RESEARCH ARTICLE

Sensitivity, Specificity and Predictive Values of Cytological and Microbiological Findings of Endometrial Biopsy, Cytobrush and Low Volume Uterine Lavage in Relation to Endometrial Histology in Barren Mares

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Abstract

This study was carried out on 10 infertile barren mares to evaluate the sensitivity, specificity, predictive values and agreement (kappa value) of cytological and microbiological findings of three diagnosing techniques of endometritis, *viz.,* endometrial biopsy (EB), cytobrush (CB) and low volume uterine lavage (LVL) in relation to endometrial histology. When histological examinations from EB were used as "the best standard," the sensitivity of cytology from EB, CB and LVL technique was 0.33, 0.50 and 0.50; specificity 0.75, 1.00 and 0.75; positive predictive value 0.66, 1.00 and 0.75, and negative predictive value was 0.42, 0.57 and 0.50, respectively. The sensitivity of bacteriology from EB, CB and LVL technique was 0.83, 0.83 and 1.00; the specificity was 0.75, 0.75 and 0.50; positive predictive value 0.83, 0.83 and 0.75, and the negative predictive value was 0.75, 0.75 and 1.00, respectively. In all the cases, the sensitivity of the bacteriology was found to be higher than the sensitivity of cytology. When the results of cytological and bacteriological examinations were combined, no any increase in the sensitivity was found. Bacteriology and cytology from CB showed the highest positive predictive value demonstrating that a positive result is an accurate indication of endometritis. Sensitivity values were always higher if smears were evaluated according to PMNs to epithelial cell ratio, and the highest values were observed in specimens collected from CB and LVL. The evaluation of cytological smears based on counting PMNs in relation to epithelial cells was a better method for diagnosis of endometritis than counting the number of PMNs per high power microscopic field (*k* value 0.07-0.47 vs. 0.00). The agreement of the diagnosis of endometritis between the three techniques of the collection was from fair to poor and between the different criteria adopted to evaluate smears was always poor. However, the agreement of the diagnosis of endometritis by the microbial culture was moderate between the three techniques of the collection (*k* value 0.55-0.58).

Keywords: Cytobrush, Endometrial Biopsy & Uterine Lavage, Endometritis, Endometrial Cytology, Mare, Microbiology, & Histology, Predictive Value, Sensitivity, Specificity.

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Introduction

Endometritis in mare has a significant economic impact as it requires intensive clinical breeding management and yet reduces the pregnancy rate (LeBlanc, 2010). The inflammatory response to the uterine challenge is characterized by an influx of fluid and polymorphonuclear neutrophils (PMNs) in the lumen. Clinical signs may vary depending on the chronicity of disease or type of bacteria involved. Endometrial cytology is a readily available diagnostic test to identify mares with endometritis. Low-volume lavage (LVL) evaluates a larger endometrial surface area (LeBlanc *et al*., 2007; Christoffersen *et al*., 201), while swabbing, CB and EB samples only a small focal area, potentially resulting in false negatives (LeBlanc *et al*., 2007). The bacteriological examination provides an indirect test for the diagnosis of endometritis in mares. The detection of PMN-cells in a histological examination of the endometrial luminal epithelium and the stratum compactum is considered to be the most accurate diagnosis of endometritis and uterine infection, and is used as "the best standard". It has long been recognized that the presence of PMN-cells is the most accurate indication of inflammation.

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But without positive bacterial culture and identification of a causing agent, it is difficult to propose a protocol for the treatment of the mare (Ricketts, 1999; Walter *et al.,* 2012). The literature on equine endometritis and diagnostic efficacy, sensitivity, specificity of different techniques in the Indian context is meager. Therefore, this study was carried out to find out the relationship of cytological and bacteriological findings of uterine samples collected by CB, EB and LVL from infertile mares to their endometrial histopathology.

