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Research Article

CLINICAL APPRAISAL OF INFERTILE MARWARI MARES USING ULTRASONOGRAPHY, ENDOMETRIAL CYTOLOGY AND BACTERIOLOGY

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ABSTRACT

Thirty infertile Marwari mares anestrusi (n=14) and repeat breeder (RB; n=16) subjected for ultrasonography, endometrial cytology, uterine bacteriology and antibiotic sensitivity test. Ultrasonographically the uterus and ovaries were evaluated for presence of intra uterine fluid, uterine oedema, and follicles on ovaries. The presence of intra uterine fluid indicated severe endometritis (6/16) in repeat breeding mares. The homogenous uterus was more ($P<0.05$) frequently observed in anestrus mares. In contrast, hyperoedematous uterus was observed more ($P<0.05$) in repeat breeding mares. The uterine oedema scores of RB were associated with oestrus and inflammation/infection. The cytological endometritis was positive in 46.66 % infertile mares. Further, the most frequently isolated bacteria were *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. in all infertile mares. The most sensitive and resistant antibiotics were found to be chloramphenicol and amoxycillin clavulanic acid, respectively. It is concluded that ultrasonography is vital in evaluating infertile mares along with cytology.

Keywords: Anestrus, Cytology, Endometritis, Mare, Repeat Breeding, Ultrasonography

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Infertility in the mare is a significant problem impairing regular foal production (Ginther, 1985). It may be caused by infection, hormone deregulation, and managemental factors (Threlfall, 1980). The *Streptococcus equi* subsp. *zooepidemicus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* accounted for endometritis (Brito and Barth, 2003). However, there is no gold standard diagnostic sample or method that is quick to perform, economical, and returns rapid results in endometritis. On clinics, the infectious endometritis in mare is screened using a guarded culture swab and cytology brush for microbial culture and cytologic evaluation, respectively. Variations in different blood biochemicals are also responsible for infertility in animals. Thus, reviewed literature revealed that infertility is a multifactorial condition requiring a planned study. Therefore, the present study was planned, to investigate infertility in Marwari mares, to know whether any etiological and clinical similarities exist between infertile conditions or not. The study outcome will help the clinician to formulate a therapeutic regimen comprising antibiotics, anti-inflammatory, ecbolics and nutritional supplements for both RB and anestrus mares.

MATERIAL AND METHODS

The study involved 30 infertile mares comprising of anestrus (n=14) and repeat breeding (RB, n=16) mares. The mares that failed to show the external visual signs of oestrus during the breeding season were considered as anestrus mares. The mares that failed to conceive after repeated live coverings by the fertile stud during the breeding season were considered as repeat breeding mares. The mean age of anoestrus (n=14) and repeat breeder (n=16) mares was 6.46 ± 0.89 (3-16) and 11.56 ± 1.36 (6-25) years, respectively. The 5th and/or 6th day of estrus was preferred for live coverings irrespective of estrus duration. All the mares were investigated using ultrasonography, endometrial cytology, uterine bacteriology, and antibiotic sensitivity. The blood serum biochemicals (organic and inorganic elements, enzymes and hormones) were also estimated.

The trans-rectal ultrasonography (Sonosite, Titan Ltd, Hitchin, United Kingdom, 5-10 MHz) was performed to evaluate the uterus for presence or absence of intrauterine fluid (IUF), oedema (No-homogenous, mild-difficult to identify the fold, normal-cartwheel and hyperoedema-hyperechoic folds) and ovaries for presence or absence of largest follicle (≥ 2.5 cm) (Fig. 1).

The samples for endometrial cytology were collected using sterile cytobrush attached with guarded stainless steel styleted catheter from the area of the uterine body by rotating the cytobrush against the endometrium. The smear was prepared by rolling the cytobrush on a clean glass microscope slide. The dried smear was fixed using methanol for one minute and allowed to air dry followed by staining with field stain (Eosin for 55 seconds and Methylene blue for 45 seconds). The dried smear was evaluated according to Riddle *et al.* (2007) to assess the cytological endometritis (Fig. 2).

