

## Diagnosis of equine endometritis

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### Introduction

Diagnosis of infertility problems in the mare by sampling of material from the uterus was first described more than 85 years ago in Kentucky, USA and Germany (1,2). Uterine disease, primarily endometritis, is still one of the major causes for infertility in the mare and a substantial problem in the modern breeding industry (3).

Uterine samples have mainly been used to diagnose infertility problems and endometritis by bacterial culture (4,5), cytology (6,5), and histology (7).

The finding of polymorph nuclear cells (PMN's) in a sample demonstrates the presence of endometrial inflammation, but without identification of the cause, it often is not possible to propose an optimal treatment protocol (4). A more differentiated diagnosis of both inflammatory and degenerative causes for endometritis and endometriosis can be obtained by a histological examination of an endometrial biopsy (7). The severity of the endometrial inflammation and degeneration found is correlated to the chance of the uterus to support an established pregnancy to term (7).

Originally material from the uterus for bacteriological and cytological examination was proposed collected through a vaginal speculum using a sterile cotton swab (1,2,4). In recent years however, alternatives to the guarded swab for collection of material from the equine endometrium have been proposed, and the sensitivity and specificity of these diagnostic tests have been evaluated and compared with established techniques (9,10). These methods include bacteriology and cytology obtained from double guarded swabs, cytological brushes, endometrial biopsies and endometrial flushings with sterile 0.9 % saline solution.

### Methods and procedures

Material from the endometrium and uterine lumen can be collected by several different techniques: A guarded sterile cotton swab (8), endometrial biopsy (9), uterine flush (10) and a cytobrush (11). The guarded swab can be used for diagnosing endometritis by cytology and bacteriology (5,9) whereas an endometrial biopsy can be used for diagnosis by cytology, bacteriology and histology (10). The uterine flush and the cytobrush can be used for both cytology and bacteriology (10,11). A stainless steel speculum was developed (Equi-Vet, Kruuse, 5550 Langeskov, Denmark) (9) to accommodate collection of a biopsy in an uncontaminated procedure (Fig. 1 and 2). The speculum, placed through the cervix of the mare, enable collection of an endometrial biopsy with low risk of contaminating the tip of the biopsy punch on the route back through the vagina and vulva. When the biopsy is collected, it can be smeared onto a blood agar for culture using a sterile pair of pincers. After the use for culture it can be smeared onto a glass slide and the cells can be stained for cytological examination. Finally the biopsy, if needed, can be fixed in 4% formaldehyde and processed in the laboratory for histological examination.

Fig. 1: From the top: Biopsy punches (Equi-Vet<sup>R</sup>; and Divisible biopsy punch); Stainless steel biopsy speculum (Equi-Vet<sup>R</sup>); Disposable guarded swab (Equi-Vet<sup>R</sup>).

Fig. 2: The Stainless steel biopsy speculum is placed in the cervix through the vagina, allowing access to the uterine lumen and the endometrium. Hereby an endometrial biopsy can be collected with a minimal risk of contamination.

### Culture

Samples are smeared on a blood agar plate (BA) (Mueller-Hinton agar added 5 % toxin free calf-blood). Following incubation in atmospheric air at 24 and 48 hours colony morphology, bacterial morphology using nigrosin stain and response to potassium hydroxide (KOH 3%) and catalase can be used for species differentiation (12). If more than 90 % of the colonies on BA are the same phenotype, the result can be recorded as positive growth and pure culture. Mixed growth with only two species of microorganisms, approximately 50 % of each, can also be recorded as significant (10).

### Cytology

Samples (Swab, cytobrush, biopsy or flush) are, after having been used for culture, smeared on a microscope glass slide. The cells are stained with Heamacolor<sup>R</sup> (Merck, Glostrup, Denmark) or Diff Quick<sup>R</sup> (Hemal Stain Co. Inc., Danbury, CT). The slides are examined by light microscopy (400X magnification) for the presence of PMN's. The samples can be considered positive for endometritis when the ratio PMN:endometrial cells  $\geq 0.5$  % (5).

### Results

To determine sensitivity and specificity of the commonly used guarded swab and the endometrial biopsy both samples were collected from the same mares, and culture and cytology results were correlated to the presence of PMN's detected on histology (used as "best" standard) (10).

Sensitivity of culture -and cytology from an endometrial biopsy was calculated 0.82 and 0.77 respectively. Sensitivity of culture from a swab was calculated 0.34.

In a study by LeBlanc *et al.* (10) sensitivity of an endometrial flush for culture and cytology determined 0.71 and 0.80 respectively. This is completely in accordance with a smaller study (33 mares) which calculated the sensitivity of the uterine flush as 0.65 (13).

In a study from 2010 results from cytology and bacteriology from a practice using endometrial swabs were compared with results from a practice using endometrial biopsies (5).

A significantly higher number of cytology positive, culture negative samples were found in the practice using swabs versus the practice using biopsies. In summary 95 % (225/237) of the sterile biopsies had negative cytology, whereas 63% (253/401) of the sterile swabs had negative cytology. In the same manner it was shown, that 67 % (76/114) of the culture positive biopsy samples had positive cytology, whereas 66 % (99/149) of the culture positive swabs were found to have positive cytology.

When *E. coli* was isolated, the number of cytology negative 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 using biopsies ( $P < 0.0001$  versus  $P < 0.05$ ).

### Discussion

Detection of stained PMN's by light microscopy is a quick and easy performed method for the diagnosis of endometritis in stud farm practice (6). There however is a potential risk of a false negative diagnosis. This is especially the case if *E. coli* is isolated from the endometrium (5). In this

study 31 % (15/47) had positive cytology when *E. coli* was isolated, whereas positive cytology appeared in 74% (160/216) of the samples when other species were isolated. These findings suggest, that *E. coli* compared to other pathogens, to a larger extent is capable of avoiding induction of an immune response in the endometrium.

Bacteriological examination of the endometrium can be done in several ways (8-10). A higher sensitivity and negative predictive value can be expected, if culture is performed from an endometrial biopsy rather than from a swab of the endometrial surface (9). The superior results of culture using an endometrial biopsy could be related to bacterial localization in the endometrium. Petersen *et al.* (14) has demonstrated that *Streptococcus equi* subspecies *zooepidemicus* is located deep within the endometrium in chronically infected mares. The specific localization of other pathogens in the endometrium still remains to be determined.

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