Diagnosis of 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 and Germany.[1,2] Endometritis, as one of the major causes for infertility in the mare, still seems to remain a problem in the modern breeding industry.[3] Samples from the uterus have mainly been used for diagnosing infertility and endometritis by bacterial culture[4, 5], cytology[6,5] and histology.[7] The combined use of cytology and bacteriology as an aid to the diagnosis of endometritis was initially proposed more than 20 years ago.[8] The finding of polymorph nuclear cells (PMN´s) in a sample shows the mare is suffering from an endometrial inflammation, but without identification of the causing agents, it is difficult to propose a treatment protocol.[4] A more differentiated diagnosis of both inflammatory and degenerative causes for endometritis and endometrosis can be obtained by a histological examination of an endometrial biopsy.[8] This method however is time consuming, as it takes time to mail, process, cut and stain a biopsy, before a diagnosis can be made - Time that is not often available in the middle of a breeding season.

Originally material from the uterus for bacteriological and cytological examination was proposed harvested with a vaginal speculum and a sterile cotton swab[1,2,4] Later this procedure was modified to the double guarded swabs, that today are widely commercially available.[9] In recent years however, new protocols for collection of material from the equine endometrium have been proposed, and their sensitivity and specificity under practice condition have been evaluated and compared with the already used methods .[10, 11] These methods includes bacteriology and cytology obtained from endometrial biopsies and endometrial flush with sterile 0.9 % saline solution.

Kenny and Doig[7] predicted expected foaling rates of a mare based on the histological conformation of the endometrium of the mares. Whether these predictions still are reliable under modern conditions has been questioned.[12] This paper shows results of pregnancy and foaling rates obtained recently in stud farm practice compared to histological groups as proposed by Kenney and Doig.[12]

Methods and procedure

Material from the endometrium and uterine lumen is described harvested by at least three different methods: A guarded sterile cotton swab[9], endometrial biopsy[10] and uterine flush[11]. These three methods as compared to each other have different advantages. The guarded swab can be used for diagnosing endometritis by cytology and bacteriology[5, 10] whereas an endometrial biopsy can be used for diagnosis by cytology, bacteriology and histology[11]. The uterine flush can be used for both cytology and bacteriology[11].

A stainless steel speculum has been developed (Equi-Vet, Kruuse, 5550 Langeskov, Denmark) [10] to make it possible to collect a biopsy in a sterile procedure with the commercially available types of biopsy punch (Fig. 1 and 2). The speculum makes it, placed through the cervix of the mare, possible to collect an endometrial biopsy without contaminating the tip of the biopsy punch on the route through the vagina.

When the biopsy is collected, it can be smeared onto a blood agar for bacteriology with a sterile pair of pincers. After the use for bacteriology it can be smeared onto a glass slide and be stained for

cytological examination. In the end the biopsy, if needed, can be fixed in 4% formaldehyde and processed in the laboratory for histological examination.

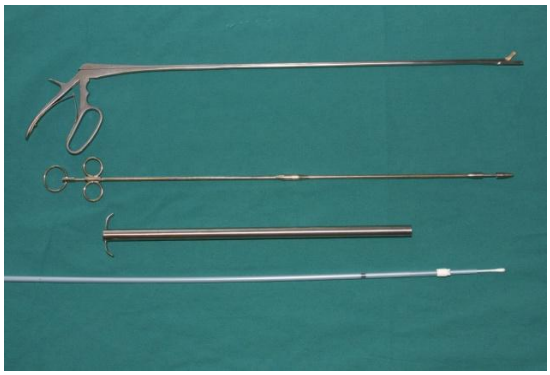

Fig. 1.

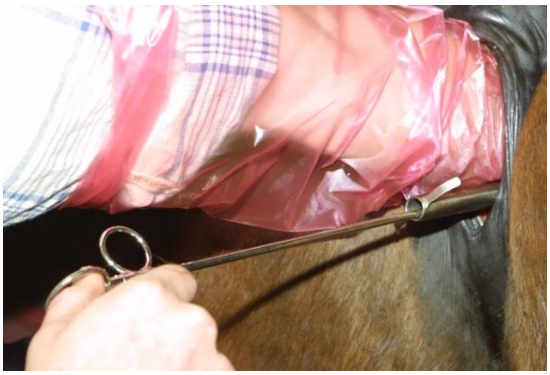

Fig.2.