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The bacteriology and antibiotic sensitivity test (ABST) samples were also collected using sterile cytobrush attached with guarded stainless steel styleted catheter. The cytobrush was immediately transferred to a sterile container for shipping to the laboratory in 30 minutes. The collected sample was streaked on brain heart infusion agar (BHI)/Nutrient Agar and kept for incubation at 37º C for 24 hours. The sample for any visible growth after incubation was further differentiated by gram staining. Based on gram staining, further selective isolation was carried out on selective media: Mac-conkey Agar, Eosin methylene blue agar, Mannitol salt agar, Nutrient agar, Sheep Blood Agar and Polymyxin-Acriflavin-Lithiumchloride-Ceftazidime-Aesculin-Mannitol (PALCAM) agar. The incubated culture plates at 37° C were examined at 24 hours and 48 hours for the presence of pathogenic organisms. The further identification of recovered isolates, i.e., gram-positive and gram-negative organisms, were subjected to biochemical tests like catalase, oxidase, indol, methylene red, voges-proskauer (VP), citrate, capsule stain, and methylene blue stain, motility test and urease test. The bacterial isolates were subjected to *in vitro* antibiotic sensitivity test as per the method of Bauer *et al.* (1966). The antibiotic discs used for antibiotic sensitive test were amikacin, amoxicillin/clavulanic acid, cefoperazone, ceftriaxone, chloramphenicol, enrofloxacin, gentamicin and levofloxacin.

The analysis of frequencies of various investigation parameters (ultrasonography findings, cytology, ABST) associated with anestrus and repeat breeding was made by chi-square test. Further, odd ratio (OR) was calculated for dichotomous variables. The mean difference in the biochemical parameters was check by Independent t-test. Significance was set at 95%. Data analysis was done with SPSS software (IBM® SPSS® statistics, version 20.0).

RESULTS AND DISCUSSION

The ultrasonographic parameters of uterus and ovaries in mares were significantly ($P<0.05$) associated with infertile conditions (RB and anestrus) (Table 1). The 37.50% RB mares had IUF while IUF was absent in all anestrus mares. The ultrasonographically homogenous uterus was more ($P<0.05$) frequently observed in anestrus, whereas hyperoedematous uterus was more ($P<0.05$) in RB (7/16) in the present study due to endometritis, infection and presented during oestrus. However, difficult to identify endometrial folds and cartwheel uterine edema (UE) differed non-significantly between anestrus and RB mares. The presence of largest follicle (Fig. 1f) was more ($P<0.05$) frequently observed in repeat breeder (16/16) as compared to anestrus (4/14). The ultrasonographic examination of anestrus mares revealed 4/14 mares with cartwheel UE and largest follicle on ovaries due to silent oestrus. The most anestrus mares (9/14) had either homogenous UE or difficult to identify uterine fold indicates true anestrus condition corroborated with Samper (2010).

The RB mares with severe endometritis showed the presence of IUF corroborated with earlier workers (Burleson *et al.*, 2010, Kouider *et al.*, 2017). The IUF 2.0 cm during the ovulatory period is associated with decreased pregnancy rates (Brinsko *et al.*, 2003). Therefore, presence of < 1.0 cm IUF may not be conclusive evidence of inflammation (De Borba *et al.*, 2012). The presence of IUF might be due to decreased myometrial contractions (Troedsson *et al.*, 1997) and limited mobility (LeBlanc, 2008). The observed uterine oedema corroborated with earlier description/classifications (Samper, 1997). The high frequency of UE in RB mares at oestrus in the present study may be due to bacterial infection and inflammation (Table 2). The endometrial edema score is a robust diagnostic indicator of subclinical infection (Al-Fatlawy, 2017). The ultrasonography can reduce the number of breeding per mare by combining the information of UE, follicle size on ovary and early diagnosis of abnormality (Samper, 1997).

