Endometritis is a major cause of sub-fertility and infertility in the mare, with prevalence of 25% to 60% in barren mares\textsuperscript{1,3}.

The pathogenesis of infectious endometritis is multifactorial and identification of risk factors, timely diagnosis and efficacious treatment is essential for a successful breeding outcome. Endometritis in the mare can be divided into subclinical, acute infectious, chronic infectious or persistent breeding-induced endometritis (PBIE)\textsuperscript{4}.

Mares show acute inflammation after semen deposition, which is characterised by an innate
immune response that includes a rapid influx of polymorphonucleated neutrophils (PMNs)\textsuperscript{5}.

It is believed this inflammatory process is necessary for an effective removal of contaminating bacteria and excess spermatozoa from the uterine lumen\textsuperscript{6}, and, in the resistant mare, resolves within 24 to 48 hours\textsuperscript{7}, leaving the uterus free of any inflammation in time for conceptus arrival. The susceptible mare, however, fails to resolve the inflammation, resulting in persistent endometritis.

If bacteria are present, the mare will develop a persistent infectious endometritis, which may affect the outcome of a pregnancy\textsuperscript{8}. Experimental studies have demonstrated the inflammatory response to spermatozoa is indistinguishable from the inflammation triggered by bacteria in the resistant mare\textsuperscript{5,6}. This inflammation appears to be limited to the uterus and no changes in blood parameters, such as acute phase proteins and leukocytes, are observed\textsuperscript{9}.

The pathogenicity of the bacteria depends on virulence factors, such as induction of inflammation, resistance to phagocytosis, adherence to epithelia and viscosity of secretions, and they vary greatly between pathogens\textsuperscript{3}. Infectious endometritis in the mare is most commonly associated with aerobic pathogens\textsuperscript{9}, but it is important to remember isolation of bacteria will not necessarily prove the presence of endometritis. Failure to isolate bacteria, meanwhile, does not eliminate the diagnosis\textsuperscript{4,10}.

### Examination methods

The sensitivity and specificity of diagnostic tests influence perception of the prevalence of endometritis and the need for treatment. Examination includes a detailed history of the mare, clinical examination and sampling of the uterus.

### Clinical examination

Transrectal palpation provides information regarding size, tone and position of the uterus and the cervix. Using ultrasonography, the diameter of uterine horns, degree of endometrial oedema, presence of intrauterine fluid (IUF), endometrial cysts, presence of air and pregnancy can be detected\textsuperscript{11}.

Oestrogen dominance will increase the degree of endometrial oedema and it will normally decrease towards ovulation\textsuperscript{12}, and excessive oedema prior to breeding has been associated with chronic \textit{Streptococcus zooepidemicus} endometritis\textsuperscript{13}. IUF, meanwhile, accumulates due to increased production and/or delayed clearance of fluid. Decreased myometrial contractility is the main reason for delayed uterine clearance\textsuperscript{14}, but high concentrations of nitric oxide (NO) and inducible nitric oxide synthase (iNOS), which are responsible for smooth muscle relaxation, are hypothesised to play a role in the pathogenesis of delayed uterine clearance\textsuperscript{15,16}.

Accumulation of IUF during oestrus is consistently associated with decreased pregnancy rates\textsuperscript{17,18},
and presence of two or more centimetres of fluid is considered a good indicator for susceptibility to PBIE\textsuperscript{19}.

IUF is not always associated with isolation of pathogens\textsuperscript{20}, which could be explained by low sensitivity of the diagnostic test used. Subclinical endometritis is defined as “failure to display expected clinical signs of endometritis”, which typically will be IUF accumulation. These mares can be difficult to identify, making an accurate diagnosis even more important.

Vaginal discharge is a clinical sign of endometritis, but many mares have no discharge. A speculum examination will show the presence of abnormal secretions in the vagina or from the cervix, and can be a valuable diagnostic tool.

Endometrial samples are most commonly obtained at the beginning to middle of oestrus\textsuperscript{21}. The uterine innate immune response is believed to be maximal during this time, so if bacteria and/or a neutrophil response are present, a true problem is likely to exist.

The risk of introducing bacteria to the uterus and inducing an iatrogen endometritis during oestrus, in the absence of progesterone, is minimal.