Materials And Methods

This work was carried out during 2018-19 on randomly selected 10 mares with a history of barrenness/infertility brought to the Veterinary Clinical Complex of the College and from other stud farms around Anand in Gujarat. After recording detailed history, the animals were examined by rectal palpation followed by cytological and microbiological and histological sampling of the endometrium through CB, EB and LVL. With all aseptic precautions, a single-guarded CB technique (Mayfair Endocervical brush, Care India Surgicals, Ludhiana, India), EB (Nelson's bovine biopsy forceps) and LVL were performed consecutively under xylazine sedation on each animal. Using standard procedures, all samples were processed for cytological and bacteriological evaluation and biopsies for histopathology.

The samples collected by all the three techniques (CB, EB, LVL) were immediately smeared onto sterile glass slides, air-dried, fixed with methanol, stained with Field's stain, and were evaluated microscopically for different cell types at a high power magnification (400x or 1000x). All the samples were streaked onto blood agar with 5 % sheep blood, MacConkey agar, Sabouraud dextrose agar, and Eosin-Methylene blue agar within 6 hours of procurement. After 24 and 48 hours of incubation at 37°C, the cultures were identified by their cultural characteristics, Gram staining, and basic tests. The EB samples were processed for histopathological examination using standard procedure. The findings obtained of different cytological and cultural/microbiological examinations of samples obtained by all three techniques were interpreted to find out their associations with histopathological changes through determining sensitivity, specificity, positive & negative predictive values and their agreement (kappa values) by adopting standard formulae (Nielsen, 2005).

Results and Discussion

The study was designed to compare the accuracy of three laboratory methods for diagnosis of endometritis with results from histological examination. The sensitivity, specificity, positive and negative predictive values of microbiological and cytological results obtained from EB, CB, and LVL are

Table 1: Sensitivity, specificity, positive and negative predictive values of cytological and cultural examinations from endometrial biopsy (EB), cytobrush (CB) and low volume uterine lavage (LVL)

Sampling technique	*Criteria used*	*Histology positive (PMN +)*	*Histology negative (PMN-)*	*Sum*	*Sensitivity*	*Specificity*	*PPV*	*NPV*
Endo-metrial biopsy (EB)	Culture positive	5	1	6	0.83	0.75	0.83	0.75
	Culture negative	1	3	4	-	-	-	-
	Sum	6	4	10	-	-	-	-
	Cytology positive	2	1	3	0.33	0.75	0.66	0.42
	Cytology negative	4	3	7	-	-	-	-
	Sum	6	4	10	-	-	-	-
Cyto-brush (CB)	Culture positive	5	1	6	0.83	0.75	0.83	0.75
	Culture negative	1	3	4	-	-	-	-
	Sum	6	4	10	-	-	-	-
	Cytology positive	3	0	3	0.5	1.00	1.00	0.57
	Cytology negative	3	4	7	-	-	-	-
	Sum	6	4	10	-	-	-	-
Low volume uterine lavage (LVL)	Culture positive	6	2	8	1.00	0.50	0.75	1.00
	Culture negative	0	2	2	-	-	-	-
	Sum	6	4	10	-	-	-	-
	Cytology positive	3	1	4	0.50	0.75	0.75	0.50
	Cytology negative	3	3	6	-	-	-	-
	Sum	6	4	10	-	-	-	-
Clear effluent of LVL	Culture positive	1	1	2	1.00	0.50	0.50	1.00
	Culture negative	0	1	1	-	-	-	-
	Sum	1	2	3	-	-	-	-

Substantial bacterial growth (+) or no growth/mixed growth of more than two organisms (-) from low volume uterine lavage (LVL) samples as well as cytological examination from the smears (+, - :≥1% PMN) tested against histological examination (+, - : ≥ 1 PMN-cell/5 high magnification fields (400X). Results from histological examination used as the best standard.

presented in Table 1. When histological examinations from EB were used as "the best standard", the sensitivity of bacteriology from an EB, CB and LVL technique was 0.83, 0.83 and 1.00, respectively. The specificity of culture/ bacteriology from EB, CB and LVL techniques was 0.75, 0.75 and 0.50; positive predictive values 0.83, 0.83 and 0.75, and the negative predictive values were 0.75, 0.75 and 1.00, respectively. Further, the sensitivity of cytology from EB, CB, and LVL techniques was 0.33, 0.5, and 0.50, respectively. Specificity of cytology was 0.75, 1.00 and 0.75; positive predictive values 0.66, 1.00 and 0.75, and negative predictive values were 0.42, 0.57 and 0.50, respectively (Table 1).