There was significant ($p<0.05$) association of cytological endometritis and infertility (Table 2). Out of 14 anestrus mares, 11 had no cytological endometritis, whereas, out of 16 RB mares, 11 mares had cytological endometritis. Further, 14.29% (2/14) anestrus mares had severe inflammation, while the corresponding figure was 50% (8/16) in RB mares. The endometrial cytology was used for diagnosis of endometritis in mares at different stages of reproduction (Riddle *et al.*, 2007, Bohn *et al.*, 2014, Kozdrowski *et al.*, 2015).

The cytological results obtained from cytobrush are similar to those obtained from an endometrial biopsy (Buczkowska *et al.*, 2014). The mares with positive cytology had bacterial infections (Knudsen, 1964). Contrarily, the negative cytology in mares with bacterial infection was also reported (Brook, 1985). Similarly, all the bacteriologically positive repeat breeder mares didn't find positive for cytology during the study. The intra-uterine fluid was associated with neutrophils on cytology and not with microorganisms (Burleson *et al.*, 2010). The severe endometritis in repeat breeder mares was due to infection under the study. The field stain technique used in

Table 1

The findings of ultrasonography of uterus and ovaries in infertile Marwari mares.

	Criteria	Anestrus (n=14)	RB (n=16)	Significance	Odd Ratio (95%) CI
IUF	Present	0% (0)[a]	37.50%(6)[b]	Chi-Square=6.563, df=1, P=0.010	0.05 (0.00-1.10)
	Absent	100.00% (14)[a]	62.50% (10)[b]		
UE	Homogenous	50.00% (7)[a]	6.25% (1)[b]	Chi-Square=13.211, df=3, P=0.004	-
	Difficult to identify fold	14.28% (2)[a]	31.25% (5)[a]		
	Cartwheel	35.72% (5)[a]	18.75% (3)[a]		
	Hyperedema	0% (0)[a]	43.75% (7)[b]		
Presence of Largest Follicle	Yes	28.57% (4)[a]	100.00% (16)[b]	Chi-Square=17.143, df=1, P=0.001	0.01 (0.00-0.27)
	No	71.43% (10)[a]	0% (0)[b]		

Note: Figures in parenthesis indicates numbers of mares: Numbers (frequency) bearing superscript (a, b) differs significantly within row (P<0.05)RB=repeat breeder; IUF= intrauterine fluid; UE=uterine edema

Table 2

The criteria and observations of endometrial cytology in Marwari mares.

Severity of inflammation	Anestrus (n=14)	RB(n=16)	Significance
No inflammation (0-2 neutrophils/high power field)	78.57% (11)[a]	31.25% (5)[b]	Chi-Square=6.747, df=2, P=0.034
Moderate inflammation (2-5 neutrophils/high power field)	7.14% (1)[a]	18.75% (3)[a]	
Severe inflammation (>5 neutrophils/high power field)	14.29% (2)[a]	50.00% (8)[b]	

Note: Figures in parenthesis indicates numbers of mares: Numbers (frequency) bearing superscript (a, b) differs significantly within row (P<0.05)

Table 3

The antibiotic sensitivity pattern of infertile Marwari mares.

Antibiotic	Anestrus (n=6)				RB (n=16)				Overall (n=22)			
	S	IM	R	Sig	S	IM	R	Sig	S (n= 62)	IM (n= 36)	R (n=78)	Sig
Amikacin	1[a]	1[a]	4[a]	Chi-Square=30.22, df=14, P=0.007	2[a]	4[a]	10[a]	Chi-Square=65.25, df=14, P=0.001	3[a]	5[ab]	14[b]	Chi-Square=90.17, df=14, P=0.001
Gentamicin	2[a]	2[a]	2[a]		7[a]	6[a]	3[a]		9[ab]	8[b]	5[a]	
AmoxyClav	0[a]	0[a]	6[a]		1[a]	0[a]	15[b]		1[a]	0[a]	21[b]	
Levofloxacin	4[a]	0[a]	2[a]		5[a]	5[a]	6[a]		9[a]	5[a]	8[a]	
Enrofloxacine	4[a]	0[a]	2[a]		10[a]	2[a]	4[a]		14[a]	2[b]	6[b]	
Ceftriazone	0[a]	2[b]	4[ab]		2[a]	6[a]	8[a]		2[a]	8[b]	12[b]	
Cefoperazone	1[a]	1[a]	4[a]		2[a]	7[b]	7[ab]		3[a]	8[b]	11[ab]	
Chloramphenicol	6[a]	0[ab]	0[b]		15[a]	0[b]	1[b]		21[a]	0[b]	1[b]	

