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EVALUATION OF VARIOUS ENDOMETRITIS DIAGNOSTIC AND ALTERNATIVE THERAPEUTIC APPROACHES IN ARABIAN MARES

ABDULRAHMAN ALHAIDER

Department of Clinical Sciences, Faculty of Veterinary Medicine, King Faisal University, AL-Ahsa, 31982, Saudi Arabia. TEL. &FAX: +966-059-095-5509

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ABSTRACT

Endometritis is a major contributing factor to impair fertility in mares. This study aimed to assess the reliability of various approaches for the diagnosis and management of endometritis in Arabian mares. Mares were subjected to a body condition score, perineal conformation, an ultrasonography examination, collection of uterine swabs and low-volume lavage swabs as well as endometrial biopsy. Mares were randomly selected for three different intrauterine treatment regimens and were serviced in the next estrus after the treatment. Results indicated that the proportion of abnormal vulval conformation increased as the age of the animals increased, along with a low body score. Bacteria and fungi were isolated from 83.64% of mares. Direct swabs were positively coincident in 36% with low-volume uterine lavage. Bacteriological and ultrasonography examinations contradicted in 71% of the cases ($P < 0.05$), while bacteriological and biopsy findings matched in 86.2% ($P < 0.05$). Swabs from low-volume lavage were more reliable than direct swabs in the diagnosis of endometritis. Cytological findings agreed with ultrasonography examination, while ultrasonography findings disagreed with biopsy grade in endometritis diagnosis ($P < 0.05$). Conception rates of mares treated with an intrauterine infusion of penicillin + gentamicin, ceftiofur, and cephapirin were 75%, 50%, and 28.6%, respectively. In conclusion, cytological and bacteriological assessment of endometrium, along with an ultrasonography examination, could be used with a high degree of confidence to diagnose endometritis in mare without the need for an endometrial biopsy. Although there is a low conception rate with one intrauterine infusion of cephapirin, it could be used successfully as a one-time intrauterine therapy in mares with endometritis.

Keywords: Mare, endometritis, cytology, bacteriology, antibiotics

INTRODUCTION

Numerous uterine conditions have been reported in horses, and they may be a significant factor in mares' decreased fertility (Brendemuehl, 2002). Endometritis is the primary cause of infertility in mares; however, there are numerous other known causes as well, such as neoplasia, immunological, inflammatory, viral, traumatic, and scarring disorders, hormonal disruption, and congenital abnormalities (Hurtgen, 2006).

Endometritis threatens fertility by initiating shortened inter-estrus intervals, early embryo losses, placentitis, abortion, and the

Corresponding author: Abdulrahman Alhaider
E-mail address: aalhaider@kfu.edu.sa
Present address: Department of Clinical Sciences, Faculty of Veterinary Medicine, King Faisal University, AL-Ahsa, 31982, Saudi Arabia.

delivery of intrauterine-infected foals (LeBlanc and Causey, 2009). Because of repeated breeding, the infusion of irritating substances, aspiration of air or urine, bacterial infections that may be caused by improper perineal conformation, delayed uterine clearance of bacteria and debris, or an innate immunological deficiency, the uterus is susceptible to this inflammatory process (Troedsson, 1997; Watson, 2000). Poor perineal conformation, which can result in self-contamination, is one of the many contributing factors to chronic endometritis, which is characterized by recurring bacterial or fungal infections (LeBlanc, 2008). Increasing the chance of pregnancy in mares with endometritis requires early detection and treatment (Card, 2005). Accurate diagnosis is also necessary for the creation of a sensible treatment plan and the implementation of an ideal management approach (Rickets and Troedsson, 2007).

To control endometritis in mares with varying success rates, numerous diagnostic and therapeutic techniques have been developed (Hurtgen, 2006; LeBlanc, 2010). The vulva is the first physical barrier to prevent contamination of the reproductive tract by pathogenic organisms; thus, an assessment of the vulva's shape and muscular tone is necessary. An alteration in the vulva's structure makes the mare more vulnerable to uterine infection (Hemberg *et al.*, 2005).

