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Ultrasonographic Monitoring and Treatment of Endometritis in Mare

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ABSTRACT

The aim of the current study was to compare between different regimens used for treatment of endometritis in mares with the help of ultrasonography. This study was carried out on 50 repeat breeding mares (35 mares with endometritis and 15 mares (control group) without endometritis). All mares were examined by trans-rectal ultrasonography and uterine swabs were obtained for laboratory diagnosis and sensitivity testing. Mares with endometritis were divided into three groups according the type of treatment; group (1) contains 12 mares were treated by I/V infusion of Enrofloxacin once daily for 5 days and I/M injection of 25 IU oxytocin, group (2) contains 12 mares were treated by uterine wash with normal saline for 3 days and I/M injection of 25 IU oxytocin, group (3) contains 11 mares were treated by uterine wash with normal saline and Gentamicin for 3 days and I/M injection of 25 IU oxytocin. The results revealed that uterine horn diameter was significantly larger ($p < 0.05$) in repeat breeding mares with endometritis (7.28 cm) than that in control (5.18 cm). The best recovery rate was obtained after using Enrofloxacin for 5 days with oxytocin, where (8/12, 66.7 %) mares became pregnant, followed by uterine wash with saline and Gentamicin for 3 days and I/M injection of 25 IU oxytocin, where (6/11, 54.5 %) mares became pregnant and finally uterine wash with saline for 3 days and I/M injection of 25 IU oxytocin, where (4/12, 33.3 %) mares became pregnant. Moreover, ultrasonography is an essential tool for diagnosis of endometritis in mares, Enrofloxacin together with uterine ecbolic as a treatment improved pregnancy rate of affected mares and Enrofloxacin may be an appropriate option for treatment of infectious endometritis associated with susceptible bacterial infection in mares.

***Key words:** Endometritis, Ultrasonography, Enrofloxacin, Mares.*

1. INTRODUCTION

Endometritis is considered a major cause of subfertility in the mare due to low pregnancy and foaling rates (LeBlanc and Causey, 2009). The predisposing factors of endometritis in mares include reproductive tract anatomical defects, history of dystocia or placental retention, and improper uterine clearance after intrauterine semen deposition during natural mating or artificial insemination (AI) (Troedsson, 2006). Bacterial species associated with equine endometritis involve *Streptococcus equi subspecies zooepidemicus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Davis, et al., 2013). The most common form of non-infectious endometritis is post-mating induced endometritis (PMIE) (Troedsson and Woodward, 2016). In susceptible mares, often due to conformational impairments, poor mechanical drainage, and/or other undetermined factors, a resurgence of bacteria and fluid accumulation may occur and persist for multiple days (Canisso, et al., 2016). Several recent reports have indicated that some Gram-negative bacteria (*E. coli*) are resistant to

commonly used antimicrobial agents, such as aminoglycosides (Albihn, et al., 2003). Transient endometritis occurs after mating and post foaling, this is a normal reaction to the foreign protein and bacteria which enter the uterus at this time and is normally resolved within 24 hours of service and within six days of foaling. This is a physiological endometritis (Portus, et al., 2005 and Katila, 2012). There are a variety of documented treatments for endometritis, for example, ecbolic drugs such as oxytocin (Woodward and Troedsson, 2013), intrauterine antibiotics such as penicillin (LeBlanc, 2009), anti-inflammatory treatments such as dexamethasone (Bucca, et al., 2008) and mucolytic compounds such as dimethyl sulfoxide and acetyl cysteine added to uterine lavage fluids (Ley, et al., 1989). Since the introduction of ultrasonography to the equine veterinary field in the early 1980's, its use as a diagnostic tool has expanded dramatically. Applications of ultrasonography in mare reproduction include monitoring follicular changes, detection of pregnancy, determination of embryonic death and evaluation of uterine pathology (Squires, et al., 1988). Techniques to diagnose infectious endometritis include trans-rectal ultrasonography, uterine culture, and endometrial cytology. Treatment commonly involves uterine lavage followed by infusion of antibiotics into the uterine lumen (Hurtgen, 2006). Fluoroquinolones, antimicrobial agents related to nalidixic acid, may be a potential option for treatment of equine uterine infections caused by resistant Gram-negative bacteria. Ciprofloxacin is a fluoroquinolone with good antimicrobial activity against most Gram-negative bacteria and some Gram-positive bacteria (Launay, et al., 2009). Therefore, the aim of this work was to compare between different regimens to determine the most suitable one that could be applied for treatment of endometritis in mares with the help of ultrasonography.