Note: Figures in parenthesis indicates numbers of mares, S=Sensitive; IM=Intermediate; R=Resistant

the present study for cytological assessment proved satisfactory. Thus, cytology is an easier and quicker method to diagnose uterine inflammation (46.66%, 14/30).

There was significant (P<0.05) association of the bacterial growth and infertile condition in mares. The 42.86% (6/14) anestrus and 100% (16/16) RB mares showed bacterial growth. In anestrus mares, *E. coli* (4/6), *Proteus* spp. (1/6) and *Staphylococcus* spp. (1/6) were isolated, whereas, in RB mares, *Bacillus* spp. (1/16), *Corynebacterium* spp. (1/16), *E. coli* (7/16), *Klebsiella* spp. (3/16), *Listeria* spp. (1/16) and *Staphylococcus* spp. (3/16) were isolated. The most frequently isolated bacteria were *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. The antibiotic sensitivity pattern was given in Table 3. The most sensitive and most resistant antibiotics were chloramphenicol and amoxycillin clavulanic acid, respectively.

The combination of gram-negative and gram-positive bacterial growth is reported in mares suffering from metritis (Ferrer and Palomares, 2018). The *E. coli* is maximally isolated bacteria from mare reproductive tract in previous

Table 4

The level of estimated biochemicals (Mean ± SE) from infertile mares.

Parameters	Anestrus (n=14)	RB (n=16)	P-value
Organic and Inorganic elements			
Total Protein (g/dL)	5.84 ± 0.22 (4.30-7.51)	6.22 ± 0.18 (5.10-7.74)	0.667
BUN (mg/dL)	17.57 ± 0.43 (14.90-20.20)	18.58 ± 0.72 (14.50-26.70)	0.233
Cholesterol (mg/dL)	89.54 ± 3.61(66.60-126.20)	88.59 ± 2.58 (65.40-98.80)	0.674
Calcium (mg/dL)	10.07 ± 0.24 (8.70-12.00)	10.51 ± 0.18 (9.00-11.70)	0.592
Phosphorus (mg/dL)	2.56 ± 0.21[a] (1.70-4.10)	2.66 ± 0.12[b] (1.80-3.30)	0.045
Enzymes			
AST (U/L)	158.15 ± 13.10	198.55 ± 15.83	0.542
ALT (U/L)	9.18 ± 0.53	9.88 ± 0.65	0.269
Hormones			
Progesterone (ng/ml)	0.81 ± 0.29	0.77 ± 0.17	0.496
Cortisol (nmol/l)	239.34 ± 17.36[a]	303.04 ± 55.06[b]	0.001

Note: Superscript (a, b) differs significantly within row(P<0.05)