Various processes are involved in uterine defense against microbial infection. Physical barrier, mechanical clearance, cellular response to bacterial challenge, and humeral response to antigenic assault are the primary defense mechanisms. When combined, these processes are quite effective in healing endometritis in normal mares; however, in mares that are prone to endometritis, they are unable to eradicate imported bacteria (Brendemuehl, 2002). To be successful, uterine therapy should correct the defects in the uterine defense, neutralize virulent bacteria, and control post-breading inflammation. This is accomplished by surgically correcting anatomical defects, improving physical drainage after insemination, modulating the inflammatory response to insemination, and inhibiting bacterial growth. A first-generation cephalosporin with broad antimicrobial action against both gram-positive and gram-negative bacteria is cephapirin (Donowitz and Mandell, 1988). Cephapirin inhibits the formation of cell walls, which has a bactericidal effect. Only cells that are actively growing can profit from its action (Chambers *et al.*, 1998).

Therefore, the purpose of the current study is to examine different methods for identifying endometritis in mares and investigate alternative medications for treating endometritis in mares, as well as the potential use of cephapirin (Metricure®) as a single intrauterine infusion antibiotic.

MATERIALS AND METHODS

Chemicals

All the chemicals used in the present study were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated.

Animals

This study was carried out at the Equine Theriogenology Clinic of the King Faisal University Veterinary Teaching Hospital in the province of Al Ahsa between March 2022 and February 2023. All experimental procedures used in the current study were approved by the guidelines of Ethics Committee at King Faisal University, Saudi Arabia. The study's patients were Arabian mares who were suffering from recurrent breeding. The age range of the mares involved was three to seventeen years old. Mares have a history of miscarrying after over two cycles of mating. Mares were treated to a range of management approaches, such as changes in diet, housing, breeding season features, and timetables for teasing. Every mare's reproductive history was recorded, including age, the circumstances surrounding last foal, the

regularity of estrous cycle, the traits of estrus secretion, the number of services, and the date of last service.

A manual vaginal and cervical examination, an evaluation of perineal conformation, a general physical examination, an ultrasound examination, a comprehensive reproductive history, and a body condition score were all part of the mare's breeding soundness assessment. Three age categories were assigned to the mares. Groups I (ages three to five), II (ages six to ten), and III (ages eleven and up). Every mare's body condition scoring (BCS) evaluation has been recorded following Carroll and Huntington's (1988). The score is between zero and five (0: very poor, 1: poor, 2: middling, 3: good, 4: fat, and 5: very fat). The vulva was assessed for the presence of discharge, conformation, slope, and muscular tone. The genital organs were examined ultrasonographically via the rectum (using a 5-7.5 MHz linear transducer, SSD-3500, ALOKA, Co., Ltd., Japan). The presence of two or more centimeters (depth) of intrauterine fluid has been considered to be diagnostic of endometritis, according to McKinnon (1993) and Reilas *et al.* (1997).

Endometrial swabs and low volume lavage (LVL) for uterine culture

A double-guarded sterile uterine swab (Equi-VET® Kruuse, Denmark) was utilized. Once the swab was retracted, it was transferred to the lab in Amies modified media (SVA, Uppsala, Sweden) within 24 h in a cold chest. LVL was performed as previously described (LeBlanc *et al.*, 2007). Briefly, a sterile silicon uterine flushing catheter (90 cm in length, Minitube, Germany) was passed trans-cervical into the uterine body, the balloon inflated with 100 ml of air, and the catheter was then seated at the internal cervical os. Two hundred and fifty ml of 0.9% sodium chloride solution (0.9% NaCl, Baxter, Mississauga, ON, Canada) was infused into the uterus. The fluid was left in the uterus for two minutes, and then 20 IU of oxytocin (Oxyto-Sure™, Vétoquinol, Lavaltrie, QC, Canada) were administered intravenously to assist in the recovery of the fluid by gravity into the sterile bag. Then the fluid was transferred into 2 sterile 50-ml conical tubes (Corning® Centrifuge Tubes, Corning Incorporated Life Science, Tewksbury, MA, USA). The gross character of the LVL fluid (normal [clear] or abnormal [cloudy, discolored, debris]) was recorded before centrifugation at 400 x g for 10 min. After centrifugation, the supernatant was discarded. A swab was extracted from the pellet and subjected to the previously mentioned processing steps.

