Journal of Veterinary Science & Medical Diagnosis

Research Article A SCITECHNOL JOURNAL

# Diagnosis of Uterine Infections in Mares: Comparative study of Uterine Cytology, Uterine Culture and Uterine Ultrasonography

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**Received date:** 26 February, 2024, Manuscript No. JVSMD-24-128289;

**Editor assigned date:** 28 February, 2024, PreQC No. JVSMD-24-128289 (PQ);

**Reviewed date:** 13 March, 2024, QC No. JVSMD-24-128289;

**Revised date:** 28 March, 2025, Manuscript No. JVSMD-24-128289 (R);

**Published date:** 04 April, 2025, DOI: 10.4172/2325-9590.1000119

## Abstract

Uterine infections and the associated endometritis are the most common cause of infertility in broodmares. Persistent Mating Induced Endometritis (PMIE) and Post-Breeding Metritis (PBM) account for the major causes of conception failure in broodmare practice. Mares that are affected are usually the best performing proven animals and therefore huge effort and money are utilized in their treatment. Timely diagnosis of such infections has a major impact on the success or otherwise of any treatment regimen followed. The present study was thus conceived to evaluate the diagnostic utility of uterine cytology, uterine culture and uterine Ultrasonography (USG) in detection of uterine infection in mares as well as to correlate the results obtained by these diagnostic techniques. The sensitivity and specificity of cytology, culture and USG in detection of uterine infections was found to be 71.42% and 69.23%, 100% and 100%, and 86.66% and50% respectively. The positive and negative predictive values for cytology, culture and USG were recorded to be 71.42% and 69.23%, 100% and 100%, 68.42% and 75.00% respectively. Individually uterine culture as a diagnostic tool was found to be most reliable for diagnosis of uterine infections, given that high considerations are given to aseptic processing milieu. The false positive and false negative results with uterine cytology and uterine ultrasonography were 28.57 and 30.76% and 31.57 and 25.00% respectively. The results of uterine cytology and uterine culture were found to be highly correlated ($r=1.000$, $p<0.01$), and so were that of cytology and ultrasonography, and culture and USG ($r=1.000$, $p<0.01$). However, the combination of uterine cytology and uterine culture was found to be most promising for routine diagnosis of uterine infections in mare, based upon $\chi^2$ test.

**Keywords:** Uterine infections; Diagnosis; Cytology; Culture; Ultrasonography

## Introduction

Uterine infection is often observed during breeding and foaling in brood mares, but can be resolved within 72 h post-breeding and 10 to 15 d post-foaling in post-partum mares. Endometritis which commonly causes infertility in mares can be categorized as acute, chronic, clinical, pre-partum, postpartum, bacterial, fungal, viral, Persistent Mating Induced Endometritis (PMIE) and Post-Breeding Metritis (PBM). PMIE and PBM occur as sequelae to poor uterine defense mechanism in infertile mares and account for the major causes of conception failure in mare reproductive practices [1]. Incidence of PMIE has been 15% in breeding mares [2].

Diagnosis of equine endometritis has been established clinically by recto-vaginal examination of reproductive tract, vaginoscopy, transrectal ultrasonography, uterine culture for bacterial and fungal pathogens, endometrial cytology, uterine biopsy and uterine endoscopy.

Uterine cytology is a quick and reliable diagnostic technique to detect uterine infection in cycling and non-cycling mares with clinical or subclinical endometritis [3]. Uterine cytological smears from normal cycling fertile mares show free epithelial cells without neutrophil infiltration. During uterine pathology, there usually is accumulation of intrauterine fluid and neutrophils are the predominant cells in these fluid accumulations. Nielsen in his study on endometrial cytology found that it has a sensitivity of 0.77 (77%) when compared to the identification rate of Polymorphonuclear Leukocytes (PMNs) from histological smears. Also a positive predictive value of 1.00 (100%) and negative predictive value of 0.62 (62%) was recorded with uterine cytology. The sensitivity and specificity values of 80.0%and 76.0% were calculated by LeBlanc, et al., for flush cytology. This clearly indicates that uterine cytology has relatively high reliability in diagnosing endometrial inflammation, although false negative cases are to be expected.

Dimock and Snyder were first to report the relationship between equine endometritis and bacterial infections. Uterine culture is of diagnostic value in detection of acute and chronic equine endometritis [4]. Collection of uterine luminal exudates without contamination is prerequisite to diagnose endometritis by uterine culture. Uterine exudates can be collected aseptically by double guarded swab, cytobrush, uterine flush. The bacterial and fungal species isolated by endometrial culture vary considerably between places and even between herds of the same area. LeBlanc, et al., reported the sensitivity and specificity of flush uterine culture in detection of uterine infections to be 0.72 and 0.86, respectively. The positive predictive values for biopsy cultures and swab cultures as observed by LeBlanc, et al., were 0.97 and 1.00 and negative predictive values were respectively as, 0.67 and 0.44.

