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DIAGNOSIS OF ENDOMETRITIS USING ULTRASOUND AND LOW – VOLUME UTERINE FLUSH IN ARABIAN MARES

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ABSTRACT

*Endometritis is one of the most common causes of infertility in mares. Many mares fail to be diagnosed despite availability of many diagnostic tests. The objectives of this study were, to diagnose endometritis in Arabian mares by ultrasonography, cytological smear and microbial culture, in addition to applying of antimicrobial susceptibility test of the isolated bacteria from mares' uteri. This study conducted on 32 Arabian mares with fertility problems. All mares firstly scanned by ultrasound then sampled by low - volume uterine flush for cytological, microbial culture examinations and susceptibility test. *E. Coli* was found to be the most frequently isolated bacteria (40.54%). While, *Proteus Spp.* was the lowest frequently isolated bacteria (2.70 %). Ciprofloxacin and enrofloxacin are the highly effective antibiotics inhibiting majority of bacterial isolates. So, the low – volume uterine flush technique is a reliable, easy, quick and practical diagnostic method for endometritis in mares when combined with ultrasonography and microbiology diagnostics.*

Key words: Endometritis, Mare, Ultrasound, Uterine cytology.

INTRODUCTION

It has suggested that the equine species inherently achieves relatively low fertility and its domestication makes the situation worse. (Ricketts and Troedsson, 2007). Endometritis was ranked in a survey of 1149 veterinarians as the third most frequently occurring reproductive problem in adult horses, and the problem continues to be one of the most economically important problems in equine reproductive management as it is a major cause of subfertility in the mare (Traub-Dargatz et al., 1991 and Watson, 2000).

Endometritis is a failure of the uterus to remove foreign contaminants (e.g. bacteria, debris, dead spermatozoa, intrauterine fluid)

resulting in inflammation of the inner lining (endometrium) of the uterus (Watson, 2000). Mares with subfertility problem often their uterus retain fluid at 24 or 48 h after breeding, while fertile mares successfully clear excess fluid within 6 or 8 h after breeding (LeBlanc, 2003).

Bacterial uterine infections induce major losses in the equine breeding populations in about 25 – 60 % of barren mares. Losses can appear as failure to conceive, early foetal losses, mid-gestational abortion, placentitis, birth of septic neonate, postpartum metritis or delays in re-breeding (Traub-Dargatz et al., 1991 and LeBlanc and Causey, 2009).

Classical symptoms of endometritis may include intrauterine fluid, unusual endometrial edema, vaginitis, vaginal discharge, abnormal estrous cycles, and cervicitis. In addition, positive endometrial cytology and a positive endometrial culture. However, some or all these signs may be absent in subclinical cases (**LeBlanc and Causy, 2009**).

The highly common and frequently isolated bacteria causing bacterial endometritis are *Streptococcus equi* subsp. *zooepidemicus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (**LeBlanc, 1999; Szeredi et al., 2003 and Frontoso et al., 2008**).

Antibiotics are usually used for endometritis treatment (**Perkins, 1999**). However, the bacterial species isolated, as well as their susceptibility to antibiotics, may change over time as well as from one population of horses to another (**Sternberg, 1999**). The purpose of the present study was to study and evaluate the common techniques used for diagnosis of the subfertile mares, define the most common bacterial species inducing endometritis in problem mares, and determine the antimicrobial sensitivity pattern of isolated bacteria.

MATERIALS AND METHODS

Animals:

Thirty two Arabian mares from different small scale breeding farms in Giza province, Egypt were included in this study. Mares aged 3 – 25 years with fertility problems and had a history of previous failing to conceive after more than three consecutive breeding cycles in

one breeding season (February 2015 – October 2017). Animals were kept and raised under quite similar nutritional and managemental systems of housing, feeding, and aspects of breeding regimes.

Reproductive examination and samples collection:

1-Reproductive history and physical examination:

A full reproductive history of mares was recorded which included the age, parity, date and circumstances of last foal, purperium, regularity of estrous cycle, characters of estrous secretion, number of previous mating without conception, date of last service. The general physical examination concentrated on systemic problems and body condition.

2. Ultrasonographic examination:

Before scanning, the mares were completely restraint and secured . All mares were examined transrectally by ultrasonography (SonoScab A5, China) with 7 MHz linear transducer for examining the reproductive tract. The degree of inflammation was demonstrated by the level of endometrial oedema and the volume and characters of retained intrauterine fluid. The presence of 2 cm or more of echogenic intrauterine fluid was taken as an indicator of endometritis.

