolates from mare endometritis [48]. Uterine fungal infections are less common than bacterial infections, and were reported to be responsible for 1–5% of all cases of endometritis [49]. In this study, most of the fungal organisms isolated from uterine samples were Candida species, followed by Aspergillus. This pattern of fungal isolation is coordinated with that recorded by [10].

Conclusion

The incidence of mare endometritis is affected by the age of the mare. Endometritis increased the uterine wall thickness, uterine wall echogenicity, and intrauterine fluid, and is associated with increased serum levels of TAC. The most common bacterial cause of endometritis was α-hemolytic streptococcus and Escherichia coli (E. coli), followed by β-hemolytic streptococcus and Staphylococcus aureus. Fungal organisms, including Candida species and Aspergillus, were isolated from uterine samples.

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Declaration of Conflict of Interest

The authors declare no conflict of Interest

Ethical of approval

The present study was carried out in Derbala Equine Clinic, Pyramids area, Giza, Egypt, during the period from February to September 2025. All procedures of animal manipulations and sample collection were authorized by the Ethics Committee of the Faculty of Veterinary Medicine, Benha University (BUFVTM 01-08-24).

TABLE 1. Classification of the uterine health condition according to the age of mares

Average age of examined mares	Total number of examined mares	Mares with a normal uterus	Mares with endometritis
		No. (%)	No. (%)
≤ 10 years	8	5 (62.5)	3 (37.5) [b]
> 10 years	14	2 (14.3)	12 (85.7) [a]
Total	22	7 (31.82)	15 (68.18)

Values with different superscripts differed significantly at P< 0.05

TABLE 2. Measurements of uterine wall thickness (UWT), uterine wall echogenicity (UWMGV), and intrauterine fluid (IUF) in mares with a normal uterus and endometritis

Group	UWT (cm)	UWMGV	IUF (cm)
Normal uterus	1.37±0.13[b]	65.03±4.05[b]	0.32±0.04[b]
Endometritis	2.87±0.22[a]	129.19±3.54[a]	2.51±0.16[a]

Values with different superscripts within the same column differed significantly at P > 0.000

TABLE 3. Serum levels of antioxidants, Lipid peroxidation, and IL-6 in mares with normal uterus and endometritis

Group	TAC	MDA	NO	IL-6
Normal uterus	0.27±0.03[a]	8.85±0.46	305.43±5.55	1.80±0.10
Endometritis	0.88±0.14[b]	8.86±0.77	312.78±2.45	3.48±0.77

Values with different superscripts within the same column differed significantly at P < 0.05

TABLE 4. Incidence of microbial isolates (bacteria and fungi) in mares with endometritis.

Microbial isolate	No. of isolates	Percentage % of each microorganism from the total no of isolated microorganisms	% of microorganisms from the total No mares with endometritis
α-hemolytic *Streptococcus spp.*	10	23.8	58.8
β-hemolytic *Streptococcus spp.*	7	16.67	41.2
E. coli	10	23.8	58.8
Staphylococcus *aureus*	6	14.29	35.3
K. pneumoniae	3	7.14	17.6
P. aeruginosa	1	2.38	5.88
Candida albicans	4	9.5	23.5
Aspergillus flavus	1	2.38	5.88
total	42	100	

The number 42 in the table is based on the assumption that the microbial isolate is the sole causative agent, not mixed with other microorganisms.

Fig. 1. (A) Normal uterus with normal wall and echogenicity. (B) Accumulation of intrauterine fluid with increased echogenicity of the uterine wall. (C) High degree of endometrial edema with high echogenicity and increased thickness of the uterine wall.