The initial concept of diagnostic application of trans-rectal USG in broodmare practice was to facilitate early pregnancy diagnosis. However, more recently ultrasonography has been used for monitoring cyclic ovarian and uterine function, uterine and ovarian pathology, study of early embryonic development and embryonic pathology [5]. Currently ultrasound is routinely being used to diagnose infertility in mares associated with delayed ovulation and anovulation, and accumulation of uterine fluid associated with conception failure due to endometrial malfunction [6]. Although evaluation of uterine size can be performed by palpation per-rectum, more precise measurements

can be made only by ultrasonography. The prominence of endometrial folds during estrus, with small fluid accumulation, should not be considered pathological and does not require uterine therapy. The presence of large quantities of uterine luminal fluid during estrus and particularly dioestrus is suggestive of acute or chronic endometritis. Highly fertile mares seldom have free intrauterine fluid after breeding. Characteristics of fluid can vary with the degree of inflammation and presence of debris and pathogens [7]. Purulent uterine discharge is often recognized as a non-echogenic fluid with sprinklings of echogenic spots. The more echogenic the fluid, the more likely is the fluid contaminated with debris including PMNs [8]. In broodmare practice linear array transducers are routinely used through trans-rectal approach in diagnosis of uterine infection and other uterine pathology. Transducers with different frequencies have been used by different researchers *viz* 3.5 MHz, 5 MHz and 8 MHz [9].

The present study was therefore an attempt to strengthen the existing literature on diagnostic aspects of equine endometritis with objectives to evaluate diagnostic utility of uterine cytology, uterine culture and uterine ultrasonography in detection of uterine infections in mares as well as to correlate the results obtained from these diagnostic techniques.

## Materials and Methods

### Animals and laboratories

Twenty seven broodmares, fertile and infertile, were identified and divided into 2 experimental groups. Group I consisted of 21 non-cycling mares with history of conception failure and acute or chronic endometritis for last 2 or 3 breeding seasons, and suffered clinical or subclinical endometritis within the current breeding season. Group II had 6 cycling mares with history of proven fertility for the last 2 to 3 breeding seasons and normal reproductive health during the current breeding season. Results of the diagnostic tools used were read independent of Mares history. Ultrasonographic studies were performed at equine breeding Stud-EBS (an equine breeding establishment of Royal Veterinary Corps of Indian Army)–Hisar; Haryana, by means of a portable ultrasound machine. Samples collected at the breeding stud were processed for uterine cytology in the departmental laboratory and uterine culture in the college central laboratory. Standardized ration, consisting of green fodder, oat hay and concentrate mixture was provided. Mares had ad lbibitumaccess to water. No antibiotic or immune-stimulating additives were allowed with the feed.

Diagnosis of uterine infections was determined by recto-vaginal examination followed by uterine ultrasonography, uterine cytology and uterine culture of individual mares. Diagnostic significance of each technique was assessed (sensitivity and specificity, predicative value, false values). Correlations between diagnostic techniques were determined to advocate standard combination of diagnostic techniques to be routinely used, in the maintenance of optimum fertility of an equine breeding stud. Mares were housed at Equine Breeding Stud-EBS, Hisar [10].

### Collection of samples

Uterine flushing's were collected by low-volume flush technique using two way Foley`s catheter and sterilized phosphate buffer saline (pH, 7.2). A disposable Foley`s catheter, with a sterile metal stillete and sanitary sheath cover, was introduced through vagina with a lubricated hand covered with sterilized glove. Cervix was opened with digital pressure to introduce the catheter beyond the cervix into the uterus. Mares with tightly closed cervix that could not yield to digital pressure were administered a single dose of PGF2α (5-10 mg IM) and were sampled after 36 hours of PGF2α administration. Uterine flushing's were collected just after initial clinical examination in Group I mares, and just before breeding to first estrus (of breeding season) in fertile mares (Group II). Sterile Phosphate Buffer Saline (PBS) (100 ml) was infused into the uterus through Foley's catheter using a sterile disposable intravenous set. The uterus was manipulated per rectum to distribute the PBS into both uterine horns and then the PBS (60-80 ml) was recovered in sterile glass test tube by gravitational flow assisted by per rectal manipulation of uterine horns. The tubes were immediately capped to prevent contamination. The samples recovered were divided into 2 parts (20 ml each) inside a safety cabinet (Laminar flow) so as to prevent contamination from ambient factors. Samples were processed separately for uterine cytology and for uterine microbiology. The portion of flush sample that was selected for microbiological examination was aseptically dispensed into a sterile disposable centrifuge tube. Uterine sample were immediately processed for cytological and microbiological examinations or stored at 4°C.

