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Therapeutic management of marwari mares suffering from bacterial endometritis vis-à-vis antimicrobial sensitivity

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ABSTRACT

Inappropriate use of antibiotics to treat infectious endometritis in mares can lead to increased antimicrobial resistance (AMR) and, ultimately, hinder the success of achieving pregnancy. This study aims to investigate the diagnosis and treatment of infectious endometritis in sub-fertile Marwari mares. The mares with a reproductive history of either failure of conception upon repeated covering or failure to exhibit estrus were investigated through trans-rectal real-time B-mode ultrasonography for intra uterine fluid-IUF, endometrial cytology-EC and bacteriology. The 61 mares (repeat breeder-50, anoestrus-9 and silent estrus-2) with bacterial uterine infection were subjected to pre-breeding treatment which comprised of sensitive antibiotics (3 g Chloramphenicol/200mg Gentamicin/3 g Ampicillin Sulbactum diluted in 50ml normal saline, intrauterine), ecbolic (25IU i/m, Inj. Oxytocin), and anti-inflammatory drugs (500mg PO, Bol. Tolfenamic Acid, bid) for three days during estrus. The mares were naturally covered by proven stallion, and pregnancy was confirmed by transrectal ultrasound examination on Day 30 post mating. The results revealed that 62.88% of mares had bacterial uterine infections and 69 isolates, with the highest frequency of *E. coli* (36.23%) and *Staphylococcus* spp. (30.43%). The chi-square analysis revealed a significant association of uterine infection with IUF and EC. The study further identified chloramphenicol and gentamicin were the most sensitive antibiotics against the bacterial isolates. The pre-breeding therapeutic management of mares resulted in a 54.01% overall pregnancy rate (48.00, 77.78 and 100.00% in RB, anoestrus and silent estrus mares, respectively). The study's findings indicate that bacterial infections are present not only in repeat breeder mares but also in mares experiencing anestrus and silent estrus. This finding highlights the widespread prevalence of bacterial infections among different types of sub-fertile mares, emphasizing the necessity for comprehensive management strategies to tackle this issue. This study highlights the importance of pre-breeding bacterial investigation and treatment to improve fertility and minimize rise of AMR in equine industry.

Keywords: Antibiotic sensitivity, Antimicrobial resistance (AMR), Endometritis, Marwari Mare, Pregnancy

The hostile uterine environment due to endometritis is the primary cause of subfertility in the mare (Liu and Troedsson 2008). Endometritis is the inflammation of the uterine endometrium, which has been attributed to bacterial colonization and infection of the uterus (Trundell 2020) and reported to be the third most common clinical disease in the equine (Troedsson 1999). The most common isolates reported in the reviewed literature were *Escherichia coli, Streptococcus* species, *Pseudomonas aeruginosa, Klebsiella* species and *Staphylococcus* species (Barbary *et al.* 2016, Díaz-Bertrana *et al.* 2021). In general, endometritis can be diagnosed using transrectal ultrasound and uterine sampling (swab/cytobrush/low-volume lavage/biopsy) for microbiological, cytological, and histological evaluations (Katila 2016). The intrauterine fluid (IUF) is a hallmark of endometritis in mares (LeBlanc 2010). Cytobrush is a fast, non-invasive, user-friendly, and inexpensive tool to collect uterine samples for microbial culture and cytology (Riddle *et al.* 2007). Bacteriology and in-vitro sensitivity tests are prerequisites to establish the most appropriate antibiotic therapy. The fundamental principles of treating infertile mares remain the same over time: correcting anatomic causes, cleaning the uterine environment, and treating pathogens. Treatment of infectious endometritis in mares involves the use of antibiotics, often in combination with other approaches such as uterine lavage and oxytocin, to facilitate uterine clearance (LeBlanc and Causey 2009, Canisso *et al.* 2020). The antimicrobial treatment modulates the aerobic vaginal microflora of infertile mares, and multi-drug resistance bacterial strains are common in equine reproductive tract infections (Singh 2009). The antimicrobial resistance (AMR) in the microbial flora of the equine reproductive tract initiates from antibiotics administered *in utero*,