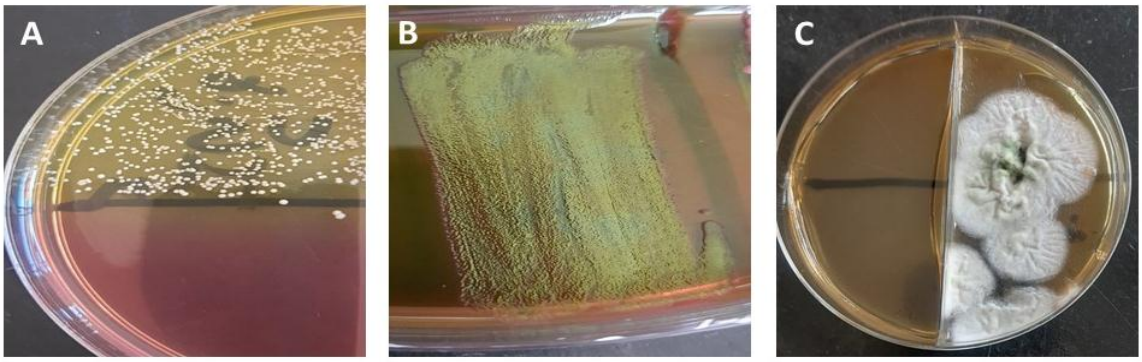

Fig. 2. (A) Streptococci on Edward's agar media. (B) Staphylococcus aureus on mannitol salt agar. (C): *Aspergillus flavus* on Sabouraud dextrose agar.

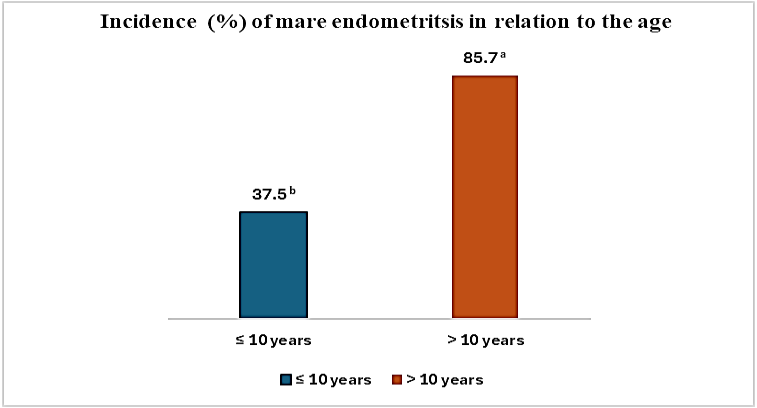

Fig. 3. The effect of age on the incidence of endometritis in the mare

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تشخيص التهاب بطانة الرحم في الأفراس العربية باستخدام التصوير بالموجات فوق الصوتية وعزل الميكروبات مع تقييم بيروكسدة الدهون ومضادات الأكسدة

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الملخص

هدفت الدراسة الحالية إلى تشخيص التهاب بطانة الرحم في الأفراس باستخدام التصوير بالموجات فوق الصوتية، وعزل وتحديد الميكروبات، بالإضافة إلى الخصائص البيوكيميائية للدم. أجريت الدراسة على إجمالي 22 فرسًا عربيًا تم تقسيمها إلى مجموعتين: مجموعة الرحم السليم (عدد=5) ومجموعة التهاب بطانة الرحم (عدد=17). وبحسب العمر، صُنفت الأفراس إلى مجموعتين (≤ 10 سنوات و 10 <سنوات). خضعت الأفراس لفحص بالموجات فوق الصوتية لتحديد سمك جدار الرحم، ووجود الوذمة، ودرجة الصدى. جُمعت عينات من بطانة الرحم (مسحات) وزُرعت لعزل وتحديد الميكروبات. كما جُمعت عينات دم، وفُصل المصل وحُلّل لتحديد مستويات السعة الكلية لمضادات الأكسدة (TAC)، وأكسيد النيتريك (NO) ، والمالونديالدهيد (MDA) ، والإنترلوكين-6.(IL-6) وعلاج المصابة منها.

الكلمات الدالة: الأفراس العربية، التهاب بطانة الرحم، التصوير بالموجات فوق الصوتية، عزل الميكروبات، المعايير البيوكيميائية.