### Transrectal ultrasonography

Mares (Group I and II) were evaluated, via ultrasound examination, to determine uterine and ovarian function, uterine fluid accumulation and infection, uterine oedema and uterine wall thickness. Ultrasonography procedures utilized were described by Squires, et al. After thorough back racking the uterus and ovaries of each mare were manually palpated per-rectum before scanning; subsequently mares were subjected to trans-rectal ultrasonographic examination using linear array probe operating at 5 and 7 MHz frequency. The probe was enveloped inside a finger of examination gloves with adequate quantity of gel, so as to remove all air between the probe and polythene lining of the glove. Inside the rectum the probe was moved across the reproductive tract from uterine body, right uterine horn, right ovary, right uterine horn, and uterine body, left uterine horn, left ovary, left uterine horn, uterine body and cervix. Due care was taken to prevent ballooning of rectum and possible intervention by gas or fluid filled bowl loops during scanning of reproductive tract. Presence of fluid in the uterine horns and/or thickened uterine wall was interpreted as positive case for uterine infection and associated endometritis. Purulent discharges often were recognized as non-echogenic fluid with sprinklings of echogenic spots (Figures 1-3).

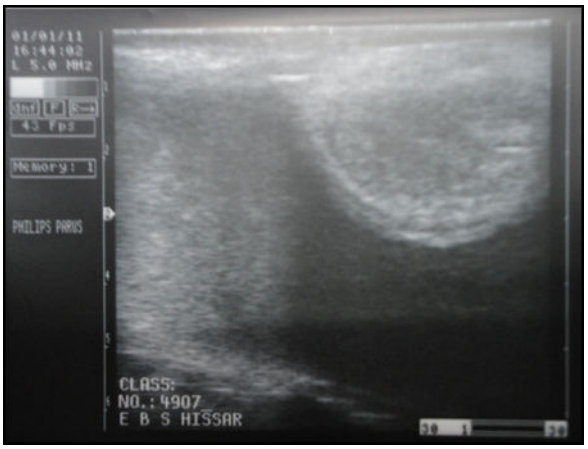



**Figure 1:** Ultrasound view of a transverse section of right uterine horn of a normal mare showing absence of uterine exudates and compact uterine wall lining.

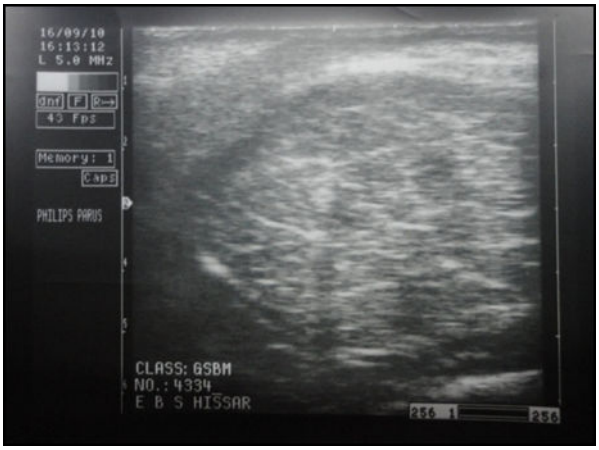



**Figure 2:** Ultrasound view uterine horn of an infertile mare showing voluminous purulent exudate with marked echogenicity.

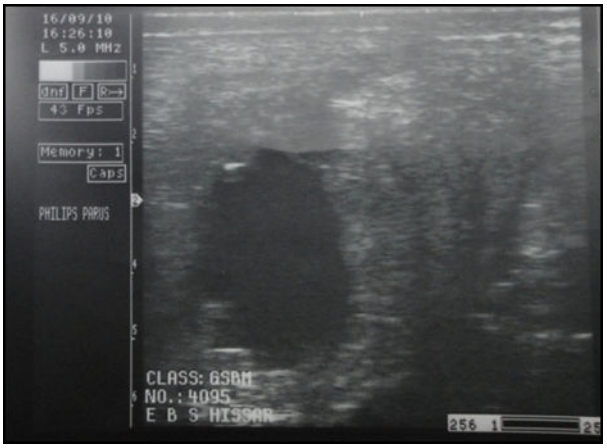



**Figure 3:** Ultrasound view of uterine body of endometritis mare showing non-echogenic uterine exudates along with distinct wall thickening.

## Uterine cytology

Samples were centrifuged in a cryo-centrifuge at 1000 rpm for 10 min at 4°C. Supernatant was discarded and the sediment re-suspended in a few drops of PBS. Smears were prepared from the re-suspended sediment, air dried and then fixed with methanol. Fixed smears were stained with Giemsa stain (1:9 dilution) for a minimum 3 hours and were then examined for Differential Leukocyte Count (DLC), under oil immersion objective (x100). Permanent mounts were prepared by using DPX mountant. Endometrial smears with ≥ 2% neutrophils (Figures 4-6) were considered positive for uterine infection [11-13].

