

Diagnosis of Endometritis in the Mare Based on Bacteriological and Cytological Examinations of the Endometrium: Comparison of Results Obtained by Swabs and Biopsies

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ABSTRACT

The objective of this study was to compare results from endometrial cytological and bacteriological examinations obtained by two different laboratories. One laboratory used endometrial swabs and the other endometrial biopsies for the examinations. A higher proportion of sterile, cytology positive cases were found in the laboratory using swabs (148/401 [37%] vs 12/237 [5%], $P < .0001$). In both laboratories it was found that positive cytology was less likely to occur when *Escherichia coli* was isolated than if other species were isolated. This tendency was significant in both laboratories ($P < .05$ vs $P < .0001$).

Keywords: Endometritis; Swab; Biopsy; Bacteriology; Cytology; *E. coli*

INTRODUCTION

Since endometrial cytology was described as a diagnostic aid in evaluating potential endometrial inflammation in the beginning of the 1960s,¹ it has become a routine tool in reproduction practice. In the following years, the method has been described and further developed by numerous authors as a useful tool in the diagnosis of endometrial inflammation and an indicator of bacterial endometritis.²⁻¹² Using this technique, uterine inflammation can quickly be assessed,

and the decision of whether to breed the mare can be made instantly, without having to wait for culture results.¹⁻¹²

Endometrial cytology has been described and related to bacteriological findings by several authors.^{3,4,9-12} It has been used both to diagnose inflammation in relation to persistent endometritis^{3,4,9-12} and in relation to postbreeding endometritis.⁶ In a recent study, identification of polymorph nuclear neutrophils (PMNs) in an endometrial cytology smear was found to have a sensitivity of 0.77 when compared to the identification rate of PMNs from a histological preparation from the same animals.¹¹ The same study showed a specificity and a positive predictive value of endometrial cytology of 1.00 and a negative predictive value of 0.62. This clearly indicates that the quick method of endometrial cytology has a relatively high reliability in diagnosing endometrial inflammation, although false negative cases are to be expected.

A recent study has related uterine cytology and bacteriological findings to pregnancy results.¹² Mares showing inflammation on cytology specimens were found to have lower pregnancy rates than mares with normal cytological findings, irrespective of culture results. Both uterine cytology and bacterial findings were found of diagnostic value; however, twice as many mares were identified by endometrial cytology than by endometrial culture.

The bacterial species found by endometrial culture vary considerably, depending on the laboratory where the examinations are done.^{4,9,11,12} A higher sensitivity of the bacteriological test has been described, when a biopsy is examined instead of a guarded swab.¹¹

The relationship between findings from cytology specimens and endometrial culture has not recently been described. The aim of this study was to compare two methods of culturing endometrial specimens including endometrial swab and biopsy, respectively, and correlate the results to the results obtained by endometrial cytology. In practice it was done by comparing findings from cytology specimens and endometrial culture in a North

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American and a European equine practice using endometrial swabs and biopsies, respectively.

MATERIALS AND METHODS

Animals and Laboratories

Ansager Large Animal Clinic in Denmark and the Hagyard-Davidson-McGee Clinic in Lexington, Kentucky, both practices with their own laboratories, participated in this study. A total of 902 samples were included in the investigation. The Danish samples were obtained during the breeding seasons 2004–2006, whereas all the American samples were from the breeding season of 2006. All samples were submitted to either laboratory for routine diagnostics independent of the individual mare's previous fertility history.

Sample Collection

Samples from the Kentucky laboratory were obtained with guarded swabs following the procedure described by Brook.⁴ Samples from the European Laboratory were obtained from an endometrial biopsy following the procedure described by Nielsen.¹¹

Microbiology

All samples, whether from biopsies or swabs, were smeared on the surface of a blood agar plate (BA) (Mueller-Hinton agar added 5% toxin free calf-blood). After 24 hours incubation in atmospheric air at 37 °C, bacterial growth was identified and evaluated. BAs with no growth were incubated for another 24 hours at 37 °C and re-evaluated.¹³ Only BAs with more than five bacterial colonies in pure culture were recorded as positive. If more than 90% of the colonies on a BA had the same phenotype, the result was considered as a positive and pure culture. Presumptive identification of bacteria and yeast was based on colony morphology and reactions with potassium hydroxide and catalase in accordance with Quinn et al.¹⁴

Cytology

The endometrial biopsy and swab was smeared on a microscope glass slide after being streaked onto to the BA. The Danish slides were stained using the Haemacolor^R-system (Merck, Glostrup, Denmark), whereas the American slides were stained using the Diff Quick^R-system (Hemal Stain Co. Inc., Danbury, CT). All slides were examined by light microscopy (400× magnification) for the presence of PMNs. The sample was considered positive for endometritis when PMNs made up $\geq 0.5\%$ of all cells.¹⁵ A minimum of 200 cells was counted in each sample.

Statistics

All comparisons were made by χ^2 test, and significance was set at $P \leq .05$.

RESULTS

Results of endometrial culture and cytology (PMN \pm) in a practice in Ansager, Denmark, 2004–2006 (352 samples), and Lexington, KY, 2006 (550 samples), are presented in Table 1.

A significantly higher number of cytology positive, culture negative samples were found in the practice using swabs (Lexington) versus the practice using biopsies (Ansager). The number of "other" bacteria isolated was also significantly higher in the Lexington practice. In summary, 95% (225/237) of the sterile biopsies had negative cytology, whereas 63% (253/401) of the sterile swabs were negative for PMNs. In the same manner it is shown that 67% (76/114) of the bacteriology positive biopsy samples had positive cytology compared to 66% (99/149) of the bacteriology positive swabs had positive cytology.