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parenterally in semen extender, and resistance genes in environmental bacteria/bacteria in personnel (Malaluang *et al.* 2021). The decrease in antibiotic efficacy and rapid development of AMR has also been associated with inappropriate use, over-prescription, overuse or inadequate antibiotic course, and blind treatments. The most preceding studies on equine endometritis have been conducted on thoroughbred (Virendra *et al.* 2023), Arabian (El-Shalofy *et al.* 2021) or warm-blood mares (Vandaele *et al.* 2008), but such studies are scarce in the Marwari mares. A clinical study on infectious endometritis from diagnosis to treatment and pregnancy in Marwari mares is required to recommend antibiotic usage and, thus, minimize and control the AMR. Therefore, the present study aimed to identify uterine microbial isolates and their antimicrobial sensitivities. In addition, efficacy of sensitive antimicrobial therapy on fertility was also evaluated.

MATERIALS AND METHODS

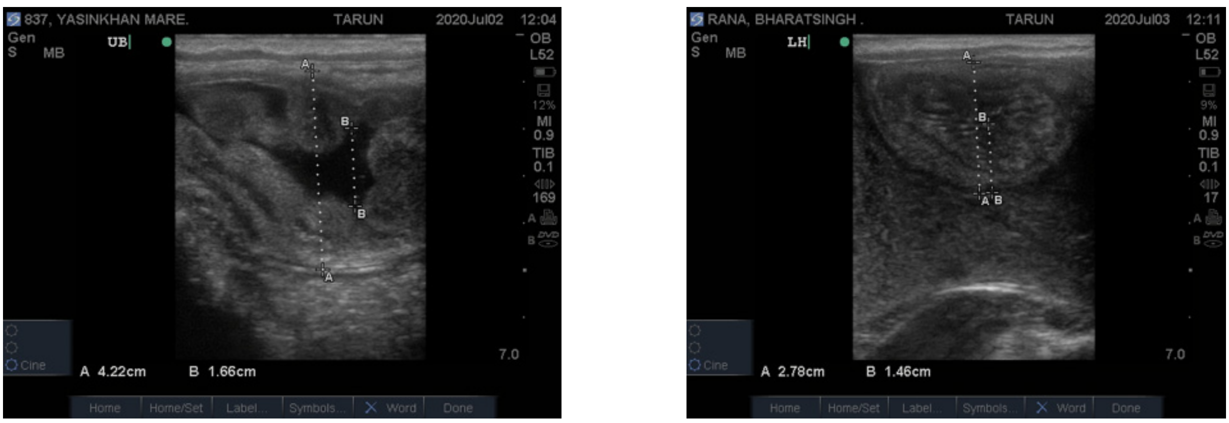
Ethical approval: The experiment was a part of clinical procedure and approved by Institute Animal Ethics Committee (IAEC) *vide no.* VETCOLL/IAEC/2019/15/Protocol-06.

Study Design: Marwari mares (n=97) presented to the university hospital for reproductive problems (Jan 2018 to December 2020) were included in the present study. All mares were aged between 2.5 and 18 years (7.59±4.03), stallfed, regularly dewormed and maintained in good body condition. The reproductive history included either failure of conception after repeated covering (repeat breeder, RB, n=64) or failure to exhibit estrus (anoestrus/silent, n=33). The mares that failed to conceive after repeated coverings during the breeding season were defined as repeat breeding. The mares that failed to show the external signs of oestrus during the breeding season were defined as anoestrus. Hence, these sub-fertile mares were investigated through trans-rectal real-time B-mode ultrasonography, endometrial cytology, and bacteriology (Fotariya *et al.* 2023) for uterine infection and other abnormalities causing sub-fertility. Further, perineal confirmation was also

Fig. 1. Measuring angle of vulvar inclination to calculate caslicks index.

recorded, and the caslick's index ranged from 17 to 275 (Fig. 1). Trans-rectal ultrasonography (10-5 MHz linear array transducer, Sonosite, Titan Ltd, Hitchin, United Kingdom) was performed to detect the intrauterine fluid (IUF, Knutti *et al.* 2000), uterine edema (UE, Samper 2010) and ovarian activity (Ginther *et al.* 2004). The mares with ≥0.50cm IUF on ultrasound were considered to be suffering from endometritis (Fig. 2). The UE showing homogenous or mild-difficult to identify the endometrial fold indicated anoestrus or diestrus stage, normal-cartwheel pattern indicated estrus and hyper-oedema with hyperechoic endometrial folds indicated inflammation and/or infection. The ovaries with follicle ≥20mm and/or *corpus luteum* were considered cyclic mares (n=64). The ovaries with follicle <20mm and absence of *corpus luteum* (CL) were considered anoestrus mares (n=25). Anoestrus mares with CL on ovaries were considered as silent estrus mares (n=8).

