Diagnostic efficacy of ultrasound, cytology, and uterine culture in mare sub-clinical endometritis

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Abstract

In the present study, the diagnostic effectiveness of endometrial cytology, uterine swab culture, and ultrasonography for endometritis in infertile mares was explored. The study was aimed to figure out endometritis in infertile mares by employing ultrasound (USG), endometrial cytology, and culture isolation of pathogens, and to estimate positive and negative predictive values, sensitivity, specificity, and degree of agreement between the diagnostic methods. Thirty infertile mares were screened using cytobrush cytology to identify polymorphonuclear neutrophils (PMNs), trans-rectal ultrasonography to assess intrauterine fluid and uterine swab culture for bacterial isolation. To estimate sensitivity, specificity, positive and negative predictive values, and Kappa coefficient, a 2 x 2 contingency table was constructed. Out of 30 infertile mares screened, USG revealed 19 (63.33%) had intrauterine fluid, endometrial cytology showed 17 (56.67%) with rich PMNs, and 12 (40%) had bacteria colonies on swab culture (p=0.175). When endometrial cytology was considered "gold standard", the sensitivity and specificity for USG were 100 and 84.61%, and for cultural isolation 70.58 and 100%, respectively. For USG, positive and negative predictive values were 89.47 and 100%, respectively, while for culture isolation, 100 and 72.22%. Kappa coefficient (κ) revealed a nearly perfect (κ=0.86) agreement between USG and cytology, a substantial (κ=0.67) agreement between endometrial cytology and cultural isolation, and a moderate (κ=0.55) agreement between USG and cultural isolation. The findings indicate that the most sensitive method for detecting subclinical endometritic mares is a combination of endometrial cytology and ultrasonography. The association between endometrial cytology and ultrasonography is nearly perfect, making it possible to identify mares with subclinical endometritis.

Keywords: Endometrial cytology, Endometrial swab culture, Endometritis, Infertile mare, Ultrasonography.

Introduction

Endometritis continues to be one of the causes of mare infertility. Early nidation and embryonic development are impeded by inflammation of endometrium, which ultimately leads to embryonic losses and failure of reproduction. Endometritis is extremely important to identify, especially if it is subclinical, since it frequently goes undetected, as it frequently lacks clear clinical signs, costing the equine industry financially (Del Prete *et al.,* 2024). In order to lessen the financial burden on the equine industry, it is crucial to accurately diagnose, determine the etiology, and treat endometritis as soon as possible. It can be challenging for an equine practitioner to diagnose endometritis since different uterine pathogens can have different clinical signs, ultrasonographic results, and laboratory results. Although ultrasonography is more sensitive and requires less experience than rectal palpation, equine practitioners typically use traditional transrectal palpation as part of clinical examination to determine the pathological condition of the uterus (Purohit, 2011; Katila, 2016). However, uterine cytology and bacteriological diagnostic techniques are used to confirm endometritis further. In addition to being simpler, more reliable, and faster than culture isolation, uterine cytology offers additional benefits (Buczkowska *et al.,* 2014).

Additionally, it has been documented that the absence of bacterial isolation neither necessarily eliminate endometritis, nor is always linked to endometrial inflammation in mares (Walter *et al.,* 2012; Buczkowska *et al.,* 2014). When comparing different methods for diagnosing endometritis in mares to the well-established gold standard test, previous studies found a wide range of sensitivity and specificity (Nielsen *et al.,* 2012; Katila, 2016; Mathew *et al.,* 2020; Teixeira-Soares *et al.,* 2022). This illustrates, currently that there is no single technique that can be used to correctly diagnose endometritis in mares. Under field conditions, the equine practitioner adapts the diagnostic test that is easy to use, yields consistent results, and operates at a faster speed (Cocchia *et al.,* 2012). Endometrial cytology to assess polymorphonuclear neutrophils (PMNs) cell infiltration in the endometrial layer, transrectal ultrasonography to assess intrauterine fluid aggregation, and bacterial isolation from uterine swabs are the most widely used diagnostic techniques for endometritis in mares. However, it is crucial to use multiple diagnostic techniques at the same time for accurate diagnosis of endometritis to prevent false positive and false negative cases. Therefore, the current study was designed to identify endometritis in infertile mares by means of culture isolation, endometrial cytology, and ultrasonography. Additionally, the strength of agreement, sensitivity, specificity, and positive and negative predictive values of various diagnostic methods were assessed.