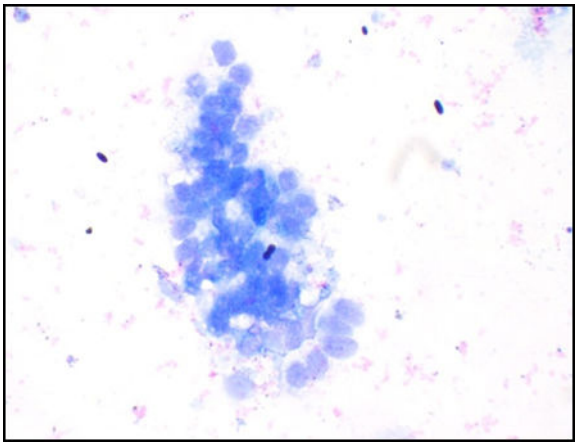
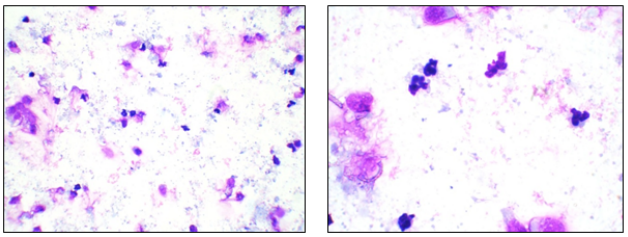


**Figure 4:** Uterine cytological smear from a normal mare showing epithelial cells without infiltration of neutrophils (x400).



*a* *b*

**Figure 5:** A) Uterine cytological smear of endometritis mare showing epithelial cells with moderate neutrophilic infiltration (x400). B) Uterine cytological smear of endometritis mare showing neutrophils and epithelial cells (x1000).

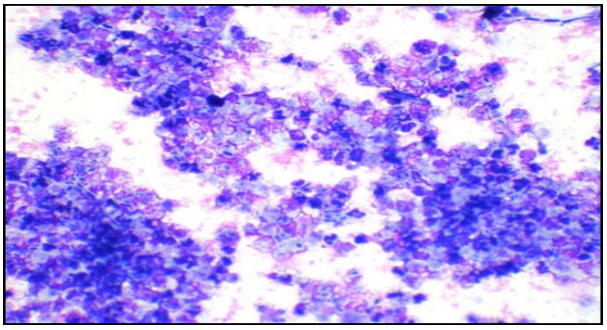


**Figure 6:** Uterine cytological smear of an infertile mare showing heavy infiltration of neutrophils (x400).

### Uterine microbiology

A second aliquot of uterine flush (20 ml) was centrifuged at 4000 rpm at 4°C for 30 min. The supernatant was decanted and the sediment was re-suspended in I ml of sterile PBS. Two to 3 drops of re-suspended sediment were used as inoculum for isolation of bacteria and fungi.

**Isolation of bacterial flora:** Each sample was inoculated on set of 3 culture media, *vis* nutrient agar, blood agar, and MacConkey`s lactose agar, and the inoculated plates were incubated at 37°C temperature for 24 to 48 h. In case of positive samples, growth was evident after overnight incubation. Smears were prepared from isolated colonies and these smears were subjected to gram staining for morphology evaluation and gram`s reaction (whether gram positive or gram negative). Further identification of bacteria into different genera and species was done on the basis of various biochemical tests.

**Isolation of fungal flora:** Re-suspended sediment used for inoculation on bacterial media, was also inoculated on Sabouraud`s Dextrose Agar (SDA) and incubated at 25°C for 2 weeks. The incubated tubes were grossly examined daily for presence of any growth. The fungal isolates obtained were examined for gross and microscopic morphology and biochemical tests [14].

Uterine flush samples yielding four or more colonies of a pathogenic organism on culture plate were considered as positive for uterine infection. Presence of colony growth involving more than three types of colonies are indicative of sampling contamination and were thus recorded as negative for uterine infection [15].

### Statistical analysis

The experimental results obtained were analyzed for correlation by computer based SPSS software (SPSS 16.0) and $\chi^2$ (*chi-square*) test was used to evaluate the relative significance of experimental data. The significance level was set at p=0.1 [16,17].

## Results

The results of trans-rectal ultrasonography, uterine cytology and uterine culture are presented in Tables 1, 2 and 3 respectively. From Table 1 it is clear that out of 21 mares of group I, that were suffering from clinical or subclinical uterine infections (as evident from their clinical history and breeding records), ultrasonographic examination revealed uterine infections in 17 of them. Moreover, out of six group II mares (as per their clinical history and breeding records) only four were declared negative for uterine infections by ultrasonography. However, two mares evidenced accumulation of uterine luminal fluid and /or uterine wall thickness and were thus considered as positive for endometritis.

As can be observed from Table 2, thirteen out of 21 mares of group I were positive for uterine infections and associated endometritis (since they evidenced >2% neutrophils in their cytological smears). Out of these 13 mares nine had moderate uterine infections (2-5% PMNs) and four were suffering from severe uterine infections (>5% PMNs). The remaining eight mares of this group had <2% PMNs in their cytological smears and were thus counted as negative for uterine infection. Of the six mares of group II, five were found to be negative. However, uterine cytology of one mare of this group evidenced >2% PMN infiltration and was thus recorded as positive for uterine infection.