2. MARERIALS AND METHODS:

2.1. Animals: This study was carried out during the period from September 2017 to December 2018. The study was carried out on 50 repeat breeding Baladi mares reared in private farms on Cairo-Alex desert road. These mares were aged from 8-15 years. All mares were fed barseem and tibn with concentrates during winter season while in summer season they were fed hay and concentrates with tibn and water provided ad-libitum. Uterine swabs were obtained for laboratory diagnosis and sensitivity testing.

2.2. Experimental design:

2.2.1. Ultrasonographic examination of mares

All mares were examined by trans-rectal ultrasonography using Portable Ultrasonic Diagnostic System (Sonoscape Co. Ltd., China) provided with trans-rectal linear transducer (5 – 7.5 MHZ) for rectal scanning. The uterine body and the uterine horns were examined for the presence of intra-luminal fluid both before and after mating also pre- and post- treatment, and follow-up during the study. The quantity of intrauterine fluid was recorded and was classified according to (Ginther and Pierson, 1984) into: Grade A- scanty (2 ml), Grade B-medium (3 up to 10 ml) or Grade C- large (more than 10ml).

2.2.2. Uterine (sample) swabbing:

Uterine swabs were collected according to (Barbary, et al., 2016). Briefly, the perineum of all mares was cleansed with betadine scrub, rinsed, dried and tail was wrapped. A double guarded swabs and media tubes (for bacterial culture) were used for swabbing. Uterine samples were examined by culture and sensitivity testing.

2.2.3. Antibiotic sensitivity test

Antibiotic sensitivity test was performed according to (Forbes, et al., 2002). In brief, colony of the organism was transferred and mixed well into a tube of sterile Muller Hinton broth. The determination by using in vitro susceptibility of the tested organism to a given antibacterial agent was determined as sensitive (S), intermediate (NT) or resistant (R) according to the type of antibacterial agent used, the type of the tested organism and the standard diameter of inhibition zones. Antibiotic discs that were used in sensitivity test are shown in **Table (1).**

Table (1): The abbreviations of antibiotic discs used in sensitivity test.

Antibiotic name	Bio-Disc code	Antibiotic name	Bio-Disc code
Ciprofloxacin	CIP	Amoxicillin/ clavulanic acid	AMC
Enrofloxacin	ENR	Ampicillin	AM
Gentamicin	CN	Chloramphenicol	C
Penicillin G	P	Cloxacillin	OB
Streptomycin	S	Erythromycin	E
Kanamycin	K	Flumequin	UB
Cefotaxim	CTX	Tetracycline	TE
		Rifampicin	RD

2.2.4. Treatment of mares with endometritis

Mares with endometritis (n = 35) were divided to three groups according the type of treatment; **group (1)** contains 12 mares were treated by I/V infusion of Enrofloxacin 2.5 mg/kg (Vil-floks®, Vilsan, Turkey) once daily for 5 days and I/M injection of 25 IU oxytocin (Oxytocin® , Adwia Co, Egypt), **group (2)** contains 12 mares were treated by uterine wash with normal saline (Sodium chloride®, 0.9 %, 500 ml solution, Nile Co. Egypt) for 3 days and I/M injection of 25 IU oxytocin, **group (3)** contains 11 mares were treated by uterine wash with normal saline and Gentamicin 2.5 mg/kg (Genta-100®, Vilsan, Turkey) for 3 days and I/M injection of 25 IU oxytocin. First heat post treatment was passed to allow the inflammatory response to subside **(LeBlanc, 2008)**. Mares were bred on the second heat by natural mating.

2.2.5. Ultrasound examination of treated mares for pregnancy diagnosis

All treated mares after sexual rest were conducted for ultrasound reproductive examination to detect the efficacy of treatment. Absence of fluid accumulation by ultrasonography was a good indication of treatment success (Causey, 2007). Pregnancy diagnosis using ultrasonography was conducted on 15- and 25-days post mating.