Materials and methods

Selection of animals

The present investigation was executed on a total of 30 infertile mares in estrus (aged between 4-18 years) presented at Veterinary Clinical Complex, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India during March 2020 and June 2022. The mares those did not conceive after natural mating with a stallion of known fertility for thrice or more cycles during the breeding season were included in this study. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the College.

Physical and ultrasonographic examination

General inspection of the vulva (horizontal angle) and perineal region was carried out to check vulvar conformation as a predisposing factor for endometritis. The mare's reproductive tract was evaluated (Fig 1A) using a real time B-mode trans-rectal ultrasound scanner equipped with an 8-10 MHz linear array rectal transducer (MINDRAY, Model: Z5 Vet USG) to determine the presence of varying degrees of fluid in the uterine lumen as a sign of endometritis.

Uterine sample collection

To avoid contamination of the reproductive tract and impeding sample collection, each mare's tail was bandaged and placed aside after being secured in the travis. The adhering debris was then removed by washing the vulva and perineal area with clean water thrice. The vulva was then gently wiped with the disposable tissue paper from the dorsal to the ventral vulvar commissure to dry it while preventing clitoral fossa contamination.

Endometrial cytology

The endometrial samples for cytology were obtained using the cytobrush assembly (Fig 1B), (Mayfair Endocervical brush, Care India Surgicals, Ludhiana, India). Duplicate slides were made from every swabbed sample. Slides were promptly transported to the lab, and stained using the Giemsa stain. Mares with more than five polymorphonuclear leukocytes (PMN) under high power (400x) microscopy fields (Fig 1C) were considered to have an active endometrial inflammation (Katila, 2016).

Endometrial swab culture

Following parting vulvar labia, a sterile cotton-tipped swab soaked in sterile saline fitted over the cytobrush assembly was passed in a manner similar to the endometrial cytology collection in order to collect the endometrial swab samples. The cotton swabbing sample was collected by gently pressing it against the uterine wall and rolling it clockwise and keeping it for 20-30 seconds. Then the swab was subsequently taken away, avoiding

contamination. Samples were then sent to the microbiology lab of the College for the isolation (Fig 1D) of the bacteria.

Statistical analysis

All laboratory and clinical findings were entered into a Microsoft Excel spreadsheet and recorded on data forms. The incidence of sub-clinical endometritis identified by different diagnostic methods was compared by Chi-square test (SPSS Version 16.0, USA) and considered as significant if '$p' \leq 0.05$. A simple 2×2 contingency table was constructed (Table 1) for binary outcomes, and then sensitivity, specificity, and positive and negative predictive values were calculated using the standard formula (Parshall, 2013; Buczkowska *et al.,* 2014). Kappa statistics (SPSS Version 16.0, USA) was carried out using a two-way contingency table to determine the strength of agreement between two diagnostic methods. The strength of agreement was interpreted based on Kappa coefficient (κ) and considered as poor if $\kappa = 0.00\text{-}0.19$, mild if $\kappa = 0.20\text{-}0.39$, moderate if $\kappa = 0.40\text{-}0.59$, substantial if $\kappa = 0.60\text{-}0.79$ and almost perfect if $\kappa = 0.80\text{-}1.00$ (Teixeira-Soares *et al.,* 2022).

Results

Clinical signs and perineal conformation

In the current study, no visible clinical sign of endometritis such as mucous or mucopurulent discharge was observed in any case. This confirmed the sub-clinical cases of endometritis in the mares. Out of thirty mares, only two (6.67%) had abnormal perineal conformation. One mare with abnormal perineal conformation was observed to be positive for ultrasonography (USG) as well as endometrial cytology, but negative for culture isolation. While the other one with abnormal perineal conformation was observed as negative for sub-clinical endometritis irrespective of the diagnostic test used.