It can be summarized from Table 3, that the uterine cultural examination of 21 mares of Group I revealed fifteen mares with uterine infections. The remaining six mares of this group were found to be negative as their uterine flush samples could not reveal any bacterial or fungal pathogens. B-hemolytic *Streptococcus* sp. was the most prevalent uterine bacterial pathogen (isolated from 7 out of 21 mares, 33.33%), followed by *Trueperella pyogenes* (isolated from 5 out of 21 mares, 23.80%). The incidence of *Escherichia coli* and yeast were the lowest with two out of 21 mares yielding *E. coli* (9.5%) and one mare yielding yeast (5%). The uterine cultures of all six mares of group II were negative for bacterial as well as fungal pathogens.

| Experimental group | Ultrasonographic detection of uterine fluid and/or uterine wall thickness | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Group I mares | 17 (80.95%) | 4 (19.05%) | 21 |
| Group II mares | 2 (33.33%) | 4 (66.66%) | 6 |
| Total | 19 (70.37%) | 8 (29.63%) | 27 |

**Table 1:** Ultrasound observations for detection of uterine infections in mares.

| Experimental Group | Uterine cytological detection of uterine infection | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Groups I mares | 13 (61.9%) | 8 (38.09%) | 21 |
| Group II mares | 1 (16.66%) | 5 (83.33%) | 6 |
| Total | 14 (51.85%) | 13 (48.14%) | 27 |

**Table 2:** Uterine cytological observations for detection of uterine infections in mares.

| Experimental Group | Uterine cultural detection of infection | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Group I mares | 15 (71.4%) | 6 (28.57%) | 21 |
| Group II mares | 0 (0.00%) | 6 (100%) | 6 |
| Total | 15 (55.55%) | 12 (44.45%) | 27 |

**Table 3:** Microbiological evaluation of uterine flush from mares.

## Relationships between ultrasonography, uterine cytology and uterine culture techniques

Total number of experimental mares that were found positive and negative for uterine infection by uterine ultrasonography, uterine cytology and uterine culture are given in the Table 4. Variations in diagnostic utility of different diagnostic techniques in detection of uterine infection were recorded in fertile as well as infertile mares. The mares that were declared positive/negative for uterine infection by one of the diagnostic techniques were necessarily not positive/negative for infection when evaluated by the other diagnostic technique.

These diagnostic variations are shown in the form of a contingency Table 5. This table indicates that individually uterine culture technique shows maximum agreement in detection of uterine infection with the other two diagnostic techniques viz, uterine cytology and uterine ultrasonography. Further, all the mares of Group II were declared as normal by uterine culture technique. Hence, the results obtained by uterine culture were taken as the gold standard. The sensitivity, specificity, false positive, false negative, positive predictive value and negative predictive value of the diagnostic techniques that were used during the investigation were worked out by means of contingency Table 6.

| Diagnostic technique | No. of mares declared positive | No. of mares declared negative | Total |
|---|---|---|---|
| Uterine ultrasonography | 19 (70.37%) | 8 (29.63%) | 27 |
| Uterine cytology | 14 (51.85%) | 13 (48.14%) | 27 |
| Uterine culture | 15 (55.55%) | 12 (44.45%) | 27 |

**Table 4:** Diagnosis of uterine infections in fertile and infertile mares by uterine ultrasonography, uterine cytology and uterine culture.

| | $USG^+$ and $cytology^+$ | $USG^-$ and $cytology^-$ | $USG^+$ and $cytology^-$ | $USG^-$ and $cytology^+$ | Total |
|---|---|---|---|---|---|
| $Culture^+$ | 10 (37%) | 1 (3.7%) | 3 (11%) | 1 (3.7%) | 15 (55.5%) |
| $Culture^-$ | 2 (7.5%) | 5 (18.5%) | 4 (15%) | 1 (3.7%) | 12 (44.5%) |
| **Total** | 12 (44.5%) | 6 (22.5%) | 7 (26%) | 2 (7.5%) | 27 (100%) |

**Table 5:** Relationships between uterine ultrasonography, uterine cytology and uterine culture in detection of uterine infection in mares.

| Diagnostic test | True +ve a | True -ve b | False +ve c | False -ve d | Sensitivity a/(a+d) × 100 | Specificity a/(b+c) × 100 | +ve predictive value a/(a+c) × 100 | -ve predictive value b/(b+d) × 100 |
|---|---|---|---|---|---|---|---|---|
| Uterine culture | 15 | 12 | 0 | 0 | 100% | 100% | 100% | 100% |
| Uterine cytology | 10 | 9 | 4 | 4 | 71.42% | 69.23% | 71.4% | 69.23% |
| Uterine ultrasound | 13 | 6 | 6 | 2 | 86.66% | 50.00% | 68.42% | 75.00% |

**Table 6:** Sensitivity, specificity and positive and negative predictive values for the different diagnostic techniques.