Fig. 1: From the top: Biopsy punch of the type Equi-Vet[R]; Divisible biopsy punch; Stainless steel biopsy speculum; Disposable Equi-Vet[R] guarded swab.

Fig. 2: The Stainless steel biopsy speculum is placed through the vagina and cervix. The access to the endometrium and uterine lumen is free. It is possible to harvest a biopsy without the risk of contamination.

Bacteriology

Presumptive identification of bacteria and yeast can be made in laboratories in most practices. It is time saving not having to mail samples for a referral laboratory. Samples (Swab, biopsy or flush) are smeared on the surface of a blood agar plate, BA, (Mueller-Hinton agar added 5 % toxin free calf-blood). Colony morphology is recorded after incubation in atmospheric air at 24 and 48 hours. If more than 90 % of the colonies on BA are the same phonotype, the result is recorded as positive and pure culture. Reactions with potassium hydroxide and catalase can be used for further species differentiation[13] (Table 1).

Table 1. Presumptive identification of bacteria and yeast based on colony morphology and reaction with potassium hydroxide and catalase.

	Colony morphology	Heamolysis	Catalase	KOH	Cell	Other
Hem. Strep.	Pin point	Beta	-	-	Coccoid chains	
S. Aureus	Circular White	Alfa, Beta	+	-	Coccoid single/pairs	
E. Coli	Smooth Grey	+/-	+	+	Rods	Odor
Pseudomonas	Smooth Non pigm.	+/-	+	+	Rods, chains	Odor, sweet

Yeast	Circular Rough	-	-	-	Ovoid Diff. size

Cytology

Samples (Swab, biopsy or flush) are smeared on a microscope glass slide after being streaked onto a blood agar. The cells are stained with Heamacolour^{R} (Merck, Glostrup, Denmark) or Diff Quick^{R} (Hemal Stain Co. Inc., Danbury, CT). The slides are examined by light microscopy (400X magnification) for the presence of PMN´s. The samples are considered positive for endometritis when PMN´s are making up $\geq$ 0.5 % of all cells[14].

Histology

Endometrial biopsies for histological examination are fixed in 4 % formaldehyde and send to a referral laboratory for preparation and staining. HE and PAS stained slides are examined by light microscopy for the presence of inflammatory cells, glandular structure, fibrosis, lymphatic lacunae and other histological abnormalities as described by Schoon et. *al*.[12]. Classification as described by Kenny and Doig[7] is made.

Results

Table 2: Histological examination of an endometrial biopsy from 48 mares. Significant Bacteriological growth (+) or no growth/mixed growth (-) from biopsy smears and endometrial surface swabs was as well as cytological examination from smears (+,- > 0.5 % PMN) tested against histological examination (+,- > one PMN-cell pr. 5 high magnification fields (X400)). Results from histological examination are used as the best standard.[10]

	Histology + (PMN +)	Histology - (PMN -)	Sum	Sensi-tivity	Speci-ficity	Positive pred. val.	Negative pred. val.
Biopsy +	29 (60%)	1 (2%)	30 (62%)	0.82	0.92	0.97	0.67
Biopsy -	6 (13%)	12 (25%)	18 (38%)				
Sum	35 (73%)	13 (27%)	48 (100%)				
Swab +	12 (25%)	0 (0%)	12 (25%)	0.34	1.00	1.00	0.44
Swab -	23 (48%)	13 (27%)	39 (75%)				
Sum	35 (73%)	13 (27%)	48 (100%)				
Cytology +	27 (56%)	0 (0%)	27 (56%)	0.77	1.00	1.00	0.62
Cytology -	8 (17%)	13 (27%)	21 (44%)				

Sum	35 (73%)	13 (27%)	48 (100%)

Using the presence of PMNs in a tissue specimen from the uterus as the "best standard" for diagnosing endometritis, the sensitivity of bacterial growth from an endometrial biopsy was 0.82. The sensitivity of bacterial growth from an endometrial surface swab was 0.34, and the sensitivity for cytology was 0.77.
Another similar Danish study comparing bacteriology from uterine flush with bacteriology from a biopsy and histology as golden standard calculated the sensitivity of the uterine flush as 0.65.[15] The same study showed a lack of ability to identify PMN´s in the flush.