Bacteriological and mycological examination

Swabs were inoculated on Columbia blood agar with 5% sheep blood (cm331; Oxoid, Basingstoke, UK) and MacConkey agar (oxoid, Basingstoke, UK) for general microbiological examination. Sabouraud Dextrose Agar (SDA) (Oxoid CM 41) was used for mycological isolation. The bacterial identification was done by the VITEK® 2 System. (David H. Pincus, BioMérieux, Inc., Hazelwood, MO, USA).

Cytological examination

Sterile uterine swabs were stained with Diff Quick (Jor Vet Dip Quick Stain, Jorgensen Labs, 1450 Van Buren Ave). Then, the number of neutrophils (X400) across ten fields was recorded. The endometrial inflammation was graded as not inflamed (0–2 neutrophils/field), moderately inflamed (≥2–5 neutrophils/field), and severe inflamed (≥5 neutrophils/field) (Riddle *et al.*, 2007).

Biopsy

An endometrial biopsy was collected as described by Nielsen (2005) using a sterilized biopsy punch instrument (Equi-VET® Kruuse, Denmark). The specimen was immediately fixed in 10% formalin and processed for staining with hematoxylin and eosin. The histopathological diagnosis of the biopsies was based on the grading scheme of Kenney and Doig (1986). The biopsy was analyzed for the stage of the estrous cycle, the number of fibrotic nests, and the presence of neutrophils, plasma cells, and

lymphoid follicles. The presence of inflammatory cells in the endometrium was graded as zero if no inflammatory cells were seen; 1 if there were a low number of cells; 2 if there were moderate numbers; and 3 if cells were abundant. Mares that had the inflammatory cells graded as zero or one were classified as negative for endometritis, while grade 2 or 3 placed mares under the category of positive for endometritis. Mares were classified as having acute endometritis if the inflammatory cells were predominantly neutrophils, chronic endometritis if the inflammatory cells were predominantly plasma cells or lymphocytes, and mixed (chronic-active) endometritis if the inflammatory cells within the biopsy included neutrophils, plasma cells, and lymphocytes.

Treatment regimen

Treatment started on the second day following the pretreatment endometrial swab and smear collection. Initially, all animals were exposed to intrauterine Lactated Ringer's via a sterile disposable uterine flushing catheter (Animal Reproduction Systems, Cal, USA). Animals (n = 23) were randomly distributed into three treatment groups. Group A (n = 8) received 17.5 ml of intrauterine (5 million unite) procaine penicillin (Norocllin, Norbrook, Northern Ireland) and 2 g/20 ml, intrauterine Gentamicin sulfate (Gentakel 10%, Kela Laboratoria N.V., Belgium) buffered with an equal volume of 8.4% sodium bicarbonate for three consecutive days. Group B (n = 8) was infused intrauterine with 20 ml of 1 g ceftiofur sodium (Excenel, Zoetis Inc., Kalamazoo, USA) in sterile water for three consecutive days. Group C (n = 7) was infused intrauterine once with 500 mg of cephapirin (Metricure®) buffered with 5 ml of 8.4% sodium bicarbonate. Bacteriological swabs and endometrial biopsies were collected in the next cycle post-treatment. The mares were serviced in the next estrus after the treatment. Then, the conception was recorded using ultrasonography on the 45th day post-insemination.

Statistical analysis

All comparisons were performed by a chi-square test using JMP software version 11.0.0 (SAS Institute, Cary, NC, USA). Sensitivity and specificity for different diagnostic tests were calculated following Nielsen (2005). Differences of $P < 0.05$ were regarded as significant.

RESULTS

Mares had an abnormal vulval conformation of 38.2%. The proportions of mares with abnormal vulval conformation were 10% in age Group I, 47.1% in Group II, and 61.1% in Group III (Table 1). According to body scores 2, 3, 4, and 5, the percentages of mares with endometritis were 10.90%, 40%, 40% and 9.1%, respectively (Table 2).

Table 1: The relationship between mare age and vulval conformation.

Vulval conformation	Age (years)			Total	P. value
	Group I (3-5)	Group II (6-10)	Group III (≥ 11)		
Abnormal	10% (n=2)	47.1% (n=8)	61.1% (n=11)	38.2% (n=21)	(P< 0.05)
Normal	90% (n=18)	53.9% (n=9)	38.9% (n=7)	61.8% (n=34)	(P< 0.05)

Table 2: The relationship between body score and affection with endometritis.