3. Low – Volume uterine flush sampling:

The low-volume uterine flush samples were collected according to **LeBlanc et al., (2007)**. The flush samples were collected using a sterile and disposable Rail's tube

(ULTRAMED Rail's tube 14 FG, Egypt) which is a feeding polyethylene tube for human patients.

4. Cytological diagnosis:

After sampling and preparing the cytological smears as described by LeBlanc et al., (2007). The cytological specimens were fixed immediately and stained within 6 hours with (DIF- QUICK III stain, VERTEX, Egypt). The smears were examined with light microscopy (Optica, 180 K, Italy) under oil immersion (1000 x). Smears were considered indicative of inflammation if there was an average of 1 - 2 neutrophils per magnification in 10 HPF fields.

5 . Bacteriological culture and identification of isolated Bacteria:

For proper bacteriological detection, samples were cultured on Nutrient agar, Sheep blood agar, MacConkey agar and EMB media. The plates were incubated aerobically for 24 – 48 h at 37 °C. The incubated plates were been examined for morphological character, pattern of hemolysis on sheep blood agar and pigment production on nutrient agar. If > 90 % of the grown colonies in plate were of one species,

the result was considered positive and pure growth in monoculture. Growth of two types of colonies in pure culture considered positive and mixed infection. Growth of more than two types of colonies considered as contaminated sample. Gram stain and biochemical tests were performed for identification of the isolated bacteria (Quinn et al., 1994 and Langoni et al., 1997).

6 . Antimicrobial susceptibility test:

The susceptibility test was done for 10 antimicrobial discs (Oxoid) commonly used for treatment of equine endometritis (Forbes et al., 1998). The interpretations of the sensitivity results were read according to National Committee for Clinical Laboratory Standards (2002).

RESULTS

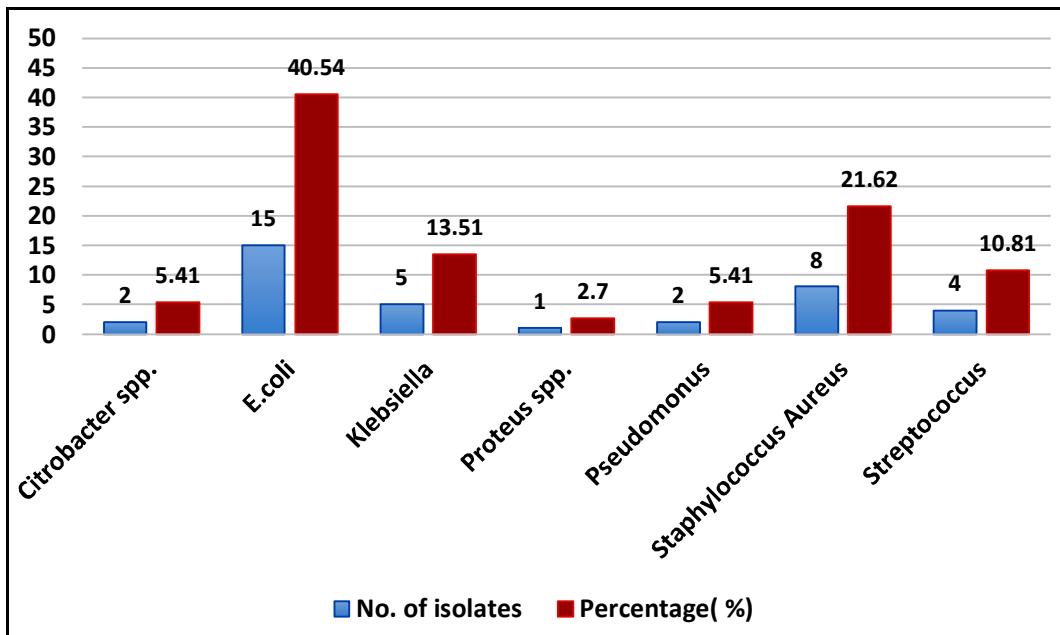
By ultrasonographical examination, 29/32 (90.63%) of mares showed endometritis. In addition, 19/32 (59.37%), 31/32 (96.87%) of mares were positive by cytological and microbial examination respectively (Table 1).

Table 1: Incidence of mares affected with endometritis by different diagnostic methods:

Diagnostic Method	Number Of Mares	Percentage %
Ultrasonography	29/32	90.63 %
Cytology	19/32	59.37 %
Microbiology	31/32	96.87 %

Types of bacteria recovered from the uterine flush samples as well as their frequency of isolation shown in (Fig 1). The results recorded positive

bacteriological culture in 96.87% (n=32). Only one uterine flush sample showed no growth 3.13% (n=32).