The endometrial cytology (EC) and bacteriology samples were collected from the uterine body using separate sterile cytobrushes as described in our previous study (Fotariya *et al.* 2023). The cytobrush was immediately transferred into the sterile container for shipping to the laboratory within 30 minutes for bacteriology and antibiotic sensitivity tests



(a) (b)

Fig. 2. Ultrasound pictures of infertile mares showing: (a) presence and (b) absence of intra uterine fluid (IUF).

(ABST). The collected cytobrush was streaked on brain heart infusion agar (BHI) / nutrient agar and incubated at 37 ºC for 24 h. The bacteria were identified based on gram staining. Further, isolation was carried out on selective media (MacConey Agar, Eosin methylene blue agar, Mannitol salt agar, Nutrient agar, Sheep Blood Agar, and PALCAM agar) and subjected to biochemical tests (catalase, oxidase, indole, methylene red, VP, citrate, capsule stain, and methylene blue stain, motility test, and urease test). The bacterial isolates were subjected to an *in vitro* antibiotic sensitivity test. The antibiotic discs were selected based on antibiotics routinely used by the field veterinarians to treat sub-fertile mares. The antibiotic discs (Himedia laboratory, India) of chloramphenicol, gentamicin, ampicillin sulbactam, enrofloxacin, levofloxacin, ceftriaxone, ciprofloxacin, amikacin, cefoperazone and amoxicillin/clavulanic acid were used. The cytology smear was prepared and stained according to Fotariya *et al.* (2023) and evaluated to assess the cytological endometritis according to Riddle *et al.* (2007) (Fig. 3).

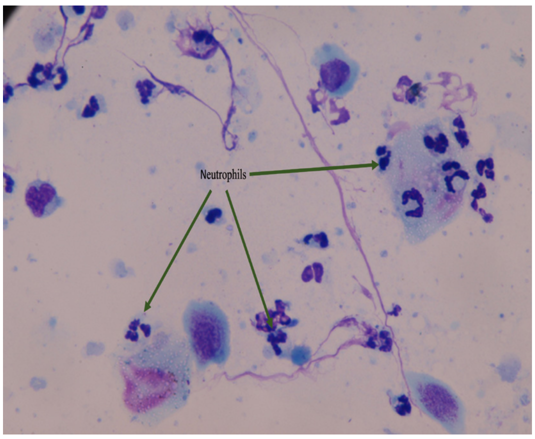
Treatment and fertility: The 61 mares with bacterial uterine infection were subjected to pre-breeding treatment during estrus. The RB mares (n=50) with uterine infection were treated with sensitive antibiotics i.e., 3g chloramphenicol or 200 mg Gentamicin or 3 g ampicillin sulbactum diluted in 50 ml normal saline, intrauterine), ecbolic (25IU i/m, Inj. Oxytocin), and anti-inflammatory drugs (500mg PO, Bol. Tolfenamic Acid, bid) for three days during estrus. The anoestrus mares with uterine infection (n=9) were treated with Buserelin Acetate (10 µg, i/m, bid for five days) and silent estrus mares (n=2) were treated with cloprostenol sodium-synthetic prostaglandin (250 µg, i/m), and further treatment and breeding management was same as for RB mares on induced estrus. All the mares were bred naturally with healthy fertile stallions in the same estrus upon notice of normal uterine cartwheel pattern and absence of IUF on 3rd/4th day of treatment. The ecbolic (25 IU i/m, Inj. Oxytocin) was injected 4-6 h post-breeding in all the mares to prevent fluid accumulation. All the mares were examined 24 hr post-breeding to rule out mating-induced endometritis. Pregnancy was confirmed on day 30th post-breeding using ultrasonography.