Body score	1	2	3	4	5
Endometritis (n=55)	0.0% (n=0)	10.9% (n=6)	40% (n=22)	40% (n=22)	9.1% (n=5)

Table 3 shows the percentage of mares in each age group that had a biopsy-confirmed diagnosis of endometritis. 6.9% of the mares' endometrium was found to be grade 1 biopsy, 62.1% of the mares had grade 2a endometrium, 20.7% had grade 2b endometrium, and 10.3% had grade 3 endometrium (Table 3).

Table 3. The relationship between the age of mares and the degree of endometritis.

Biopsy grades	Age (years)			Total	P. value
	Group I (3-5)	Group II (6-10)	Group III (≥ 11)		
Endometritis	20.7% (n=6)	24.1% (n=7)	55.2% (n=16)	(n=29)	
Biopsy grades					
1	50.0% (n=1)	0.0% (n=0)	50% (n=1)	6.9% (n=2)	(P< 0.05)
2a	27.8% (n=5)	27.8% (n=5)	44.4% (n=8)	62.1% (n=18)	(P< 0.05)
2b	0.0% (n=0)	33.3% (n=2)	66.6% (n=4)	20.7% (n=6)	(P< 0.05)
3	0.0% (n=0)	0.0% (n=0)	100% (n=3)	10.3% (n=3)	(P< 0.05)

A mycological investigation revealed that fungi had been identified in 5.5% of mares with endometritis (Table 4). A microbiological examination showed that 30 distinct types of colonies were growing (Table 4). Single and double bacterial infections had been identified in 83.7% and 16.3% of the infected samples, respectively. 7% of the infected samples had a mixed bacterial and fungal infection. *Escherichia coli* (*E. coli*)(n = 21; 36.8%), *Staphylococcus spp*. (n = 10; 17.54%), and Beta-hemolytic *Streptococcus* (n = 8; 14.0%) were the most frequently isolated bacterial species. In 32.6% of mares with abnormal vulval conformation, bacteria were successfully isolated (Table 5).

The bacteriological examination was found to be positively coincided with cytological (48%) and ultrasonography findings (12.7%), and disagreed in 32% and 71%, respectively (P < 0.05) (Table 6). Also, bacteriological and biopsy were positively coincident in 79.3% and disagreed in 13.8% (P < 0.05). Cytological results were found to be positively coincident with ultrasound findings (16%) and biopsy findings (54.6%) and disagreed in 32% and 36.4%, respectively (P < 0.05) (Table 6). The ultrasound findings disagreed in 75.86% with biopsy (P< 0.05). The comparisons between direct swabs and low-volume uterine lavage showed that 44% of the samples were positive for low-volume lavage. Conversely, 72% of direct bacterial swabs proved positive. Of the samples, both approaches disagreed in 44% and were positively coincident in 36% (P < 0.05) (Table 6). The swab from low-volume lavage was more reliable than the swab for the diagnosis of endometritis.

Table 4: Results of the mares' bacteriological endometrium culture.

Types of isolates		Frequency of isolation	Percentage
Yeast		1	1.8%
Aspergillus spp.		2	3.6%
Alloiococcus otitis		1	1.8%
Bacillus spp.		1	1.8%
E.coli		21	36.8%
Enterobacter kobei		1	1.8%
Enterococcus casseliflavus		1	1.8%
Enterococcus columbae		1	1.8%
Granulicatella adiacens		1	1.8%
Kocuria kristinae		1	1.8%
Kocuria rhizophila		1	1.8%
Lactococcus garvieae		1	1.8%
Lactococcus lactis subsp. cremoris		1	1.8%
Raoultella ornithinolytica		1	1.8%
Sphingomonas paucimobilis		3	5.4%
Staphylococcus spp.	*Staphylococcus aureus*	1	17.5%
	Staphylococcus agalatiae	1	
	Staphylococcus chromogenes	1	
	Staphylococcus hyicus	1	
	Staphylococcus schleiferi	3	
	Staphylococcus suis	1	
	Staphylococcus vitulinus	2	
Stenotrophomonas maltophilia		1	1.8%
Streptococcus equi subspecies zooepidemicus		3	5.3%
Beta-hemolytic Streptococcus spp.	*Streptococcus pluranimalium*	1	14.0%
	Streptococcus pseudoporcinus	1	
	Streptococcus suis	1	
	Streptococcus dys. equisimilis	1	
	Streptococcus uberis	1	
	Streptococcus canis	1	
	Streptococcus constellatus	1	
	Streptococcus porcinus	1	

Table 5: Relationship between vulval conformation and bacterial isolation.