3. Statistical Analysis

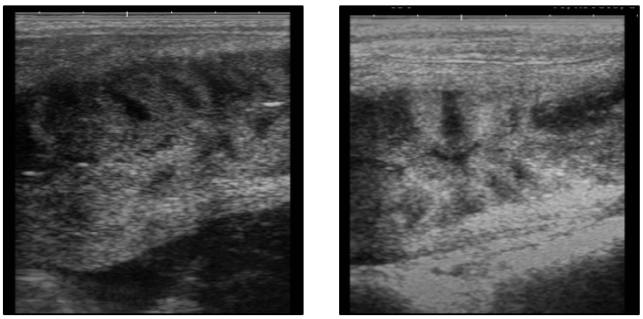
The collected data were analyzed with **SPSS** software **(Chicago, 2001)**. Chi Square test (X2) was used to determine the differences in the reproductive performance among treated groups.

4. RESULTS:

4.1. Scanning of uterus using ultrasonography.

In the current study, uterine horn diameter was clearly different in repeat breeding mares with endometritis (35/50, 70 %) compared to mares without endometritis (15/50, 30 %). The diameter was significantly larger ($p < 0.05$) in repeat breeding mares with endometritis (7.28 cm) than that in mares without endometritis (5.18 cm) as presented in **Image (1) and in Table (2).**

A)



B)

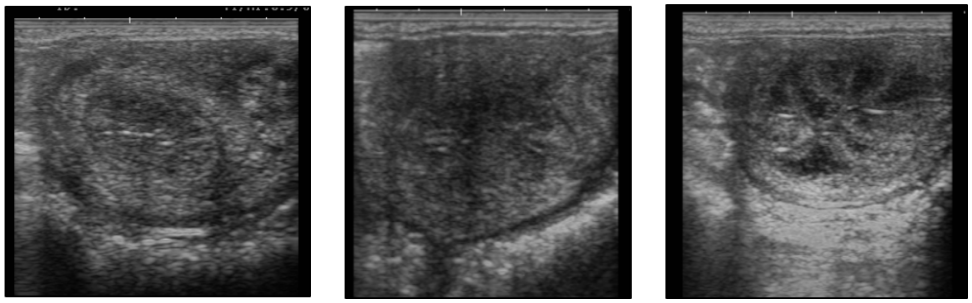


Image 1): Ultrasonographic appearance of normal mare's uterus. **A)** longitudinal section. **B)** cross section.

Table (2): Show the thickness of the uterine wall, the thickness of the endometrial edema and the echogenicity of the intra-uterine fluid in both mares with endometritis and those without endometritis.

Criterion	Animal group (No)	Endometritis n = 35	Control n = 15
	Uterine horn Φ cm	**7.28[a]**	**5.18[b]**
Uterine wall thickness in cm	1 – 2 cm	5 (14%)	10 (67%)
	2 – 3 cm	12 (34%)	5 (33%)
	3 – 4 cm	12 (34%)	----
	4 – 5 cm	6 (18%)	------
Uterine endometrium thickness	No edema (1–2 cm)	5 (14%)	12 (80%)
	Slight edema (2–3 cm)	15 (43%)	3 (20%)
	Moderate edema (3–4 cm)	10 (29%)	------
	Severe edema (4 – 5)	5 (14%)	-------
Intra-uterine fluid echogenicity	AE	10 (29%)	5 (33%)
	HO	20 (57%)	-------
	ME	5 (14%)	--------

AE = anechoic, clear fluid (black in color). HO = hypo-echoic fluid with hyper-echoic particles or slightly cloudy fluid (grayish in color). ME = moderately echogenic fluid or moderate cloudy fluid (gray-whitish in color). Values with different superscripts were significantly different ($p < 0.05$).

The mean thickness of uterine wall was thicker ($p < 0.05$) in mares with endometritis (2.37 cm) compared to control (1.93 cm), **Table (2) and Image (1 & 2)**. Moreover, mean uterine endometrial thickness was larger ($p < 0.05$) (edematous) in mares with endometritis (3.96 cm) than control (1.72 cm).

Depending on the amount of uterine exudate, ultrasonography was shown in 3 grades. Scanty grade (2 ml) appeared as black clear fluid (anechoic), medium grade, (3-5 ml) (hypo-echoic) free exudates clear fluid (anechoic black echogenicity). Large grade (more than 5 ml) the luminal fluid was appeared hypo-echoic with hyper-echoic particles, as presented in **Image (2).**

Accumulations of intrauterine fluid (mm^3) were detected by ultrasound in all mares. The results revealed that the amount of uterine fluids before treatment were less than 20 mm^3, 20 – 40 mm^3 and more than 40 mm^3 in about (20/35, 57%), (6/35, 17%) and (9/35, 26%), respectively of repeat breeding mares with endometritis. Reversely, repeat breeding mares without endometritis, the intra-luminal exudates were about 20 mm^3 and 20 - 40 mm^3 for (12/15, 80%) and (3/15, 20%), respectively.