By taking uterine culture technique as the gold standard, its sensitivity and specificity becomes 100% and so does the positive and negative predictive values. The sensitivity and specificity for uterine cytology and uterine ultrasound were 71.42% and 69.23% and 86.66% and 50%, respectively. The positive predictive value for uterine cytology was 71.4% and that for ultrasound was 68.42%. The negative predictive value for uterine cytology and ultrasonography were 69.23% and 75%, respectively. The false positive and false negative results that were to be expected with uterine cytology were 28.57%and 30.76% respectively. The false positive and false negative results expected for ultrasonography were 31.57% and 25% respectively.

## Diagnostic correlations between ultrasonography, uterine cytology and uterine culture techniques

The observations recorded by uterine ultrasonography, uterine cytology and uterine culture in all the experimental mares including group I and group II are summarized in Table 7. The correlations Table that was obtained is given in Table 8.

| Diagnostic technique | Experimental mares (group I and group II ) | | Total |
|---|---|---|---|
| | Positive (No.) | Negative (No.) | |
| Uterine ultrasonography | Group I: 17<br>Group II: 2 | Group I: 4<br>Group II: 4 | 27 |
| | Subtotal: 19 | Subtotal: 8 | |
| Uterine cytology | Group I: 13<br>Group II: 1 | Group I: 8<br>Group II: 5 | 27 |
| | Subtotal: 14 | Subtotal: 13 | |
| Uterine culture | Group I: 15<br>Group II: 0 | Group I: 6<br>Group II: 6 | 27 |
| | Subtotal: 15 | Subtotal: 12 | |
| Total | 48 | 33 | 81 |

**Table 7:** Diagnostic utility of uterine ultrasonography, uterine cytology and uterine culture in diagnosis of uterine infection in fertile and infertile mares.

| | Uterine ultrasonography | Uterine cytology | Uterine culture |
|---|---|---|---|
| Uterine-Pearson correlation sig.<br>Ultrasonography-(2-tailed) | 1<br>2 | 1.000**<br>2 | 1.000**<br>2 |
| Uterine cytology-pearson correlation sig. (2-tailed) N | 1.000**<br>2 | 1<br>2 | 1.000**<br>2 |
| Uterine culture pearson correlation sig. (2-tailed) N | 1.000**<br>2 | 1.000**<br>2 | 1<br>2 |
| **Note:** **Correlation at 0.01 level of significance (2-tailed) | | | |

**Table 8:** Pearson correlation table showing correlations between different combinations of diagnostic techniques.

From the correlations table (Table 8), it is clear that there was high correlation between uterine ultrasonography and uterine cytology (1.00 at P<0.01) and between uterine ultrasonography and uterine culture (1.00 at P<0.01). Also high correlation between uterine cytology and uterine culture (1.00 at P<0.01) was recorded. Thus based on the correlation studies, uterine culture can be used in any combination (either along with uterine cytology or with ultrasound) for routine diagnosis of uterine infection in mares, and no combinations has a marked advantage over another. In pursuit to decide upon the better combination of these diagnostic techniques, the results of uterine ultrasound, uterine cytology and uterine culture were further evaluated by using $\chi^2$ test (*chi-square*). The results of $\chi^2$ test are given in the Table 9.

Table 9 indicates that results obtained from any combination of two diagnostic techniques are significantly related. Further, the most desired combination of diagnostic techniques for detection of uterine infection, based on magnitude of significance is that of uterine cytology and uterine culture, followed by uterine ultrasound and uterine cytology and uterine ultrasound and uterine culture in decreasing order of preference.

| Combination of diagnostic techniques | Value of $\chi^2$ (*chi-square*) | Degrees of freedom=1<br>Table value:<br>at 0.01=6.63 |
|---|---|---|
| Uterine ultrasound and uterine cytology | 11.35** | |

| | | |
|---|---|---|
| Uterine ultrasound and uterine culture | 8.57* | at 0.05=3.84 |
| Uterine cytology and uterine culture | 18.21*** | |

**Note:** *significant, **highly significant, ***very highly significant

**Table 9:** Evaluation of combination of diagnostic techniques in detection of uterine infection (*chi-square* test).

## Discussion

The presence of uterine exudates and uterine wall thickness in endometritis mares concurs with the previous reports in equine endometritis. The occurrence of uterine luminal ecogenic fluids with mild endometrial oedema in clinically normal mares can be attributed to either prolonged progesterone dominant luteal phase of estrous cycle or persistent subclinical uterine infection. Further, accumulation of uterine fluid is associated with inadequate uterine cellular and humoral defense mechanisms, impaired cervical function, damaged uterus, impaired lymphatic drainage, PMIE and PBM in infertile mares. Recorded sensitivity (86.66%) and specificity (50%) values of ultrasonography in diagnosis of uterine infections in mares was in variation with reported values (sensitivity, 57.2% and specificity, 78%by Barlund, et al., Further the diagnostic sensitivity of 3.90% and specificity of 80.95% in equine endometritis associated with endometrial oedema and thickness reported by Barlund, et al., are at variation with the current diagnostic sensitivity and specificity values. In the present study both accumulation of uterine luminal fluid as well as presence of wall thickness was considered for diagnosis of infection. Nielsen also suggested that diagnostic efficiency (sensitivity and specificity) of ultrasound improves when both endometrial fluid accumulations as well as uterine wall thickness is considered during examination as compared to single evaluation criterion. The lower positive predictive value of ultrasonography is essentially due to its higher sensitivity to detect even minute quantities of luminal exudates present in uterus, which is often wrongly interpreted as a positive case for uterine infection.