The sensitivity, specificity, positive and negative predictive values of bacteriology obtained from EB and LVL techniques were found to be similar and the values were higher than those obtained by Buczkowska *et al.* (2014). The sensitivity of bacteriology obtained from EB was, however, similar to that obtained by Nielsen (2005), but the sensitivity of cytology was much lower. In contrast, the sensitivity of cytology and microbiology obtained from EB and CB was much higher than in the study conducted by Overbeck *et al*. (2011). Whereas the sensitivity and negative predictive value of bacteriology obtained from lavage technique were found to be superior to the other two methods. The sensitivity of bacteriology from LVL was found higher. Therefore, flush culture increased the ability to detect infected mares based on culture alone. The improved sensitivity appeared to result from improved detection of gram-negative organisms as the recovery of β-hemolytic *Streptococcus* from uterine flush did not differ from the other methods. On the other hand, specificity and positive predictive value of bacteriology obtained from lavage technique were found inferior to the other two methods, most likely due to the increased risk of the sample contamination while collecting by LVL technique.

The sensitivity of cytology obtained from EB (0.33) was inferior to CB and lavage techniques (0.50 each), whereas, sensitivity, positive and negative predictive values of cytology obtained from CB was found to be superior to the other methods, while the same for cytology obtained from EB were found to be the lowest (Table 1). The present sensitivity of cytology obtained from EB and CB were much lower and not in agreement with the results obtained by Buczkowska *et al*. (2014). In all the cases, the sensitivity of the bacteriology was found to be higher than the sensitivity of cytology. This demonstrates that bacteriological diagnosis could be improved under field conditions so that both diagnoses of the infection and detection of the agents could be achieved within a short time (few days) with a very high degree of sensitivity.

When the results of cytological and bacteriological examinations were combined, we couldn't find any noticeable increase in sensitivity (Table 2). This was in contrast to the study conducted by Overbeck *et al*. (2011), where they could notice an increase in the sensitivity with the combined results.

"The best standard" is considered to be "the real truth". However, no test that is chosen as "the best standard" is 100 % accurate, and can consequently always be improved. Results obtained from this study suggest that the sensitivity of microbiological examinations of the equine endometrium can be increased by obtaining a sample from a lavage technique. A swab or a CB touches only a very small surface area, and therefore local inflammatory foci may remain undetected, which could explain the higher sensitivity of LVL (Christoffersen *et al*., 2015; LeBlanc *et al*.. 2007) as compared with swab or biopsy, both for culture and cytology. *E. coli* and some fungi can occur as local plaques, suggesting that the use of lavage allows the culture of *E. coli* more readily as compared with the swab (LeBlanc *et al*., 2007). Bacteriology and cytology from CB showed the highest positive predictive value and demonstrated that a positive result in any given sample is an accurate indication of endometritis, based on the presence of PMN cells in tissue samples. Morel *et al*. (2013) reported significantly reduced foaling rates associated with a threshold value of ≥1 % PMNs, the culture of a single bacterial isolate, and a combination of both.

The sensitivity, specificity, positive and negative predictive values and *k* values (agreement) of cytological and bacteriological examinations from EB, CB, and LVL in relation

Table 2: Sensitivity, specificity, positive and negative predictive values of the combination of cytological and bacteriological examinations from EB, CB, and LVL