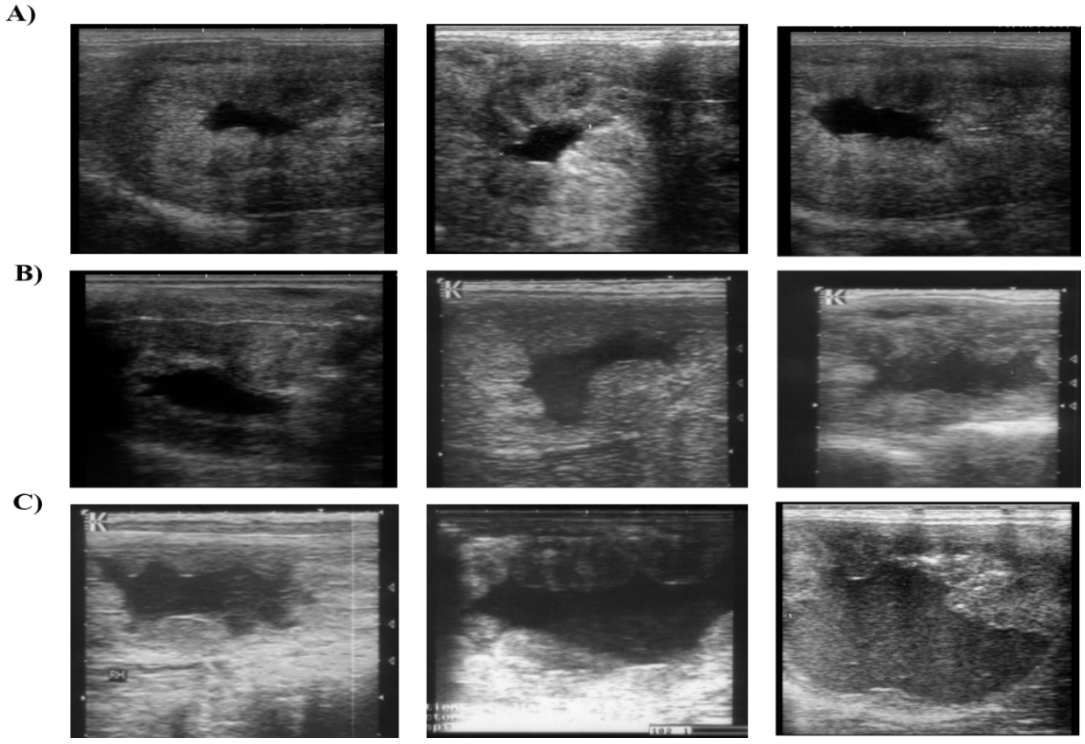


Image 2): Ultrasonographic characteristics of mare's uterus with different degrees of endometritis. **A)** Slight amount of anechoic fluid (black). **B)** Moderate amount of hypoechoic fluid (gray). **C)** Large or severe amount of hyperechoic fluid (black with white particles)

4.2. Uterine swabs findings:

The bacteriological examinations of uterine swabs were shown in table (3). A highly increased scores ($p < 0.05$) of bacterial growth was recorded in repeat breeding mares with endometritis (score, 7.56) than control (score, 3.07) as presented in **Table (3).**

Table 3): Uterine swabs for bacterial (degree of growth) in repeat breeding mares with and without endometritis (Mean ± SEM).

Group	Bacterial score*
Mares with endometritis n = 35	$7.56^{a} \pm 1.09$
Mares without endometritis n = 15	$3.07^{b} \pm 0.55$
Total n = 50	

*Bacterial scores: no growth ≤ 2, scanty growth = 3-4, moderate growth = 5-6 and heavy growth > 6.Values with different superscripts were significantly different at ($p < 0.05$).

The most isolated microorganisms from repeat breeding mares with endometritis were *Escherichia coli* (33.33%), *streptococci spp.*, *klebsiella spp.* (15.15% for each), *staphylococcus spp* (9.09%), *β-hemolytic streptococci* (6.06%), *β-hemolytic staphylococci* (3.03%) and finally *pseudomonas aerogenosa (3.03%).* Enrofloxacin antibiotic was the most effective against most of isolated microorganisms, followed by Ciprofloxacin, Gentamicin and Norfloxacin.