Bacterial isolation	**Vulval conformation**		**P. value**
	Normal	**Abnormal**	
Positive	67.4% (n=31)	32.6% (n=15)	(P< 0.05)
Negative	33.3% (n=3)	66.7% (n=6)	(P< 0.05)

Table 6: Comparison of the results of diagnosis of endometritis.

	Disagree	Coincident	Positively Coincident	Negatively Coincident	P. value
Bacteriology *vs.* cytology	32% (n=8)	68% (n=17)	48% (n=12)	20% (n=5)	(P< 0.05)
Bacteriology *vs.* sonography	71% (n=39)	29.0% (n=16)	12.7% (n=7)	20% (n=5)	(P< 0.05)
Bacteriology *vs.* biopsy	13.8% (n=4)	86.2% (n=25)	79.3% (n=23)	6.9% (n=2)	(P< 0.05)
Cytology *vs.* sonography	32% (n=8)	68% (n=17)	16% (n=4)	52% (n=13)	(P< 0.05)
Cytology *vs.* biopsy	36.4% (n=8)	63.6% (n=14	54.6% (n=12)	9.1% (n=2)	(P< 0.05)
Sonography *vs.* biopsy	75.9% (n=22)	24.1% (n=7)	17.2% (n=5)	6.9% (n=2)	(P< 0.05)
Swabs *vs. lavage*	44% (n=11)	68% (n=17)	36% (n=9)	20% (n=5)	(P< 0.05)

The comparisons between the direct swab and the low-volume lavage swab for the cytological diagnosis of endometritis are set in Table (7). Three and five animals were found to have moderate endometritis using direct swabs and low-volume lavage, respectively. According to direct swab results and low-volume lavage results, respectively, two and six mares were found to have severe inflammation.

Table 7: Comparison between cytological swabs and uterine lavage for the diagnosis of endometritis.

Endometritis	Swab (n=25)	Lavage (n=25)	P. value
Normal	80% (n=20)	56% (n=14)	(P< 0.05)
Moderate inflammation	12% (n=3)	20% (n=5)	(P< 0.05)
Sever inflammation	8% (n=2)	24% (n=6)	(P< 0.05)

Table (8) compares the histological evaluation of endometritis with the sensitivity and specificity of cytological, microbiological, and ultrasonography examinations. The results of the sonographic, microbiological, and cytological examinations showed sensitivity rates of 14.8%, 66.7%, and 44.4%, respectively. When compared to histological examination, there was no significant difference in the combined sensitivity and specificity of the three techniques.

Table 8: Sensitivity and specificity of cytological, microbiological, and sonographic examination

Type of investigation	Sensitivity	Specificity	P. value
Cytological	44.4% (n=12)	100% (n=2)	(P< 0.05)
Microbiological	66.7% (n=18)	50% (n=1)	(P< 0.05)
Sonographic	14.8% (n=4)	100% (n=2)	(P< 0.05)

The conception rates of mares treated with an intrauterine infusion of procaine penicillin plus Gentamicin sulfate, Ceftiofur sodium, and Metricure® were 75%, 50%, and 28.6%, respectively (Table 9). In the Metricure group, although they did not conceive, bacteriological swabs from 28.6% of mares were negative after treatment. Moreover, biopsies taken post-treatment did not show any deterioration of the endometrial architecture.

Table 9: Treatment results of mares with endometritis.