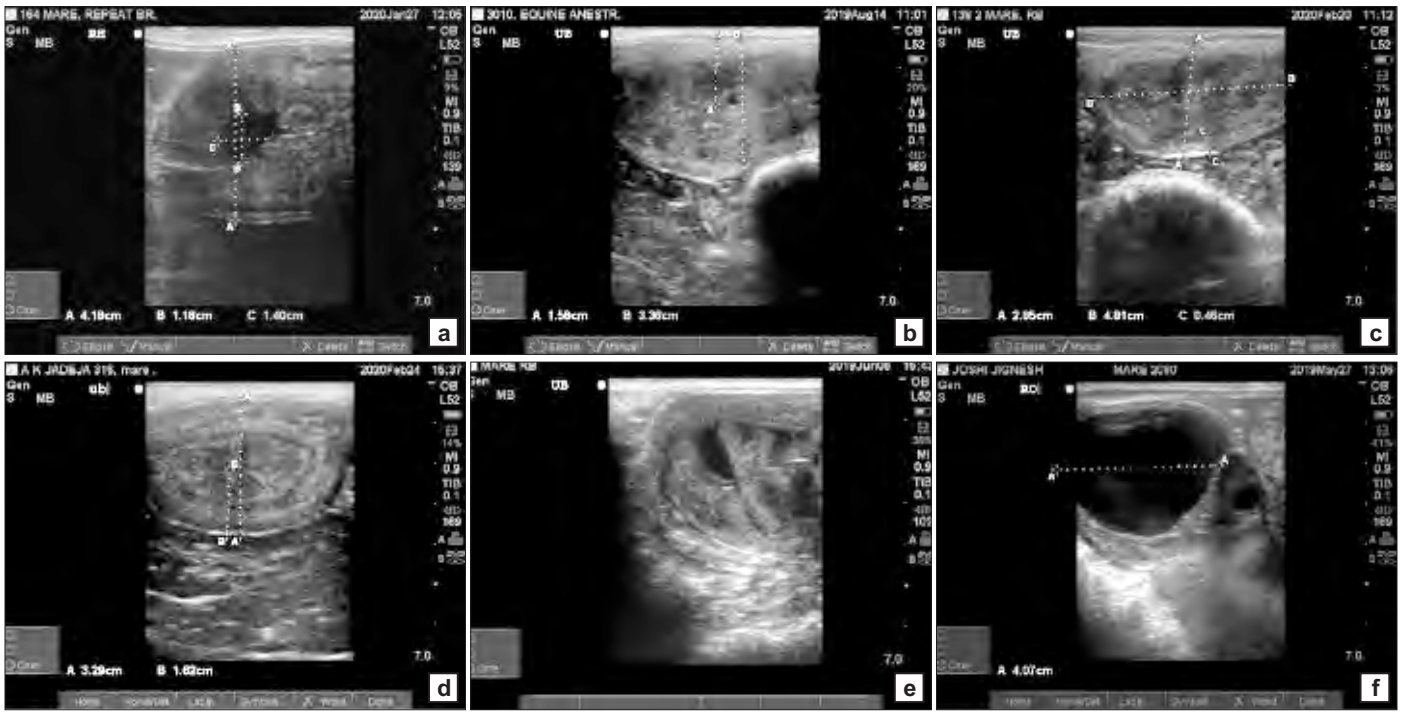

Fig. 1. The ultrasonographic findings of uterus and ovary **(a)** Intra uterine fluid (IUF); **(b)** No (homogenous) uterine oedema; **(c)** Mild uterine edema (difficult to identify folds); **(d)** Normal (cartwheel) uterine edema; **(e)** Hyper uterine edema; **(f)** Presence of largest follicle

study (Davis *et al.*, 2013). The intra-uterine fluid was more frequently linked with beta-haemolytic *Streptococcus*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, or yeast (Burleson *et al.*, 2010). The gram-positive and negative bacteria showed high susceptibility to antibiotics such as cefotaxime, marbofloxacin and enrofloxacin (Benko *et al.*, 2015). The uterine contamination with pathogenic microflora negatively affects the hormonal regulation of oestrus and result infertility in mare (Benko *et al.*, 2015). The serum biochemicals differed non significantly except significantly (P<0.05) higher cortisol in RB than anestrus mares (Table 4) in the study.

CONCLUSION

The intrauterine fluid and higher uterine edema were most corresponding to RB compared to anestrus mares. Further, ultrasonography helped to identify the silently cycling mares based on presence of ovarian follicle and uterine edema. The repeat breeding mares with positive bacterial isolates were not necessarily positive for cytology. The most common bacterial isolate was *E. coli* and the most sensitive antibiotic was observed to be chloramphenicol in infertile Marwari mares.