	Histology positive (PMN +)	*Histology negative (PMN-)*	*Sum*	*Sensitivity*	*Specificity*	*PPV*	*NPV*
Culture and cytology of EB positive	2	1	3	0.67	0.67	0.67	0.67
Culture and cytology of EB negative	1	2	3	-	-	-	-
Sum	3	3	6	-	-	-	-
Culture and cytology of CB positive	3	0	3	0.75	1.00	1.00	0.75
Culture and cytology of CB negative	1	3	4	-	-	-	-
Sum	4	3	7	-	-	-	-
Culture and cytology of LVL positive	3	1	4	1.00	0.67	0.75	1.00
Culture and cytology of LVL negative	0	2	2	-	-	-	-
Sum	3	3	6	-	-	-	-

Table 3: Sensitivity, specificity, positive and negative predictive values (PPV and NPV) and the agreement (*k*) between the number of PMNs infiltrated into the luminal epithelium and stratum compactum and cytology results for smears obtained from EB, CB and LVL

	Sensitivity	Specificity	PPV	NPV	k
Cytology of EB[a]	0.33	0.75	0.66	0.42	0.07
Cytology of CB[a]	0.50	1.00	1.00	0.57	0.44
Cytology of LVL[a]	0.50	0.75	0.75	0.50	0.23
Cytology of EB[b]	0	1.00	0	0.40	0.00
Cytology of CB[b]	0	1.00	0	0.40	0.00
Cytology of LVL[b]	0	1.00	0	0.40	0.00

[a]Smears were regarded as indicative of inflammation if the amount of PMNs was ≥ 1%.
[b]Smears were regarded as indicative of inflammation if ≥ 1 PMNs were found /HPF.
Kappa values and strength of agreement- $k \leq 0.2$ (poor); 0.21-0.4 (fair); 0.41-0.6 (moderate); 0.61-0.8 (substantial); and $k > 0.8$ (good)

Table 4: Sensitivity, specificity, positive and negative predictive values (PPV and NPV) and the agreement (*k*) between the number of PMNs infiltrated into the luminal epithelium and stratum compactum and culture/bacteriology results for samples obtained from EB, CB and LVL

	Sensitivity	*Specificity*	*PPV*	*NPV*	*k*
Culture of EB	0.83	0.75	0.83	0.75	0.58
Culture of CB	0.83	0.75	0.83	0.75	0.58
Culture of LVL	1.00	0.50	0.75	1.00	0.55

to the method (histology) applied to smear evaluation are presented in Tables 3-4.

The sensitivity values were always higher if smears were evaluated according to criterion II (PMNs to epithelial cells ratio), and the highest values were observed in specimens collected from CB and LVL. The agreement (*k* value) between the number of PMNs infiltrated into the endometrial luminal epithelium, and stratum compactum and cytology results for smears evaluated according to criterion II was usually poor or fair. However, when cytological smears were evaluated according to criterion I (PMNs per HPF), the agreement was always poor. These observations were in agreement with those made by Kozdrowski *et al*. (2013, 2015). In contrast, this study has shown same values for sensitivity, specificity, positive and negative predictive values, and *k* values while evaluated using criterion I, because, none of samples had positive cytology in any of the three methods when evaluated using criterion I. These results indicate that evaluation of cytological smears based on counting PMNs in relation to epithelial cells is a better method for diagnosis of endometritis than counting the number of PMNs per HPF. In the study conducted by Cocchia *et al*. (2012), an agreement between the different criteria adopted to evaluate smears obtained with the same technique was also poor. These data indicate that we cannot expect similar results using different evaluation methods.

In our study, samples from EB were smeared on a glass slide for cytological examination, and they were obtained from the same place as reference biopsy samples, however, the samples obtained for cytology using the CB technique were most likely not identical to the biopsy samples due to different sampling locations. Despite the differences in the locations of sampling for cytology between the EB and CB techniques, agreement between the numbers of PMNs infiltrated into the endometrial luminal epithelium and stratum compactum and the cytology results was usually poor or fair for both sample collection techniques. The agreement of the diagnosis of endometritis between the three techniques of the collection was from fair to poor, and between the different criteria adopted to evaluate smears obtained with the same technique was always poor (Table 3). On the other side, the agreement of the diagnosis of endometritis by the microbial culture was moderate between the three techniques of collection (Table 4).

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