Statistical Analysis: Data were collated and presented as percentages for bacterial isolation, cytology, ABST, and pregnancy. Data were analysed with a statistical package (IBM® SPSS® v. 20.0). Response of different antibiotics toward the bacterial isolates was categorised as resistant, intermediate, and sensitive. Proportions of these three categories were compared by chi-square and Z-test with Bonferroni correction. The level of significance was set at $p \leq 0.05$.

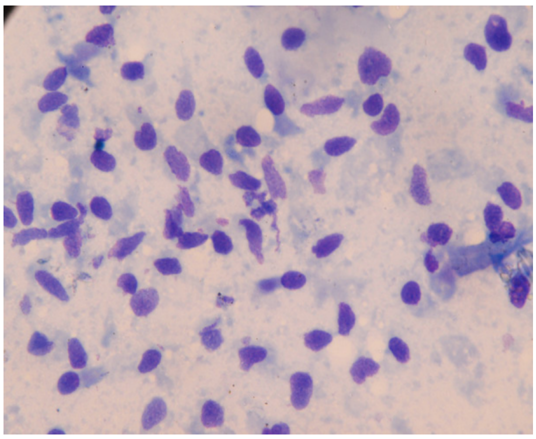
RESULTS AND DISCUSSION

The investigation of sub-fertile Marwari mares revealed 62.88% (61/97) cases having bacterial uterine infections with 69 isolates, 50.52% (49/97) cases of cytological endometritis, and 17.53% (17/97) cases of higher intra-uterine fluid, indicating the importance of each test. In the present study, the incidence of bacterial endometritis was higher (62.88%) compared to the previous study by Virendra *et al.* (2023) who reported 31.25% bacterial endometritis in Thoroughbred mares from various stud farms in India. However, earlier reports by Riddle *et al.* (2007) and Davis *et al.* (2013) have shown that the incidence of bacterial endometritis ranges between 25 and 60%. The higher percentage of bacterial endometritis in the present study might be due to a study on clinical cases referred to clinics rather than from the field. However, the mares from various commercial farms have also been reported to have a very high occurrence of uterine infection (89%) (Díaz-Bertrana *et al.* 2021).

The ultrasonographic parameters of uterus (edema and IUF) and ovaries (follicles and CL) in sub-fertile mares (n=97) were found to be associated with sub-fertile conditions i.e., RB, anestrus and silent estrus. The normal-cartwheel pattern and hyper-oedema endometrial folds with pre-ovulatory follicle on either ovaries were observed in RB mares (n=64) being presented during estrus. The homogenous uterus with inactive ovaries was observed in anoestrus mares (n=25). The 08 anestrus mare revealed



(a)



(b)

Fig. 3. The endometrial cytology : (a) presence of neutrophils-endometritis and (b) absence of neutrophil-no inflammation.

homogenous uterus and CL on ovaries due to silent estrus. Out of 64 RB mares, only 17 (26.56%, 17/64) mares had IUF. None of anoestrus (n=25) and silent estrus (n=8) mares had IUF. The study revealed that 50 out of 64 RB mares had uterine infection which included 15 mares with IUF and 35 mares without IUF. The study also found that 88.24% (15/17) of RB mares with IUF had uterine infection, compared to 74.46% (35/47) of RB mares without IUF. Further, 9/25 anoestrus and 2/8 silent estrus mares also had uterine infection. Thus, total 61 subfertile (50 RB, 9 Anoestrus, 2 Silent estrus) mares had uterine infection. The incidence of bacterial infection was 88.24% and 74.46% in mares with IUF and without IUF in the study. Similarly, Chiba *et al.* (2019) found that there was a notable increase in bacterial prevalence in mares with IUF. Mares with uterine infection of *E. coli* were less likely to have IUF, while uterine infections caused by β hemolytic *Streptococcus*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, or yeast had a higher incidence of IUF (Burleson *et al.* 2010). All mares affected with endometritis do not show IUF on ultrasound screening (Canisso *et al.* 2020). Sometimes, the IUF may be present during estrus without infection or inflammation, possibly due to increased endometrial mucus secretion (Knutti *et al.* 2000). Pathogens associated with IUF were more likely to have neutrophils on cytology, while pathogens not associated with IUF tended to be negative for neutrophils on cytology (LeBlanc 2010). Mares positive for *E. coli, S. aureus* and *Pseudomonas spp*. had fewer cytology positives than mares positive for *Streptococcus* or *Klebsiella* (Burleson *et al.* 2010, Riddle *et al.* 2007). All the cytologically positive mares were also positive for microbial cultures (Mathew *et al.* 2020, Fotariya *et al.* 2023). In contrast to the present study, Díaz-Bertrana *et al.* (2021) has also reported positive cytology but negative bacteriology.