**Figure 1:** Number and percentage (%) of different types of bacteria isolated from the uterine flush samples (n=32)

Ciprofloxacin and enrofloxacin inhibited the majority of isolated bacteria (E. Coli, Staph. Aureus, Klebsiella, Streptococcus,

Pseudomonas, Citrobacter spp. and proteus spp.) as shown in (Table 2).

Table 2 : Show the results of antibiotic sensitivity test of isolated bacteria to 10 types of antibacterial agents:

Antibiotic Discs	E. coli			Klebsiella			Pseudomonas			Proteus			Citobacter			Staph. aureus			Streptococcus		
	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
AMC	0	100	0	0	60	4	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0
AM	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0
K	73.3	26.7	0	80	20	0	0	100	0	0	100	0	50	50	0	0	100	0	0	100	0
E	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0
SXT	80	20	0	40	60	0	0	100	0	10	0	0	50	50	0	50	50	0	0	100	0
T	46.7	53.3	0	0	100	0	0	100	0	10	0	0	100	0	0	25	50	25	100	0	0
ENR	86.7	13.3	0	100	0	0	100	0	0	10	0	0	100	0	0	75	25	0	100	0	0
CIP	100	0	0	100	0	0	100	0	0	10	0	0	100	0	0	87.5	12.5	0	100	0	0
CN	73.3	26.7	0	60	40	0	100	0	0	10	0	0	100	0	0	100	0	0	100	0	0
P	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	75	25	25	75	0

AMC: Amoxicillin Clavulanic Acid. AM: Ampicillin. K: kanamycin. E: Erythromycin. SXT: Sulphamethoxazole Trimethoprim. T: Tetracycline. ENR: Enrofloxacin. CIP: Ciprofloxacin. CN: Gentamycin. P: penicillin G. (S = Sensitive / R = Resistant / I = Intermediate)

DISCUSSION

The result of this study revealed that 31 (96.87%) mares, were suffered from bacterial endometritis, which was confirmed with the support of ultrasonography and cytology test. Therefore, bacterial endometritis still a main field problem in the mare's breeding practice. These results were mentioned by **Traub-Dargatz et al. (1991) and Nikolakopoulos and Watson (1999)**, where they revealed that bacterial endometritis is the most common cause of subfertility problem and induce major losses in the broodmare practice in 25 – 60 % of barren mares.

Due to the disadvantages of using one diagnostic technique alone for diagnosing endometritis in equine breeding practice, the diagnosis was achieved by Ultrasonography, Microbial culture and Cytology to decrease the susceptibility of occurrence of either false negative or false positive results. This come in agreement with **Riddle et al. (2007); LeBlanc and Causey (2009) and Overbeck et al. (2011)**. They hypothesized in their studies that combining diagnostic methods into reproductive examination sheet would increase confidence in diagnosing mares with endometritis. Also, they stated that both uterine culture and cytology are common diagnostic techniques for diagnosing equine endometritis through their respective detection of uterine bacterial infection and inflammatory cells (neutrophils). Using low- volume uterine flush for bacteriological and cytological examinations other than uterine swabs. This was stated by **LeBlanc et al. (2007) and Diel De Amorium, M. et al. (2016)**, where they concluded in their study that the low- volume flush technique was a rapid, sensitive and practical test for diagnosing endometritis in chronically infertile mares based on its

sensitivity and specificity estimates 0.75 % and 0.9 % respectively.

The usage of ultrasonography became an important and valuable diagnostic tool for diagnosing equine endometritis. This finding was in agreement with **Liu and Troedsson (2008)**. They found before the use of ultrasonography, it was difficult to diagnose the presence, character or the amount of the accumulated uterine fluid.