Subclinical-endometritis and diagnostic methods

The outcomes of different diagnostic sampling methods in infertile mares are presented in Table 2. Amongst the 30 infertile mares screened, trans-rectal USG examination of the uterus showed varying degrees of anechoic intrauterine fluid (Fig 1C) accumulations in 19 (63.33%) mares. The endometrial cytological examination revealed richness of PMNs (>5/hpf) in 17 (56.67%) mares; whereas, 13 (43.33%) mares didn't show any PMNs in endometrial cytology. Culture isolation of bacteria from the endometrial swabs of infertile mares exhibited growth of bacterial colony (Fig 1D) in 12 cases (40.00%). Among the 12 positive cases, 5 (41.66%) had *Klebsiella* spp., 2 (16.67%) had *Streptococci* spp., and 5 (41.66 %) had *Gram negative bacilli.* The incidence of sub-clinical endometritis was higher with USG followed by endometrial cytology and cultural isolation (Table 2). However, the difference was statistically not significant (p=0.175).

Sensitivity, specificity, and predictive value of diagnostic methods

The sensitivity, specificity, and positive and negative predictive values of the different diagnostic methods for sub-clinical endometritis are depicted in Table 3. Out of 30 mares, 17 had both intrauterine fluid accumulation as well as richness of PMNs (>5/hpf) in endometrial cytology. However, in two mares there was intrauterine fluid accumulation, but negative for endometrial cytology. Furthermore, bacterial growth was observed in samples from 12 (70.59%) mares out of 17 and these mares were also positive for the cytology. However, samples from the remaining 5 (29.41%) endometrial cytology positive mares didn't show any bacterial growth in the culture medium. Considering endometrial cytology as the "Gold Standard", the sensitivity and specificity for USG were 100 and 84.61%, respectively; while, for cultural isolation were 70.58 and 100%, respectively. Further, the positive and negative predictive values for the identification of sub-clinical endometritis were 89.47 and 100%, respectively by USG, and 100 and 72.22%, respectively by culture isolation.

Kappa statistics

The strength of agreement between diagnostic methods for sub-clinical endometritis was evaluated by Kappa statistics (Table 4). In the current study, the Kappa coefficient (κ) showed almost perfect ($\kappa = 0.86$) agreement between USG and cytology for the identification of sub-clinical endometritis. While there was substantial agreement ($\kappa = 0.67$) between endometrial cytology and cultural isolation and moderate agreement ($\kappa = 0.55$) between USG and cultural isolation for the identification of sub-clinical endometritis.

Table 1: Contingency table (2×2) for binary outcomes

Diagnostic test outcome	Gold standard test outcome		Rows total
	Positive	Negative	
Positive	True positive (TP)	False positive (FP)	Test positive (TP+FP)
Negative	False negative (FN)	True negative (TN)	Test negative (FN+TN)
Columns total	Outcome present based on gold standard (TP+FN)	Outcome absent based on gold standard (FP+TN)	Total (TP+FN+ FP+TN)

Table 2: Results of different diagnostic sampling methods in infertile mares (n=30)

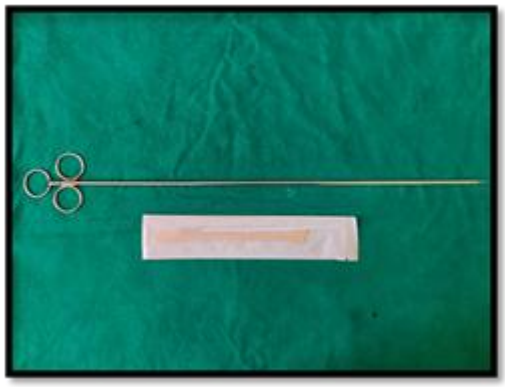
Diagnostic method	Results positive (+ve)			Results negative (-ve)	
	Number	Percentage		Number	Percentage
Ultrasonographic	19	63.33		11	36.67
Endometrial cytology	17	56.67		13	43.33
Culture isolation	12	40.00		18	60.00
Chi-square value	3.482				
Degree of freedom	2				
p-value	0.175				