4.3. Treatment of mares with endometritis

The best recovery rate of mares with endometritis was obtained after using Enrofloxacin for 5 days with oxytocin, where (8/12, 67 %) mares become pregnant as shown in **Image (3).** Followed by using uterine wash with saline and Gentamicin for 3 days and I/M injection of oxytocin, where (6/11, 55 %) mares became pregnant and finally uterine wash with saline for 3 days and I/M injection of oxytocin, where (4/12, 33%) mares became pregnant.

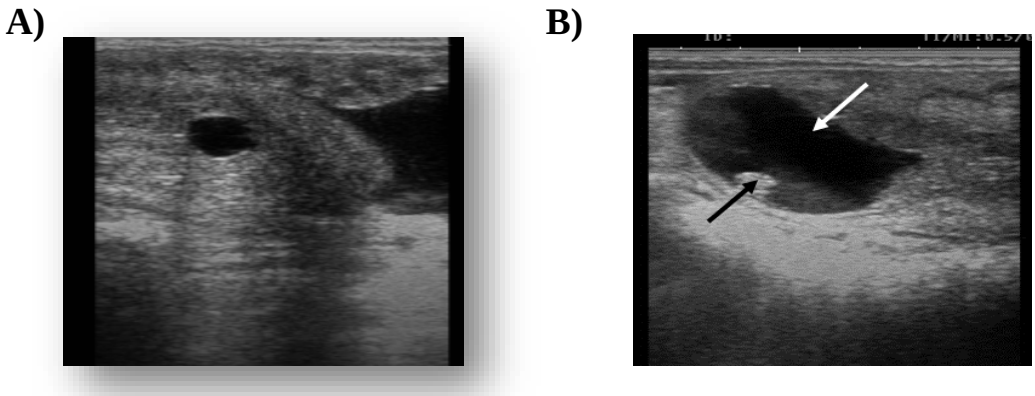


Image 3): Ultrasonographic examination of mare's uterus, showing, **A)** amniotic vesicle anechoic black clear fluid (pregnancy, 15 days) and **B)** at 25 days pregnancy: notice embryo proper (black arrow) and yolk sac (white arrow).

5. DISCUSSION:

The ability to keep healthy uterine environment suitable for embryonic and fetal life is critical for reproductive efficiency in mares. Unfortunately, uterine environment is easily disturbed by an inflammatory process following bacterial invasion, which can might occur during mating, artificial insemination (AI), reproductive evaluation, parturition and mostly during cleaning of the uterus as a result of poor conformation (Samper and Tibary, 2006).

In the current study, the percentage of endometritis in mares diagnosed by ultrasonographic evaluation was 70%. This percentage seems somewhat corresponding to 25–60% that was reported by (Nikolakopoulos and Watson, 1999). Contrary, this value is higher comparing with 15% obtained by (Troedsson, 1999). This might be attributed to difference in number of animals used in each study.

In the present study, the diagnosis of endometritis in mare was confirmed by suing the ultrasonography through the presence of intrauterine fluid. However, (LeBlanc and Causey, 2009, LeBlanc, 2010) used endometrial cytology and/or culture positive endometrial biopsy for confirmation of diagnosis of endometritis in mare.

The most common microorganism, which isolated from repeat breeding mare in the present study, was *E. coli* are (In 33.3 % of collected 50 uterine swabs). This result agrees with that reported by Barbary, et al., (2016) and LeBlanc, et al., (2007). However, Frontoso, et al., (2008) recorded that the most commonly bacteria isolated in mare endometritis were *ß-haemolytic streptococci* followed by E coli.

In the current work, we reported that treatment of endometritis in mares via systemic administration of Enrofloxacin together with uterine ecbolic (oxytocin) improved pregnancy rate of affected mares. This is in agreement with (Barbary, et al., 2016), where ciprofloxacin and Enrofloxacin were the best effective antimicrobial therapy inhibiting bacterial endometritis in mares. Moreover, Abidine and Bouabdellah (2018) mentioned treatment of endometritis by intrauterine infusions of 70% honey solution enhanced the uterine drainage.

CONCLUSION:

The most important findings from this study of repeat breeding mares were firstly: ultrasonography is indispensable tool for diagnosis of endometritis. Secondly: susceptible mares accumulated intrauterine fluids which predispose to increased incidence of endometritis. Thirdly: Enrofloxacin and

oxytocin were the most effective regimen for improving conception in mares.

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