The present study includes 32 mares with fertility problems, where 31 (96.87%) of the examined mares yield positive bacteriological results. **Frontoso et al. (2008)** stated that bacterial pathogens are considered a main causes of a mare's failure to conceive to a known and fertile stallion in a well- managed breeding equine farm during one or more consecutive breeding cycle in the same breeding season. In addition, **Redaelli and Codazza (1977) and Ricketts et al. (1993)** recorded values of 30 % and 39 % of bacterial isolation respectively. While recently, **Baranski et al. (2003) and Frontoso et al. (2008)** reported values of 66.2 % and 49 % respectively. No growth was recorded in one sample (3.13 %) of flush samples examined. E.coli was found to be the dominant and most common uterine pathogen (15 isolates, 40.54 %) followed by Staph. aureus (8 isolates, 21.7 %), Klebsiella pneumoniae (5 isolates, 13.51 %), Streptococcus spp. (4 isolates, 10.18 %), Pseudomonas aruginosa and Citrobacter spp. are (2 isolates, 5.41 %) for each and lastly Proteus spp. (1 isolate, 2.70 %). However, E.coli was found to be more associated with repeat breeding endometritis without clinical signs. These findings were in agreement with results of **Albihn (1998); Albihn et al. (2003); Ghasemzadeh-nava et al. (2004); LeBlanc et al. (2007) and Kwon et al. (2012)**. They mentioned that E.coli is the overall and most frequently isolated pathogen from uteri of

repeat breeding mares and mostly accompanied by repeat breeding problems other than clinical symptoms. While, the result of this study was disagreed with the results of **Asbury and Lyhe (1993); Langoni et al. (1997); Szeredi et al. (2003) and Frontoso et al. (2008)**, where, *Streptococcus zooepidemicus* was the dominant pathogen causing bacterial endometritis in mares, followed by *E.coli*. These disagreed results may be attributed to the different study area and horse population.

Staphylococcus aureus was found to be the second isolated bacteria from mare's uteri with fertility problems (8 isolates, 21.62%). By **Shin et al. (1979) and Ricketts et al. (1993)** *Staphylococcus aureus* was found to be a rather frequently isolated bacterium from uteri of normal population mares. Also, **Frontoso et al. (2008)** mentioned that *Staph. aureus* may lead to fertility problems in mare's uterus. But, it was concluded by **Allen and Pycock (1989) and Asbury and Lyle (1993)** that *Staph. aureus* is a less common cause of equine endometritis.

Cytological diagnosis for detection of polymorphonuclear cells (PMNs) in a stained smear from the surface of the endometrium considered an easy technique for rapid diagnosis of uterine inflammation under practical field conditions (**LeBlanc et al., 2007 and Diel de Amorim, M. et al., 2016**). Cytological diagnosis yielded positive in 59.37% (n=19) and negative in 40.63 % (n=13) of suspected mares to have endometritis.

The usage of antibiotic drugs for treating mare with endometritis is a common field approach, where using these antibiotics by the conventional route of treatment became clearly unsuccessful. Therefore, the antibiotic susceptibility test was performed to pathogens isolated from the bacteriological cultures (**Albihn et al., 2003 and Frontoso et al., 2008**). The present study's findings of antibiotic sensitivity revealed that, 15 *E. coli*

isolates showed an obvious resistance to each amoxicillin/Clavulanic acid, Erythromycin, Ampicillin and penicillin G antibiotic agents. While the clear and highest sensitivity was recorded for Ciprofloxacin, Enrofloxacin and Sulphamethoxazole /Trimethoprim with 100 %, 86.7% and 80 % respectively. This result was in a complete agreement with the result of the retrospective study of **Frontoso et al. (2008)**. They found Enrofloxacin was the only of tested 10 antibiotics no resistance was recorded for *E. coli* isolates. They recorded from the 64 *E. coli* isolates of their study, 73.5 % were inhibited by Enrofloxacin. Also, *E. coli* was susceptible to Kanamycin (67.2 % isolates inhibited) and Gentamycin (73.5 % isolates inhibited). Moreover, the four isolates of hemolytic *E. coli* were highly susceptible to Enrofloxacin, Kanamycin, Gentamycin and Trimethoprim/ sulphamethoxazole and being highly resistant to Ampicillin and Penicillin G antibiotics. *Staph. aureus* susceptibility results showed that Ciprofloxacin, Enrofloxacin and Sulphamethoxazole/Trimethoprim were found the highly susceptible and effective antibacterial agents with 87.5 % and 75 % respectively. While, *Staph. aureus* was found to be highly resistant to Amoxicillin/Clavulanic acid, Ampicillin, Kanamycin, Erythromycin, Gentamycin and Penicillin G. **Frontoso et al. (2008)** reported that the 26 *Staph. aureus* isolates were inhibited, 96.2 %, 92.4 %, 92.3 % and 88.6 % by Amoxicillin/Clavulanic acid, Enrofloxacin, Gentamycin and Rifampicin respectively. The results disagreed with **Shin et al. (1979)**. They found that all streptococcal isolates were highly susceptible to β -lactam antibiotics. Also, **Albihn et al. (2003)** reported that among 31 β - haemolytic streptococcal isolates resistance was clear to Gentamycin and Oxtetracycline. While, isolates were sensitive to β -lactam antibiotics (Penicillin G and Ampicillin and also to Erythromycin and chloramphenicol). The two *Pseudomonas aeruginosa* isolates were resistant to the