Table 3: Sensitivity, specificity and positive and negative predictive values of the different diagnostic tests for sub-clinical endometritis

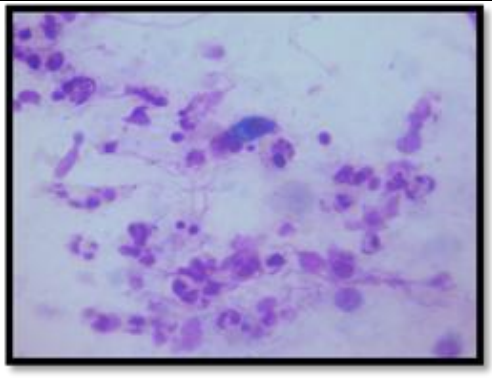
Statistics	Cytology	Ultrasonography	Cultural isolation
True positive	17	17	12
True negative	13	11	13
False positive	0	2	0
False negative	0	0	5
Sensitivity (%)	100	100	70.59
95% CI for Sensitivity	77.07-100	77.07-100	44.04-88.62
Specificity (%)	100	84.62	100
95% CI for Specificity	71.65-100	53.66-97.28	71.65-100
PPV (%)	100	89.47	100
95% CI for PPV	77.07-100	65.46-98.15	69.87-100
NPV (%)	100	100	72.22
95% CI for NPV	71.67-100	67.85-100	46.40-89.28

CI: Confidence Interval; PPV: Positive predictive value; NPV: Negative predictive value

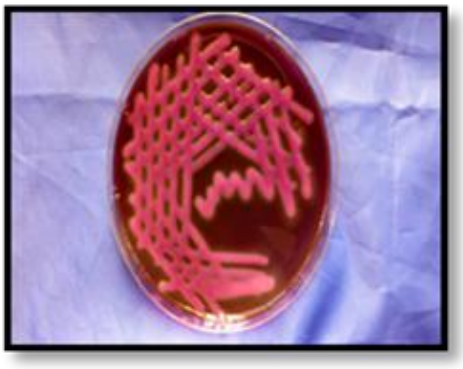
Fig 1A Accumulation of fluid in the uterus of an infertile mare



1B Cytobrush Assembly



1C PMNs cells in endometrial cytology



1D Bacterial isolation from the uterine swab

Table 4: Kappa statistics of the different diagnostic tests for sub-clinical endometritis

Diagnostic methods	Kappa coefficient (κ)	95% Confidence Interval for 'κ'	Strength of agreement
Cytology and Ultrasonography	0.86	0.67-1.0	Almost perfect
Cytology and Cultural isolation	0.67	0.47-0.87	Substantial
Ultrasonography and Cultural isolation	0.55	0.29-0.81	Moderate

Discussion

One of the causes of infertility in mares is endometritis, which severely hinders their ability to reproduce. The infection is often left untreated in animals that do not exhibit the peculiar signs of the infection, such as mucous or mucopurulent discharge. Hence, a clinician must employ multiple diagnostic methods simultaneously to diagnose the condition that does not exhibit the classical signs of inflammation and exudation accurately. Although, previous studies on mares reported abnormal perineal conformation as a predisposing factor for endometritis (Morris *et al.,* 2020), in the current study no such relationship was observed. The findings of the current study regarding the varying levels of anechoic to echogenic fluid accumulation in the uterus of endometritic mares are consistent with the previous studies (Hamouda *et al.,* 2012; Ibrahim *et al.,* 2015; Barbary *et al.,* 2016; EI-Atafi *et al.,* 2018), who documented different grades of uterine fluid accumulation in endometritic mares. In the current study, 63.33% of infertile mares showed uterine fluid accumulation, which was comparable to Assad *et al.* (2025), who reported 70.37% by USG, but lower than the 82% reported by Hamouda *et al.* (2012). In contrast, El-Atafi *et al.* (2018) observed a higher prevalence of endometritis (90.63%) using USG. Although intrauterine fluid during estrus is physiological, impaired uterine clearance increases susceptibility to endometritis (Katila, 2016). Thus, uterine fluid accumulation during different reproductive phases can serve as a useful indicator of endometritis in infertile mares. However, Katila (2016) emphasized that its presence alone does not confirm endometritis, while its absence does not necessarily indicate a healthy uterus.