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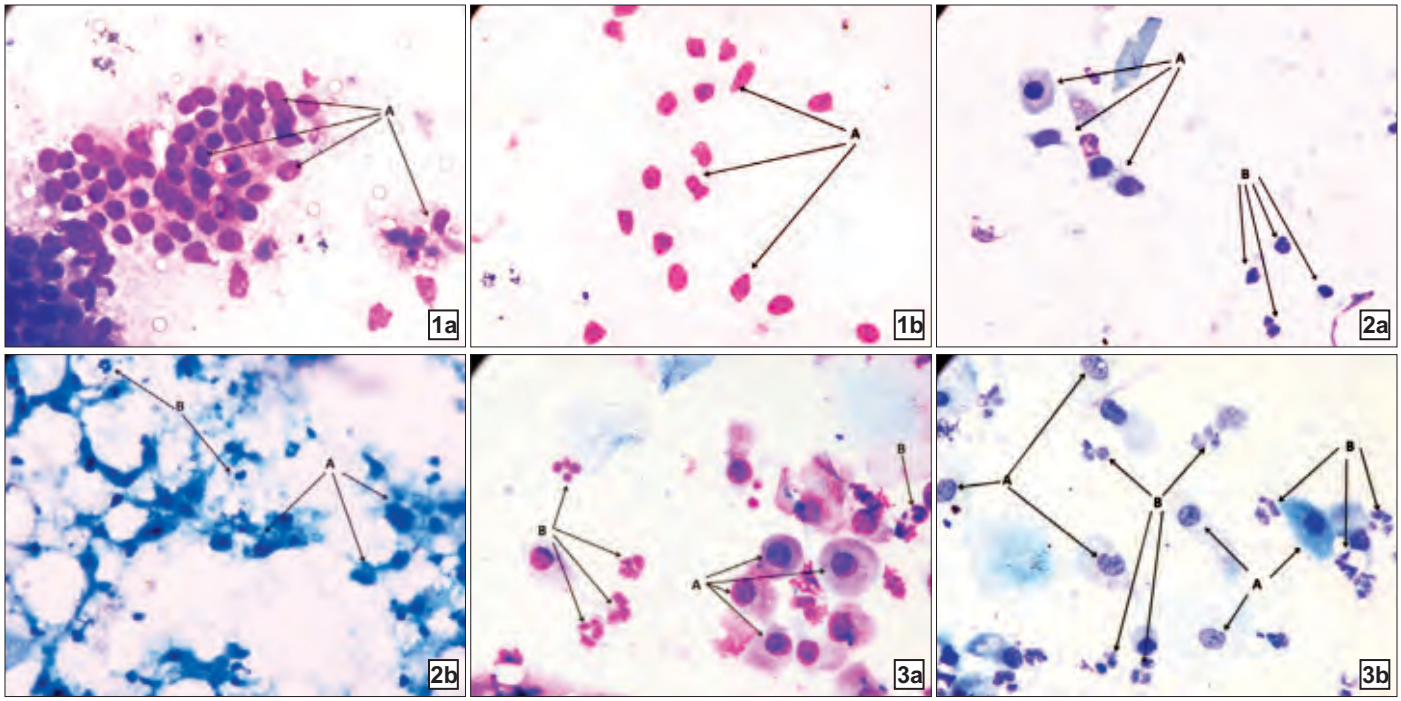

Fig. 2. The cytological assessment (100X) of endometrial smear of anestrus (a) and repeat breeder (b) mares for inflammation (1-No Inflammation; 2- Moderate Inflammation; 3-Severe Inflammation) **Note:** A-Endometrial Cells; B-Polymorphonuclear cells

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REFERENCES

Al-Fatlawy, H.J. (2017). Prevalence of pneumovagina among subfertile Arabian mares in Al-Najaf province/Iraq. *Mirror Res. Vet. Sci. Anim.* **6(3)**: 40-45.

Bauer, M.D., Kirby, M.D. and Turck, M.D. (1966). Antibiotic Susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**: 493-496.

Benko, T., Boldizar, M., Novotny, F., Hura, V., Valocky, I., Dudrikova, K. Karamanova, M. and Petrovic, V. (2015). Incidence of bacterial pathogens in equine uterine swabs, their antibiotic resistance patterns, and selected reproductive indices in English thoroughbred mares during the foal heat cycle. *Vet. Med.* **60(11)**: 613-620.