The less than 0.5 to 2.0% PMN infiltration recorded on uterine cytology from clinically normal mares could be assigned to resident population of neutrophils in uterine lumen that are associated with uterine defense mechanisms. However, presence of subclinical infections can`t be ruled out. The sensitivity and specificity for the uterine flush cytology in detection of uterine infection in infertile mares was 71.42% and 69.23%, respectively and confirmed earlier reports on uterine cytological diagnosis in infertile mares. However, other workers have reported higher sensitivity and specificity values of uterine flush cytology as well as lower sensitivity and specificity values. Also, the positive and negative predictive values for uterine flush cytology (71.4% and 69.23%, respectively) don't agree with the earlier reported positive predictive value (100%) and negative predictive value (62%) in mares. Variations in sensitivity and specificity of cytological diagnosis of uterine infection in mares could be accounted to adoption of different standards of PMN infiltration by different workers in diagnosis of uterine infections in mares. A second reason is the cellular damage that occurs during centrifugation process of uterine sample.

All the bacterial and fungal pathogens that were isolated during the investigation are commonly reported uterine pathogens [18]. The recorded negative uterine culture for bacterial and fungal infections in clinically normal mares is in agreement with the previous reports. Interestingly, both the mares that yielded *E. coli* on uterine flush culture were declared negative on cytological examination. Similar observations in this regard were recorded by Bindslev, et.al, Riddle et al., and Nielsen et al. Recorded 100% sensitivity and specificity of uterine culture in diagnosis of uterine infection concurs with earlier reported sensitivity value of 92% and specificity value of 97% and 100% specificity value in mares. However, LeBlanc et al. recorded lower sensitivity and specificity (71% and 86%, respectively) of uterine culture in diagnosis of uterine infection [19]. The higher sensitivity and specificity of uterine culture recorded during present investigation may be assigned to sterile collection of uterine exudate by uterine flush sampling technique as well as aseptic processing milieu. Further, Nielsen also confirmed higher diagnostic sensitivity and specificity of uterine culture in mares with uterine biopsy sampling technique (which collects uterine sample in a more sterile manner) than guarded swab sampling technique. The positive and negative predictive values for uterine culture in diagnosis of uterine infection (100% positive predictive value and 100% negative predictive value) simulates observations of Nielsen, et al. [20] The diagnostic variations between uterine ultrasonography and uterine cytology in detection of uterine infections in mares are due to difference in uterine inflammatory response associated with uterine luminal fluid. While one category of mares has a vivid cellular response with little uterine luminal fluid, the other category of mares exhibit inadequate cellular response despite of voluminous uterine fluid accumulation. Further, uterine ultrasound detects presence of uterine luminal fluid associated with delayed uterine clearance without uterine infection. Observed differences in diagnosis of uterine infection by uterine cytology and culture examination occur due to differences in uterine cellular response to different uterine bacterial infections. Poor uterine cellular response was associated with *Escherichia coli* infection, whereas adequate cellular response was associated with *Streptococcus* sp. infection causing variation in cytological diagnosis of uterine infection. The possible reason for different uterine cellular response with *E. coli* is consequential prevention of activation of uterine immune response by *E.coli* as well as its non-chemotactic nature to PMNs.

The false positive and false negative results for detection of uterine infection by uterine ultrasonography, uterine cytology and uterine culture were 31.57% and 25%, 28.57% and 30.76%, and 0.00 and 0.00%, respectively. Recorded observations of false positive and false negative results of uterine culture do not agree with reported values (false positive, 14.28% and false negative, 29.16%) in mares. However, LeBlanc et al., found that false positive results of uterine cytology may be upto 70%. Uterine culture did not record false positive and false negative results in detection of uterine infections and proved most reliable diagnostic technique as compared to uterine cytology and uterine ultrasonography and concurs with the observations of Ball, et al., It must be noted that any diagnostic test for endometritis will have less than 100% sensitivity when measured against reproductive performance, because there are numerous unaccountable, independent reasons for which animals fail to become pregnant, resulting in false negative results.