Out of 69 microbial isolates from 61 sub-fertile mares, 58 from RB, 09 from anoestrus and 02 from silent estrus mares were isolated. The uterine samples from 53/61 mares (86.88%) yielded a pure culture whereas only 8/61 mares (13.11%, 7 RB + 01 anoestrus) yielded mixed growth. *Escherichia coli* was isolated in all eight mares, bearing mixed infection with *Staphylococcus* in 07 and *Klebsiella* in 01 cases. The gram-positive (+) and negative (-) bacteria were observed in 24 and 45 isolates, respectively (Fig. 4). The prevalence was highest for *E. coli* (36.23%, 25/69), followed by *Staphylococcus* spp. (30.43%, 21/69), gram negative coccobacilli and *Klebsiella* (Table 1). Díaz-Bertrana *et al.* (2021) also previously observed similar pattern of prevalence with *E. coli* (17.3%), followed by *Staphylococcus* spp. and *Streptococcus* spp. non-haemolytic (15.6% and 13.5%, respectively) from subfertile mares. From the repeat breeder (RB) mares also, the isolated bacterial species were *E. coli* (40.0%), *Staphylococcus* (26.7%), *Streptococcus* (20.0%), and *Klebsiella* (13.3%) by Virendra *et al.* (2023) and β-haemolytic *Streptococci* and *E. coli* by Mathew *et al.* (2020). Thus, most frequent bacterial isolates from problem mares were *Streptococcus spp., Staphylococcus spp.,* and *E. coli* (Díaz-Bertrana *et al.* 2021, Virendra *et al.* 2023). However, *Streptococcus* was not isolated from RB mares in a study by Berwal *et al.* (2006),

Table 1. Frequency of bacterial isolates and cytology in mares

Bacterial Isolate	No. of Isolate (N)	Mares with Positive Cytology in bacterial Uterine infection (%)
E. Coli 17		13 (76.47)
Staphylococcus Spp.	14	14 (100.00)
Gram -vecocobacilli	9	7 (77.78)
Klebsiella Spp.	4	4 (100.00)
Gramm -ve bacilli	2	1 (50.00)
Proteus Spp.	3	2 (66.67)
Bacillus Spp.	1	0 (0.00)
Corynebacterium Spp.	1	1 (100.00)
Listeria Spp.	1	1 (100.00)
Pseudomonas Spp.	1	1 (100.00)
E. Coli + *Staphylococcus* Spp.	7	4 (57.14)
E. Coli + *Klebsiella* Spp.	1	1 (100.00)
Total	69	49 (71.01%)