Evaluation of endometrial cytology by the cytobrush method revealed PMN cells (>5/hpf) in 56.67% of infertile mares, comparable to previous reports of 52–65% (Hamouda *et al.*, 2012; Diel de Amorim *et al.*, 2016; El-Atafi *et al.*, 2018; Assad *et al.*, 2025). Cocchia *et al.* (2012) identified endometritic changes in 25% of cytobrush samples, while Carvalho *et al.* (2025) reported inflammation in 42.2% of mares. In contrast, Soliman *et al.* (2021) recorded 80% cytological positivity. Such variation may be due to differences in cytological methods and PMN threshold levels used to define positive cases (Katila, 2016).

Endometrial swab cultures in the current study revealed bacterial growth in 40% of cases, comparable to Buczkowska *et al.* (2014), who reported 43% positivity. However, higher rates were reported by Hamouda *et al.* (2012) (82%), El-Atafy *et al.* (2018) (97%), Assad *et al.* (2025) (55%), and Carvalho *et al.* (2025) (65%). Among the positive cultures, 41.66% each were identified as Klebsiella spp. and Gram-negative bacilli, while 16.67% were Streptococci spp. In contrast, several studies reported Streptococci spp., E. coli, and other bacteria as predominant isolates in infertile mares with endometritis (Hamouda *et al.*, 2012; Barbary *et al.*, 2016; El-Atafy *et al.*, 2018; Soliman *et al.*, 2021; Assad *et al.*, 2025; Carvalho *et al.*, 2025). Streptococci spp. are also common components of the normal genital flora of mares, which may explain their frequent isolation (Katila, 2016).

Among 30 infertile mares, 17 showed both intrauterine fluid accumulation and cytological inflammation (true positive), whereas 2 were USG-positive but cytology-negative (false positive). Considering cytology as the gold standard, USG showed 100% sensitivity and 84.61% specificity for subclinical endometritis, with positive and negative predictive values (PPV and NPV) of 89.47% and 100%, respectively. Of 17 cytology-positive mares, 12 also showed bacterial growth (true positive), while 5 were culture-negative (false negative), resulting in 70.58% sensitivity and 100% specificity. The PPV and NPV of culture were 100% and 72.22%, respectively. Assad *et al.* (2025) reported 71.4% sensitivity and 69.23% specificity for culture, while Buczkowska *et al.* (2014) reported 50% and 73%, respectively. Differences may be attributed to variations in gold standards and diagnostic criteria among studies (Katila, 2016), making direct comparisons difficult. Carvalho *et al.* (2025) also found discordance between cytology and culture results. Therefore, culture alone should not be considered an indicator of endometritis but should be supported by cytological evidence of PMNs. Culture is valuable for identifying the causative organism and guiding appropriate treatment, while positive cytology should ideally be complemented by culture for etiological diagnosis.

In the current study, the strength of agreement between diagnostic methods for sub-clinical endometritis ranged from moderate to almost perfect (k=0.55–0.86), comparable to previous reports (k=0.13–0.79) (Nielsen *et al.*, 2012; Diel de Amorim *et al.*, 2016; Katila, 2016; Teixeira-Soares *et al.*, 2022). Variations in agreement may be attributed to differences in the gold standard used to define endometritis. In the present study, cytobrush cytology was used as the gold standard, whereas previous studies primarily used endometrial histology. Assad *et al.* (2025) also reported significant ($p<0.01$) correlations among uterine cytology, culture, and USG for detecting endometritis in mares.

Conclusion

The results of the present study show that a single diagnostic test is not perfect for identifying sub-clinical endometritis in mares. Moreover, a combination of ultrasonography and endometrial cytology is most sensitive for identifying mares with sub-clinical endometritis. The strength of the association between ultrasonography and endometrial cytology is almost perfect for identifying mares affected with sub-clinical endometritis which showed that these diagnostic methods are more accurate to identify endometritis.

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