Bohn, A.A., Ferris, R.A. and McCue, P.M. (2014). Comparison of equine endometrial cytology samples collected with uterine swab, uterine brush, and low volume lavage from healthy mares. *Vet. Clin. Pathol.* **43(4)**: 594-600.

Brinsko, S.P., Rigby, S.L., Varner, D.D. and Blanchard, J.L. (2003). A practical method for recognizing mares susceptible to post-breeding endometritis. *Ame. Assoc. Equ. Pract.* **49**: 363-365.

Brito, L.F.C. and Barth, A.D. (2003). Endometritis in mares. *Large Anim. Vet. Rounds.* **3(9)**: 1-6.

Brook, D. (1985). Cytological and bacteriological examination of mares endometrium. *J. Equine Vet. Sci.* **5**: 16-22.

Buczkowska, J, Kozdrowski, R., Nowak, M., Ras, A. and Mrowiec, J. (2014). Endometrosis significance for horse reproduction, pathogenesis, diagnosis, and proposed therapeutic methods. *Pol. J. Vet. Sci.* **17(3)**: 547-554.

Burleson, M.D., LeBlanc, M.M., Riddle, W.T. and Hendricks, K.E.M. (2010). Endometrial microbial isolates are associated with different ultrasonographic and endometrial cytology findings in Thoroughbred mares. *Anim. Reprod. Sci.* **121(1-2)**: 103.

Davis, H.A., Stanton, M.B., Thungrat, K. and Boothe, D.M. (2013). Uterine bacterial isolates from mares and their resistance to antimicrobials: 8,296 cases (2003-2008). *J. Am. Vet. Med. Assoc.* **242(7)**: 977-983.

De Borba, E.V.C., Camozzato, G.C., Malschitzky, E., Bustamante-Fillro, I.C., Martins, A.A., Mattos, R.C. and Neves, A.P. (2012). Is the presence of uterine fluid a reliable indicator of endometrial inflammation. *Pferdeheilkunde.* **28(1)**: 27-29.

Ferrer, M.S. and Palomares, R. (2018). Aerobic uterine isolates and antimicrobial susceptibility in mares with post-partum metritis. *Equine Vet. J.* **50(2)**: 202-207.

Ginther, O.J. (1985). Dynamic physical interactions between the equine embryo and uterus. *Equine Vet. J.* **3**: 41-47.

Knudsen, O. (1964). Endometrial cytology as a diagnostic aid in mares. *Cornell Vet.* **54**: 415-422.

Kouider, Z. El. A., Benallou, B. and Houari, H. (2017). Ultrasonographic and cytological diagnosis of endometritis in the mare. *Glob. Vet.* **19(4)**: 586-589.

Kozdrowski, R., Sikora, M., Buczkowska, J., Nowak, M., Ras, A. and Dzieciol, M. (2015). Effects of cycle stage and sampling procedure on interpretation of endometrial cytology in mares. *Anim. Reprod. Sci.* **154**: 56-62.

LeBlanc, M.M. (2008). When to refer an infertile mare to a theriogenologist. *Theriogenol.* **70**: 421-429.

Riddle, W.T., Leblanc, M.M. and Stromberg, A.J. (2007). Relationship between uterine culture, cytology and pregnancy rates in a thoroughbred practice. *Theriogenol.* **68**: 395-402.

Samper, J.C. (1997). Ultrasonographic appearance and the pattern of uterine edema to time ovulation in mares. *Procedding- Ame. Assoc. Equ. Pract.* **42**: 189-191.

Samper, J.C. (2010). A review of a practitioner's perspective on endometrial edema. *Pferdeheilkunde.* **26(1)**: 14–18. https://doi.org/10.21836/PEM20100103.

Threlfall, W.R. (1980). Diagnosis and treatment of infertility in the mare. *Vet. Med. Small Anim. Clin.* **75(3)**: 483-486.

Troedsson, M.H.T. (1997). Therapeutic considerations for mating-induced endometriti. *Pferdeheilkun.* **13**: 516-520.