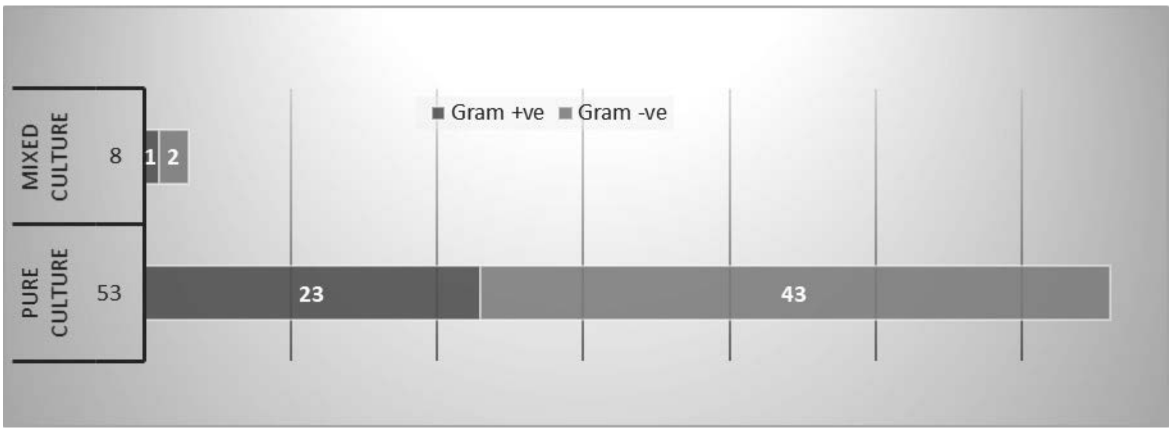


Fig. 4. Bacterial colony and Gram staining (+ve or -ve) of endometrial cytobrush from sub-fertile Marwari mares.

although *E. coli, Klebsiella spp. and Micrococcus spp.* were isolated and identified as causal agents. The uterine culture of the subfertile mares also yielded *Corynebacterium spp.* in the study by Assad and Pandey (2012). Further, Jones (2017) reported the dominant genus in the equine uterus was *Corynebacterium*, followed by *Porphyromonas*, *Enterobacteriaceae*, and *Streptococcus*. In line with this, the *Streptococcus spp*. was also not recovered in the present study. The earlier treatment with antibiotics might have localized the bacterial load or cured the *Streptococcus spp*. infection from the studied mares is also supported by McDonnell and Watson (1992). The localized infection of *Streptococcus spp*. in uterus may not have been collected during the sampling by cytobrush in the study resulting in negative growth for *Streptococcus*. Moreover, it is also possible that the initial culture media used in the study (BHI) might not have allowed *Streptococcus* to grow due to its fastidious nature. The use of bacterial growth media (bActivate) improves sensitivity of traditional diagnostics (Petersen *et al.* 2015) by activating dormant bacteria or in case of improper/poor sampling. Although, *E. coli* and *Staphylococcus spp*. were the most common bacterial isolates in our study. Regarding mixed cultures, the results of the present study are corroborated by Davis *et al.* (2013), who observed *E. coli* as the most frequently isolate along with other microorganisms. In contrast, Díaz-Bertrana *et al.* (2021) reported that *Staphylococcus* was mainly associated with mixed infection. Disparities in frequently isolated bacteria from mares between different studies have been attributed to geographic locations, diverse mare populations, and exposure to various antimicrobial agents (Davis *et al.* 2013). *E. coli* was the most common cause of equine endometritis in the present study and this is very well supported by Virendra *et al.* (2023) and Fotariya *et al.* (2023). All the earlier studies were conducted in repeat breeder mares of different breeds and revealed the presence of bacterial infection but the present study unveils that the anoestrus as well as silent estrus mares are also harbouring the bacterial infection.

According to the results of the present study, chloramphenicol showed the highest sensitivity at 88.41%, followed by gentamicin at 60.87%, and ampicillin sulbactum at 59.42% (Table 2, Fig. 5). On the other hand, the antibiotics with the lowest sensitivity rates were amoxicillin/clavulanic acid at 20.29%, cefoperazone at 21.74%, and amikacin at 28.99%. The sensitivity rates of the other antibiotics tested were enrofloxacin at 53.62%, levofloxacin at 44.93%, ceftriazone at 36.23%, ciprofloxacin at 30.43%, and amikacin at 28.99%. Variations in the sensitivity patterns of isolates from subfertile mares have also earlier been reported and cannot be comparable due to the different geographical locations of the studies and antibiotic usage in those areas (Davis *et al.* 2013, Díaz-Bertrana *et al.* 2021). A study conducted in Maharashtra, India, showed that gentamicin, amikacin, netilmicin sulfate, and ampicillin/sulbactam were the most sensitive (Virendra *et al.* 2023), while a study conducted in North India reported chloramphenicol was most sensitive antibiotic in endometritis mare (Berwal *et al.* 2006). Díaz-Bertrana *et al.* (2021) reported that the most sensitive antibiotics were amikacin (57.3%), cefoxitin (48.6%), and gentamicin (48.3%) in a study conducted on endometriotic mares of Spain. Our previous study on the investigation of infertile Marwari mares revealed that the most sensitive antibiotics were chloramphenicol, enrofloxacin, gentamicin, and levofloxacin (Fotariya *et al.* 2023). The highest sensitivity of bacterial isolates from sub-fertile thoroughbred mares was towards marbofloxacin and cefotaxime (Benko *et al.* 2015). Ciprofloxacin and enrofloxacin were the most effective antimicrobial agents in curing bacterial endometritis, with a 44.40% (8/18) pregnancy rate in Arabian mares (Barbary *et al.* 2016). However, several of the organisms commonly isolated from the uterus of mares with endometritis, such as *Pseudomonas, Klebsiella* and