Excenel® (Ceftiofur sodium)			**Norocillin® + Genta-kel® 5%,** (Penicillin G procaine+Gentamicin sulfate)			**Metricure®** (Cephapirin 500mg) **+ 5 ml Sodium bicarbonate**		
Isolated bacteria	Cytological	Pregnancy	Isolated bacteria	Cytological	Pregnancy	Isolated bacteria	Cytological	Pregnancy
Str. equi zooepidemicus	Inflamed	-ve	*Alloiococcus otitis*	Normal	+ve	*Str. puranimalium*	Inflamed	+ve
Sphingomonas paucimobillis	Normal	+ve	-ve	Normal	+ve nt	*E. coli*	Inflamed	-ve
Staph. vitulinus	Inflamed	+ve	*E. coli*	Normal	+ve	*E. coli*	Inflamed	-ve
Str. suis	Normal	+ve	*E. coli*	Normal	+ve	*Staph. vitulinus + Enterobacter kobei*	Inflamed	
Staph. schleiferi	Inflamed	+ve	*Str.dys. equisimilis + E. coli*	Inflamed	-ve	*Staph. chromogenes*	Normal	+ve
E. coli	---------	-ve	*Lactococcus garvieae +Str. uberis*	Normal	+ve	*Bacillus*	Normal	-ve
E. coli	----------	-ve	*Staph. vitulinus*	Inflamed	-ve	*E. Coli*	---	-ve
Strep. porcinus	inflamed	-ve	*Lactococcus lactisspcremoris + E. coli*	Normal	+ve	*Str. porcinus*	inflamed	-ve
50% (n=4)			75% (n=6)			28.57% (n=7)		

DISCUSSION

Endometritis in the mare is known to cause major losses in broodmare practice, as its prevalence ranges from 25 to 60% in barren mares (Causey, 2006). This supports the current results, which proved that 80.88% of repeat breeding mares suffered from endometritis. Precise diagnosis and efficacious treatment are therefore essential for a successful breeding outcome (Witte *et al.*, 2010). Pre-breeding diagnosis of endometritis should include clinical examination, transrectal palpation, and ultrasonography of the reproductive tract, vaginal examination, uterine culture, cytology, and endometrial biopsy (Bourke *et al.*, 1997).

This study recorded that 38.2% of mares had abnormal vulval conformation. Previous studies reported that the vulva, vestibule, vagina, and cervix normally act as physical barriers protecting the uterus from external contamination (Katila, 1996; Troedsson, 1999; Rambags, 2003). Abnormality in this region can affect the barrier function, causing air, feces, and urine to enter the reproductive tract (Hurtgen, 2006). The current study reported a connection between low body scores and endometritis. The justification of the association of endometritis with a low body score was reported by Newcombe (2011), who explained that the pressure gradient from the uterus through the vagina to the exterior is found in the mares with a high body score, whereas the case was reversed in the mares with a low body score. This will hinder the evacuation of uterine fluid, leading to a persistent uterine infection.

The current study verified that age was a significant predisposing factor for endometritis, as older age is connected with delayed uterine clearance (Rigby *et al.*, 2001). A direct association between age and endometritis in mares has been reported (Asbury and Lyhe, 1993; Hurtgen, 2006).

In the present study, 83.6% of the samples were microbiologically positive. It was found that bacterial infection in the uterus is considered the main cause of endometritis in mares (Hinrichs *et al.*, 1989; Reiswig *et al.*, 1993). Microbiological analysis proved that 16.4% of the samples were negative for microbiological investigation. Endometritis may occur in the presence or absence of a uterine infection (Asbury, 1986; Brito and Barth, 2003). Mycological investigation of the samples confirmed that 5.5% of the infected animals were positive for fungus; this percentage is parallel with that reported by Dascanio *et al.* (2000). *E. coli* was the most isolated bacteria in the current study. This result was parallel to that of Albihn *et al.* (2003). On the other hand, LeBlanc (1999) and Szeredi *et al.* (2003) found that *Streptococcus equi* was the most common potential uterine bacterial pathogen. The uterine swab touches only a small area of the endometrium, and focal infections might be missed (Ball *et al.*, 1988). The use of low-volume lavage was more efficient for microbiological investigation in our study. We found that not all types of isolated bacteria caused cytological changes in the endometrium. *Pseudomonas*, *Proteus*, and coliforms like *E. coli* were unlikely to have cytological evidence of inflammation. These bacteria produce mucus of high viscosity and slip through the immune response by circumventing the cellular defense system and hindering cilia function (LeBlanc and Causey, 2009). Another study demonstrated that in only 12% of uterine culture samples positive for anaerobes, cytological evidence of inflammation existed (Ricketts and Mackintosh, 1987).