The correlation between uterine ultrasonography and uterine cytology, between uterine ultrasonography and uterine culture, and uterine cytology and uterine culture ($p<0.01$) was 1.00. Present results

thus indicate that the findings of all the three diagnostic techniques were significantly correlated with each other in detection of uterine infections in mares. The positive correlation between uterine cytology and uterine culture results was also reported by Ball, et al., Narwal and Monaga, Blanchard, et al. As per the reports of Digby and Ricketts, and Reiswig, et al., the correlation between uterine cytology and uterine culture ranged between 0.76 to 0.88 in equine endometritis. Mateus, et al., found that the uterine luminal fluid detected by ultrasonography was positively and significantly correlated with uterine culture results in mares. Kasimanickam, et al., reported that positive endometrial cytology and in utero fluid as detected by ultrasound were associated with a more detrimental milieu of uterus than positive results of either of the techniques.

## Conclusion

In conclusion, it may be mentioned that despite a high correlation between all the three diagnostic techniques in the diagnosis of equine endometritis, the combination of diagnostic techniques to be recommended for routine diagnosis of endometritis are uterine cytology and uterine culture. This is recommended on the basis of *Chi-square* test. The same combination has been recommended by Nielsen, who advocated that a combination of uterine culture and uterine cytology techniques was most accurate approach to diagnose equine endometritis it must be added that uterine ultrasound should be used as a supplementary technique in evaluation of quantity of uterine fluid accumulation well as assessment of endometrial oedema/uterine wall thickening in prediction of fertility.

## Acknowledgements

Sincere thanks to the administration of EBS-Hisar for providing us the mares and assistance in terms of manpower. Special thanks are due to Lt. Col (Dr) A H Haldar and Lt. Col (Dr) J Taneja for their valuable cooperation. Dr. Tariq Abdullah Ahmad Gassim deserves a particular mention for the valuable inputs with respect to statistical evaluation. Dr. Mark Crenshaw for reviews and editing the manuscript.

## References

1. Ajello L, Georg LK, Kaplan W, Kaufman L (1962) Laboratory manual for medical mycology. 1st Edition. US Department of health, education and welfare, Atlanta, Georgia.
2. Ball BA, Shin SJ, Patten VH, Lein DH, Woods GL (1988) Use of a low-volume uterine flush for microbiologic and cytologic examination of the mare's endometrium. Theriogenology 29: 1269-1283.
3. Barlund CS, Carruthers TD, Waldner CL, Palmer CW (2008) A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology 69: 714-723.
4. Berwal SS, Bugalia NS, Kapoor PK, Garg DN, Monga DP, et al. (2006) Cytological and microbiological evaluation of uterine flush of fertile and repeat breeder mares and post-treatment fertility. Haryana Vet 45: 15-17.
5. Bindslev MM, Villumsen MH, Petersen MM, Nielsen JM, Bogh IB, et al. (2008) Genetic diversity of *S. equi* subspecies *Zooepidemicus* and *E. coli* isolated from the reproductive tract of the mare. InReproduction in domestic animals. 43: 110-111.
6. Blanchard TL, Cummings MR, Garcia MC, Hurtgen JP, Kenney RM (1981) Comparison between two techniques for endometrial swab culture and between biopsy and culture in barren mares. Theriogenology 16: 541-552.
7. Bourke M, Mills JN, Barnes AL (1997) Collection of endometrial cells in the mare. Aust Vet J 75: 755-758.
8. Bracher V, Mathias S, Allen WR (1992) Videoendoscopic evaluation of the mare's uterus: II. Findings in subfertile mares. Eq Vet J 24: 279-284.
9. Brinsko SP, Rigby SL, Varner DD, Blanchard TL (2003) A practical method for recognizing mares susceptible to post-breeding endometritis. In: Proceedings of 49th conference of American Association of Equine Practitioners, New Orleans, LA. p. 363-365.
10. Brook D (1985) Cytological and bacteriological examination of the mare's endometrium. J Eq Vet Sci 5: 16-22.
11. Brook D (1993) Uterine cytology. Eq Reprod 246-254.
12. Burns T, Pierson RA, Card CE (2000) Subjective and quantitative assessments of endometrial changes in mares inseminated with cryopreserved semen. Proc Soc Theriogenol 2000; 47.
13. Card C (1997) Current therapy in large animal theriogenology. 1st Edition. WB Saunders Co. p. 151-153.
14. Card C (2005) Post-breeding inflammation and endometrial cytology in mares. Theriogenology 64: 580-588.
15. Card C, Carley S, Green J, Chirino-Trejo M (2004) Endometrial cytology in mares bred with frozen semen. In: Proceedings of 50th conference of American Association of Equine Practitioners, Denver, CO. p. 505-509.
16. Causey RC (2006) Making sense of equine uterine infections: The many faces of physical clearance. Vet J 172: 405-421.
17. Cowan ST (1974) Manual for the identification of medical bacteria. 2nd edition. University press Cambridge.
18. Crickman JA, Pugh DG (186) Equine endometrial cytology: A review of techniques and interpretations. Vet Med 650-656.
19. Digby NW (1978) The technique and clinical application of endometrial cytology in mares. Equine Vet J 10: 167-170.
20. Digby NW, Ricketts SW (1982) Results of concurrent bacteriological and cytological examinations of the endometrium of mares in routine study farm practice (1978-81). J Reprod Fertil 32: 181.