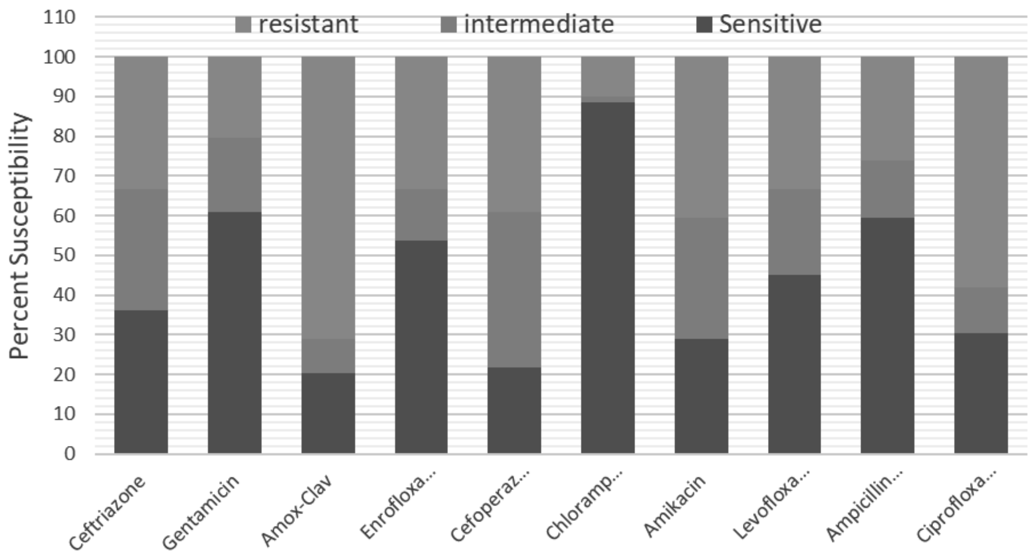


Fig. 5. The frequency of antibiotics tested for sensitivity pattern (%). Chi-Square=159.73, df=18, P<0.001

Table 2. The frequency of antibiotics tested for sensitivity pattern

Antibiotic	Sensitive		Intermediate		Resistant		Total	Sig.
	F	%	F	%	F	%		
Ceftriazone	25[a]	36.23	21[b]	30.43	23[ab]	33.33	69	Chi-Square=159.73, df=18, p<0.001
Gentamicin	42[a]	60.87	13[ab]	18.84	14[b]	20.29	69	
Amox-Clav	14[a]	20.29	6[a]	8.70	49[b]	71.01	69	
Enrofloxacin	37[a]	53.62	9[a]	13.04	23[a]	33.33	69	
Cefoperazone	15[a]	21.74	27[b]	39.13	27[c]	39.13	69	
Chloramphenicol	61[a]	88.41	1[b]	1.45	7[b]	10.14	69	
Amikacin	20[a]	28.99	21[b]	30.43	28[ab]	40.58	69	
Levofloxacin	31[a]	44.93	15[a]	21.74	23[a]	33.33	69	
Ampicillin Salbactum	41[a]	59.42	10[a]	14.49	18[a]	26.09	69	
Ciprofloxacin	21[a]	30.43	8[a]	11.59	40[b]	57.97	69	