Table 3: Results of endometrial culture and endometrial cytology (PMN +/-) in a practice in Ansager, Denmark 2004-2006 (352 samples) and Lexington, Kentucky 2006 (550 samples).[5]

	Ansager		Lexington	
	Cytology (+)	Cytology (-)	Cytology (+)	Cytology (-)
Bacterial species identified	No. samples (%)	No. samples (%)	No. samples (%)	No. samples (%)
Sterile	12 (3)	225 (64)	148 (26)	253 (46)[a]
β-hemolytic *Streptococcus* spp.	53 (15)	15 (4)	55 (10)	24 (4)
E. coli	3 (1)	20 (6)	12 (2)	12 (2)[b]
Staphylococcus aureus	8 (2)	0 (0)	0 (0)	0 (0)
Yeast	2 (<1)	3 (1)	8 (1)	4 (<1)
Micrococcus spp.	5 (1)	0 (0)	3 (<1)	0 (0)
Enterococcus spp.	3 (1)	0 (0)	3 (<1)	1 (<1)
Pseudomonas 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<0.0001, [b]P<0.01.

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 PMN´s. 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.

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 ($P<0.0001$ versus $P<0.05$).

Table 4: Pregnancy and live foal percentage related to histological quality of the endometrium expressed by classification group as described by Kenney and Doig[7] in 74 mares at Ansager Dyrehospital, Denmark 2001-02.

Kenney Group	Number of mares	% pregnant 17 day check	% live foal
I	11	100	100
IIa	22	82	67
IIb	20	55	35
III	21	28	14

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] The results from Ansager and Lexington displayed in table 3, however, shows that the result of what can be expected of a routine cytological examination depends on which bacteria is isolated. If E. coli is isolated from the endometrium, positive cytology is found in 31 % (15/47) of the samples. If another agent is isolated, positive cytology is likely to appear in 74% (160/216) of the samples. Similar numbers have recently been reported in a study from University of Copenhagen and University of Kentucky. [16] These finding suggest that E. coli is unable to induce the same immune response as other bacteria and yeast in the endometrium. The difference in the ability to detect endometritis, depending on which bacteria is the cause, also suggests that the cytological test always should be interpreted together with a bacteriological examination of the same material from the endometrium.

Bacteriological examination of the endometrium can be done in several ways. [9-11] The results in table 2 show that the best result regarding sensitivity and negative predictive value can be expected if bacteriology is performed from a biopsy of the endometrium rather than from a swab of the endometrial surface. Similar better results have been reported if bacteriology is performed from an endometrial flush with saline.[15] The superior results of the bacteriological examination of a biopsy could relate to either the fact that more material is harvested with a biopsy, or that the bacteria is residing deeper in the tissue. The theory that bacteria is residing deeper in the tissue has been supported by a paper by Petersen *et al*.[17] in 2009. Streptococcus equi subspecies zooepidemicus was found to be present deep in the endometrial crypts or intercellular in the endometrial epithelium, when visualized by Fluorescent In Situ Hybridization (FISH).

Histological examination of an endometrial biopsy is capable of providing far more information than the more simple cytological examination. It is however time consuming and requires sophisticated laboratory facilities to cut, slice and stain the slides. Histological examination will, this in mind, often be used, if more severe problems are suspected or as a control on barren mares in the end of a breeding season.[14] Some authors[12] have questioned the value of the classification system set up by Kenney and Diog[7]. These authors[12] suggest that the classification system is too simple and does not take all histological changes in the endometrium under consideration. These authors therefore suggest, that more groups should be added to the system, or that the mares' endometrium is solitary evaluated from a histological description of the found histological changes.

The result in table 4 however shows that results similar to those obtained by Kenny and Doig still can be expected in modern stud farm practice. There is a wide range of histological changes, the Kenney and Doig classification system does not consider. The system is therefore under practice conditions recommended used, together with a more differentiated description of the histological abnormalities found. The system however still predicts a reliable picture, of what the chances of pregnancy will be for the examined mare.

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