Examining uterine cells harvested through uterine lavage or cytological brushes has gained importance in diagnosing endometritis in mares (Bourke *et al.*, 1997). The assessment of microbiological and cytological analysis revealed that the results were significantly coincident. A similar relationship between bacterial growth and the presence of positive cytology was reported in previous studies (Nielsen *et al.*, 2010; Riddle *et al.*, 2007). The causes of discrepancies may be due to the fact that some pathogens do not produce a neutrophilic response (LeBlanc, 2010) or the presence of a pathogen that was missed during sampling (Ball *et al.*, 1988). If neutrophils are present in the absence of bacteria, there can be sterile inflammation or irritation (Katila, 2016). A neutrophilic uterine response is a reflex of several causes, including pneumovagina, refluxing of urine into the uterus, semen, and excessive production of endometrial mucus (LeBlanc, 2010). The combination of cytological and bacteriological examination leads to a higher sensitivity in diagnosing endometritis (Overbeck *et al.*, 2011).

Ultrasonography has proven a valuable diagnostic tool for endometritis since it has been demonstrated that intrauterine fluid accumulation during diestrus is associated with endometritis (Pycock and Newcombe, 1996; Watson, 2000; LeBlanc, 2003). Additionally, an ultrasound scan may help to demonstrate the severity of inflammation by measuring the degree of endometrial edema and the volume and appearance of retained luminal fluid (Ricketts and Troedsson, 2007). The significant contradiction recorded between the microbiological and sonographic examination may be attributed to the fact that not all uterine pathogens cause the endometrium to produce intrauterine fluid (LeBlanc, 2010) or the fact that there are other causes than bacteria for endometritis (Kotilainen *et al.*, 1994; Troedsson *et al.*, 1995).

The bacteriological examination and biopsy results were significantly matched in this study, which proved that most of the cases of endometritis are due to bacterial infection in mares (Hinrichs *et al.*, 1989). Cytological

investigation for neutrophils in a stained smear obtained from the surface of the endometrium is a simple tool for rapid diagnosis of uterine inflammation under practical field conditions (Reiswig *et al.,* 1993). In this study, both cytological and histopathological examinations were different by 36.4%. The disappearance of an inflammatory process starts with the elimination of bacteria from the uterine lumen, followed by the cessation of intraluminal leukocyte migration, and finally the elimination of the leukocytes from the endometrium (Henary *et al.,* 1982). The current study revealed that low-volume lavage was more reliable than swabs for the diagnosis of endometritis. The low-volume lavage evaluates a larger endometrial surface area (Ball *et al.,* 1988; LeBlanc *et al.,* 2007), while swabbing the endometrium only samples a small focal area, potentially resulting in false negatives (LeBlanc *et al.,* 2007). However, cytology samples prepared from low-volume lavage sometimes exhibit poor cell preservation and more debris, making interpretation difficult (LeBlanc *et al.,* 2007; Diel de Amorim, 2016).

In biopsy, the presence of polymorphonuclear neutrophils in the stratum compactum of the endometrium is commonly used as the 'best standard' to indicate endometritis (Bourke *et al.,* 1997). However, endometrial biopsy is an invasive technique that requires special equipment and laboratories. In this study, the contrast between the diagnosis of endometritis with a bacteriological examination and a biopsy was significant. Furthermore, there was no significant difference between the three methods combined in sensitivity and specificity versus histopathological examination. Combining the diagnostic methods would increase confidence in correctly diagnosing mares with endometritis (Riddle *et al.,* 2007; Overbeck *et al.,* 2011).

As a pretreatment with antibiotics, intrauterine lavage was performed in the current study. Uterine lavage is a popular treatment for endometritis. Antibiotic infusions should be preceded by uterine irrigation because exudate in the uterine lumen may inactivate or dilute an infused antibiotic (LeBlanc and Causey, 2009). Besides promoting physical clearance, uterine lavage provokes endometrial stimulation with the subsequent rise of neutrophil migration to the uterine lumen (Asbury and Lyle, 1993). Intrauterine antibiotic treatments should have a broad spectrum to successfully treat all components of the mixed aerobic and anaerobic systems (Ricketts and Mackintosh, 1987). Used antibiotics should be water-soluble, completely absorbable, and non-irritant, so as not to encourage colonization with more resistant organisms such as *Pseudomonas spp*. or fungi (Ricketts, 1995). Antibiotics are one component often used in the treatment of endometritis (Perkin, 1999). In this study, 28.6% of the animals treated with Metricure® (Cephapirin 500mg) were pregnant. Cephapirin is a first-generation cephalosporin that has a wide spectrum of activity against gram-positive and gram-negative organisms (Donowitz and Mandell, 1988). Cephalosporins are semi-synthetic derivatives of metabolic products of the fungus *Cephalosporium acremonium* (Papich, 1984). Cephapirin produces its bactericidal effect by inhibiting cell wall synthesis. Its action is only effective in actively growing cells (Chambers *et al.,* 1998). To the best of our knowledge, this is the first record for using cephapirin for the treatment of endometritis in equine. The acidic pH of the preparation was buffered by the addition of 5 ml of 8.4% sodium bicarbonate.