F: frequency, Sig: Significance, %: Percentage

E. coli, are considered to be highly resistant to antibiotics such as penicillin (Benko *et al.* 2015). Cefepime may be the choice of antibiotic for most cases of bacterial endometritis in Arabian mares with 100% pregnancy, in the opinion of El-Shalofy *et al.* (2021). The inflamed and infected uterine environment is incompatible for the survival of the embryo when it descends into the uterine lumen at day 6 (LeBlanc and Causey 2009) but if bacteria is detected and treated, the mare's chance of becoming pregnant enhances (Nielsen *et al.* 2008). So, in this study the pre-breeding therapy of sub-fertile mares having bacterial infection resulted in only 54.01% pregnancy rate. The remaining did not conceive and were repeat breeders due to persistent uterine infection. The reduced pregnancy in some treated mares might be due to presence of microorganisms in the cervix even after it is eliminated from the uterus (Chiba *et al.* 2019) or reinfection due to mating or the presence of bacteria from normal genital flora (Hinrichs *et al.* 1990).

The chi-square analysis revealed significant (p<0.05) association of uterine infection with IUF and EC (Table 3). The 80.33% (49/61) endometrial cytology was positive for uterine bacterial infections. The cytology was positive in 40/50 (80.00%) RB mares, 7/9 (77.78%) anoestrus mares and 2/2 (100%) silent mares with uterine infection. The pregnancy rate of 48.00% (24/50), 77.78% (7/9) and 100.00% (2/2) was recorded in RB, anoestrus and silent estrus mares with uterine infection, respectively. The pre-breeding therapeutic management of sub-fertile mares with bacterial uterine infection resulted in 54.01% (33/61) overall pregnancy rate. The remaining 26 RB mares and 2 anoestrus mares did not conceive and were repeat breeders. All the mares of this study with uterine infections were treated with sensitive antibiotics, oxytocin to avoid IUF and an anti-inflammatory drug to minimise uterine inflammation. Typically, three days of intrauterine therapy is sufficient to see a positive outcome to treatment (Trundell 2020). Following contamination/infection, the mare experienced poor uterine evacuation/clearance; hence, the administration of ecbolic agents is required. Oxytocin, a potent ecbolic agent that promotes strong but short-lived uterine contractions, is often used for uterine clearance (Paccamonti *et al.* 1999). Prostaglandin synthesis from endometrial inflammation is inhibited by NSAID (Wolf *et al.* 2012). Beneficial effects of NSAID treatment before breeding with firocoxib (Friso *et al.* 2019) or vedaprofen (Rojer and Aurich 2010) have also been reported. The post-AI treatment of mares with flunixine reduced inflammation, but the pregnancy rate didn't differ between the control and treatment groups (Risco *et al.* 2009). Dexamethasone (Vandaele *et al.* 2008, LeBlanc and Causey 2009) and prednisolone (Dell'Aqua *et al.* 2006), an anti-inflammatory drug could also be used to modulate the uterine inflammatory response to breeding in susceptible mares. Instilling the uterus with antibiotics is also a usual practice, although it may, under some circumstances, irritate the endometrium and interfere with local defence mechanisms (Causey 2006). Previous studies demonstrated intrauterine antibiotic therapy's efficiency in improving pregnancy rates when administered before or after mating or AI (Ricketts 1997).

In the current study, *E. coli* was found to be the most common cause of equine endometritis. Chloramphenicol and gentamicin could be used as primary treatments for equine infectious endometritis while awaiting antimicrobial susceptibility test results. The varying sensitivity patterns highlight the significance of bacteriology and AST prior to treating subfertile mares, as this helps in choosing the appropriate antibiotics for improved outcomes and prevents an increase in antimicrobial resistance.

Table 3. Association of intrauterine fluid (IUF) and endometrial cytology (EC) with uterine infection in sub-fertile mares

Variables Yes (n=17)		Intra uterine fluid (IUF)		Endometrial Cytology (EC)	
		No (n=80)	Yes (n=49)	No (n=48)	
Uterine Infection	Yes (n=61)	15	46	49	12
	No (n=36)	2	34	0	36
Significance		Chi-square=5.67, df=1, p=0.017		Chi-square=58.43, df=1, p=0.001	

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