majority of the tested antibacterial agents (Amoxicillin/Clavulanic acid, Ampicillin, Kanamycin, Erythromycin, Sulphamethoxazole Trimethoprim, Tetracycline and Penicillin G), except the 3 (Enrofloxacin, Ciprofloxacin and Gentamycin) antibacterial agents with 100 % for all. This result was agreed with that of **Frontoso et al. (2008)**. They recorded 14 *P. aeruginosa* isolates that were resistant to the majority of the tested antimicrobial agents except to Gentamycin. The results of the antimicrobial sensitivity test indicates that Ciprofloxacin ranked all over the 10 tested antibiotics. This was confirmed by study to **Trundell et al., (2016)**. They concluded that, intrauterine infusion of 600 mg of Ciprofloxacin resulted in endometrial tissue and intraluminal concentrations that exceeded minimal inhibitory concentrations of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. equi* subsp. *Zoo epidemicus*. No local or systemic adverse effects after intrauterine infusion were recorded and recommended that Ciprofloxacin administration be limited to cases in which the organism is resistant to other antimicrobial agents. Moreover, enrofloxacin which not recommended for use as intrauterine therapies in equine reproductive practice. As both drugs are very irritant to the genital tract, they were the drugs of choice as a result of antibacterial susceptibility.

The present study concluded that the combined use of ultrasonographic, cytological and bacteriological diagnostic techniques is more sensitive and beneficial for accurate diagnosis of fertility problems, *E.coli* is the dominant and most frequently isolated bacterium species that mostly associated with repeat breeding problems than clinical signs, Ciprofloxacin and Enrofloxacin were the only antimicrobial to which the majority of common endometritis pathogens had no resistance. With respect to gentamicin which inhibit some isolated bacterial species of uterine infections.

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الملخص العربي

تشخيص التهاب بطانة الرحم باستخدام الموجات فوق الصوتية والغسيل الرحمي في الأفراس العربية

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الالتهاب الرحمي هو أكثر الأسباب لنقص الخصوبه فى الأفراس و ذلك بسب عدم امكانية تشخيص ذلك الالتهاب فى بعض الأحيان على الرغم من توافر العديد من وسائل التشخيص. و كانت أهداف تلك الدراسة هى تشخيص التهاب بطانة الرحم البكتيري فى الأفراس بوسطة كلا من الموجات فوق صوتيه، الفحص السيتولوجى لخلايا التهاب بطانة الرحم كذلك عزل و تصنيف لتلك الميكروبات و اجراء اختبار حساسيه المضادات الحيويه المستخدمة فى علاج ذلك الالتهاب. و أجريت تلك الدراسة فى عدد ٣٢ من أفراس الخيل العربي الأصيل التى لديها مشاكل فى الخصوبه و تم تشخيصها بواسطة أولاً الموجات فوق صوتيه ثم أخذ عينات رحميه بطريقة لو فوليم يترين فلاش لعمل اختبار الزرع البكتيري و الفحص السيتولوجى لخلايا التهاب بطانة الرحم. لقد وجد ميكروب الايسيرشيا كولاي هو الميكروب السائد و الأكثر تكرارا بعدد ١٥ معزوله بنسبة (٤٠،٥%) تبعه الميكروب العنقودى (استاف أوريس) بعدد ٨ معزولات بنسبة (٤١،٦%) ثم كلام من ميكروب الكلبسيلانيمونى ، الميكروب السبجى (ستربتوكوس) و كلام من ميكروب سودومونس سيروبكتر بعدد ٥ ، ٤ و ٢ معزولات و نسبة (١٣،٥٧%) ، (١٠،٨١%) و (٥،٤١%) على التوالى. و كان ميكروب البروتيس الأقل تكرارا بعدد ١ معزوله و نسبة (٢،٧%). كما أظهر اختبار الحساسيه أن كلام سيبروفلوكساسين و الأنروفلوكساسين هما المضادان الحيويان الأكثر تأثيرا على الميكروبات المعزولة. كذلك أظهرت النتائج أن الغسيل الرحمي بكميات صغيرة تعتبر طريقه سهلة و سريعة لتشخيص الالتهاب الرحمي فى الأفراس العربية بالإضافة الى استخدام الموجات فوق الصوتية.