Presence of PMNs during infections with *Escherichia coli* (*E. coli*) versus other agents and comparison of results obtained by the European practice in Ansager, Denmark, and the American practice in Lexington, are presented in Table 2.

The number of cytology negative *E. coli* samples was significantly higher in both practices compared to cytology negative samples when other bacterial species were isolated. This was particularly the case in the practice (Ansager) using biopsies ($P < .0001$ vs $P < .05$).

DISCUSSION

The significant higher number of culture-negative, cytology-positive samples found in the Kentucky laboratory could be explained by several possible factors. One reason could be a different lower limit of when to record a sample positive in the two laboratories, when only a few PMNs were found. This could be supported by the fact that the proportion of cytology positive samples was higher in the Kentucky than the Ansager laboratory (45% vs 25%). However, detection of PMNs in a cytology sample is quite simple, and the theoretical lower detection limit was the same in the two laboratories; this leaves the human factor as a likely explanation to this difference together with the fact that it is two different horse populations that are being compared. Another, and to us more plausible explanation however, lies in the type of sample obtained in the Ansager laboratory as opposed to the endometrial swabs from the Kentucky laboratory. It has previously been described that bacterial culture obtained from swabs has a lower sensitivity and a lower negative predictive value than bacterial culture obtained from an endometrial biopsy.¹¹ The same lack of sensitivity and negative predictive value was likely reflected in this study (Table 1), where 148 of 401 sterile samples (37%) based on swabs were found to contain PMNs, whereas only 12 of 237 culture-negative samples (5%) based on biopsies contained PMN's ($P < .0001$).

Table 1. Results of endometrial culture and endometrial cytology (PMN \pm) in a practice in Ansager, Denmark 2004-2006 (352 samples) and Lexington, Kentucky 2006 (550 samples)

Bacterial species identified	Ansager		Lexington	
	Cytology (+)	Cytology (-)	Cytology (+)	Cytology (-)
	No. samples (%)	No. samples (%)	No. samples (%)	No. samples (%)
Sterile	12 (3)	225 (64)	148 (26)	253 (46) ^a
β -hemolytic <i>Streptococcus</i> spp.	53 (15)	15 (4)	55 (10)	24 (4)
<i>E. coli</i>	3 (1)	20 (6)	12 (2)	12 (2) ^b
<i>Staphylococcus aureus</i>	8 (2)	0 (0)	0 (0)	0 (0)
Yeast	2 (<1)	3 (1)	8 (1)	4 (<1)
<i>Micrococcus</i> spp.	5 (1)	0 (0)	3 (<1)	0 (0)
<i>Enterococcus</i> spp.	3 (1)	0 (0)	3 (<1)	1 (<1)
<i>Pseudomonas</i> spp.	2 (<1)	0 (0)	4 (<1)	4 (<1)
Other	0 (0)	0 (0)	14 (3)	5 (1) ^b
Total	88 (25)	264 (75)	247 (45)	303 (55) ^b

^a $P < .0001$.^b $P < .01$.**Table 2.** Presence of PMNs during infections with *E. coli* versus other agents. Comparison of results obtained by the European practice in Ansager, Denmark, and the American practice in Lexington, KY

Agent	Ansager			Lexington		
	Cytology (+)	Cytology (-)	Total	Cytology (+)	Cytology (-)	Total
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
<i>E. coli</i>	3 ^a (1)	20 (17)	23 (20)	12 ^b (8)	12 (8)	24 (16)
Other	73 (63)	18 (17)	91 (80)	87 (58)	38 (26)	125 (84)
Total	76 (66)	38 (34)	114 (100)	99 (66)	50 (34)	149 (100)

^a $P < .0001$.^b $P < .05$.

Again, as the samples compared were obtained by two different laboratories and from two different horse populations, part of the reason for the difference may lie here. The ultimate test would have been if both laboratories had evaluated the same tests. This, however, is not possible in a retrospective study like this, and in addition, due to simplicity of the procedures, we do expect the major difference observed to originate from the sample type rather than a mare or human-related factor.

Interestingly, in both laboratories, a significant number of samples culturing positive for *E. coli* were not associated with the presence of PMNs (Table 2). This was highly significant from the Ansager laboratory data compared to the data from the Kentucky laboratory ($P < .0001$ vs $P < .05$), again indicating a lower sensitivity of swab cultures for detection of *E. coli*. We observed similar results in a recent study by Bindslev et al,¹⁶ where presence of PMNs in

a sample with *E. coli* was much less likely than if *Streptococcus equi* subsp. *zooepidemicus* was cultured. Riddle et al¹² made a similar observation in a study of endometritis in Thoroughbreds. We can merely speculate on the reasons for these findings, which may be due to an ability to prevent activation of the immune response, possibly by the *E. coli* not being as chemotactic to PMNs in the endometrium as other bacteria or yeast. This, however, has to be investigated in detail in future studies.

The examinations in this study were made retrospectively, meaning that there was no information available in the records whether they were a result of routine investigations or made on clinical indication. If genetically different strains of *E. coli* would give rise to different levels of PMNs in the endometrium, the clinical outcome of endometritis could be of significant importance to know in a study like this. This hypothesis, however, was not supported by the

work of Bindslev et al,¹⁶ who were unable to identify specific types of *E. coli* associated with negative cytology.

In conclusion, the results in this study clearly show the importance of performing both bacteriological and cytological examinations of the endometrium, as it is not sufficient to rely on either method alone, a suggestion that has been raised by several studies in the past.^{9,11-12} This is particularly true in relation to *E. coli* infections, where a diagnosis based on positive cytology is likely to result in a considerable number of false negative samples.

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