The conception rates of mares treated with an intrauterine infusion of procaine penicillin+Gentamicin sulfate, ceftiofur sodium, and Metricure® were 75%, 50%, and 28.6%, respectively. Ceftiofur is a late-generation cephalosporin that is active against a wide range of bacterial pathogens. Ceftiofur sodium has demonstrated activity against aerobic bacterial isolates from mares with endometritis (Luchsinger and Ricketts,

2000). In the Metricure group, although mare did not conceive, bacteriological swabs from two mares (28.6%) were negative after treatment. Moreover, biopsies taken post-treatment did not show any deterioration of the endometrial architecture.

CONCLUSION

Endometrial biopsy is not always necessary to diagnose endometritis in mares. Instead, a high degree of reliability can be given to the use of cytological and bacteriological assessments of the endometrium in addition to ultrasonography assessments. Results show that cephapirin (Metricure®) might be used successfully as a one-time infusion antibiotic intrauterine therapy in mares with endometritis.

DECLARATION OF INTERESTS

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

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تقييم طرق التشخيص والعلاج البديلة المختلفة لالتهاب بطانة الرحم في الأفراس العربية

عبدالرحمن الحيدر

الملخص العربي

هدفت هذه الدراسة إلى تقييم مدى موثوقية الأساليب المختلفة لتشخيص وعلاج التهاب بطانة الرحم في الأفراس العربية. تم إخضاع الأفراس لتقييم حالة الجسم، وتشكل العجان، وفحص الموجات فوق الصوتية، وجمع مسحات الرحم ومسحات غسل منخفضة الحجم بالإضافة إلى خزعة بطانة الرحم. تم اختيار الأفراس عشوائيًا لثلاثة أنظمة علاجية مختلفة داخل الرحم وتمت خدمتهم في الشبق التالي بعد العلاج. أشارت النتائج إلى أن نسبة التشكل غير الطبيعي للفرج تزداد مع زيادة عمر الحيوانات، إلى جانب انخفاض درجة الجسم. تم عزل البكتيريا والفطريات من 83.64% من الأفراس العربية. وكانت المسحات المباشرة متطابقة بشكل إيجابي في 36٪ مع غسل الرحم منخفض الحجم. تناقضت الفحوصات البكتريولوجية والموجات فوق الصوتية في 71% من الحالات (P <0.05)، بينما تطابقت النتائج البكتريولوجية والخزعة في 86.2 .(P <0.05) %كانت المسحات المأخوذة من الغسل منخفض الحجم أكثر موثوقية من المسحات المباشرة في تشخيص التهاب بطانة الرحم. اتفقت النتائج الخلوية مع فحص الموجات فوق الصوتية، في حين اختلفت نتائج الموجات فوق الصوتية مع درجة الخزعة في تشخيص التهاب بطانة الرحم .(P <0.05)كانت معدلات الحمل للأفراس المعالجة بالتسريب داخل الرحم من البنسلين + الجنتاميسين والسيفتيوفور والسيفابيرين 75% و50% و28.6% على التوالي. في الختام، يمكن استخدام التقييم الخلوي والبكتريولوجي لبطانة الرحم، إلى جانب فحص الموجات فوق الصوتية، بدرجة عالية من الثقة لتشخيص التهاب بطانة الرحم في الفرس دون الحاجة إلى خزعة بطانة الرحم. على الرغم من أن معدل الحمل منخفض عند حقن سيفابيرين مرة واحدة داخل الرحم، إلا أنه يمكن استخدامه بنجاح كعلاج داخل الرحم لمرة واحدة في الأفراس المصابة بالتهاب بطانة الرحم.

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