**Uterine sampling**

**Double-guarded swabs/cytobrushes**

Double-guarded swabs/cytobrushes have two tubes through which the inner swab/brush is pushed when the instrument is in the uterus. The swab/brush is then pushed against the endometrial wall for a minimum of 30 seconds before being retracted from the uterine lumen.

**Biopsies**

An endometrial biopsy can be used for histology, bacteriology and cytology. To prevent contamination from the external orifice and canal of the cervix, a sterile steel speculum can be used as described by Nielsen\textsuperscript{22}.

**Low-volume lavage**
Figure 1. Double-guarded lavage equipment ready for use.

Low-volume lavage (LVL) can be performed using a double-guarded or non-guarded technique. The unguarded approach initially suggested by Ball et al\textsuperscript{23}, and later modified by LeBlanc et al\textsuperscript{24}, includes the use of a uterine catheter with an infusion of 60ml sterile saline. An evaluation of the efflux fluid and the amount of debris on cytology was included in the handling of the sample to reduce the risk of false-positive diagnosis caused by contamination from the vagina and cervix\textsuperscript{24}.

The author et al developed a double-guarded technique for collecting lavage samples by the use of a sterile steel speculum covered by a sterile sanitary sleeve, through which a disposable uterine flushing tube was guided. A 250ml sterile saline fluid bag was then attached and the uterus flushed\textsuperscript{25} (Figure 1).

Laboratory analyses

Microbial culture

Uterine samples should be inoculated directly on to blood agar plates in an environment clean and free from sources of plate contamination. They should be incubated aerobically at 37°C and evaluated after 24 hours and 48 hours.

Identification is based on morphology, colony size and haemolysis. Simple diagnostic tests, such as catalase and potassium hydroxide (3%), can help further identification of bacteria, while colonies need to be identified as potentially pathogenic or non-pathogenic. The criteria for positive pathogenic growth diverge, with the threshold for positive pure cultures varying from one to greater than 10 colonies per plate. Growth of more than three different pathogens has been considered as contamination\textsuperscript{22,25}.

Interpretation of microbiological results is based on the organism isolated, but also on the clinical history of the mare and the results of gynaecological examination. Endometrial cytological examination should be used routinely together with microbial culture to improve the accuracy of interpretation of routine endometrial bacteriological results\textsuperscript{10,22,26}. The most common isolated pathogens are \textit{Escherichia coli} (Figure 2) and \?-haemolytic streptococci, but also \textit{Staphylococci} species, \textit{Klebsiella} species, \textit{Pseudomonas aeruginosa}, yeast and fungi cause endometritis\textsuperscript{10}.

Cytology

Endometrial cytology has become a routine tool for diagnosing endometrial inflammation in the mare.
Neutrophils play a key role in the elimination of pathogens; they are remarkably short-lived (circulating half life of six to eight hours)\textsuperscript{27}, which makes them ideal as a biomarker for acute inflammation. Used in conjunction with a gynaecological examination, mares with acute endometritis can be identified quickly, meaning treatment can be initiated and decisions regarding reproductive management made without having to wait for culture results.

Not all uterine pathogens are associated with an inflammatory cytological response, however, so it is of great importance to correlate laboratory findings with clinical findings before treatment is initiated\textsuperscript{22,24,25}.

The cytological smear can be stained with Diff-Quik or Hemacolor, and will reveal the presence of epithelial cells, PMNs, red blood cells, mucus, hyphae, yeast, debris and bacteria. It is most ideal to make the cytological smear immediately to prevent the cells drying on the sample. Slides can easily be air-dried before staining. The uterine biopsy can be smeared with the luminal surface against the microscopic glass slide. The LVL have to be centrifuged (10 minutes × 400g)\textsuperscript{10} and the pellet resuspended\textsuperscript{25} before a sterile cotton swab can be used for obtaining a sample, which will be rolled on to a glass slide.

The evaluation of endometrial cytology is based on the presence of PMNs indicating an active inflammation. The smears should be evaluated for cellularity, cell morphology, cell type and debris\textsuperscript{24}, first at low magnification (100×) under bright-field microscopy, and then a high magnification (×400).

Cellularity is quantified by counting the number of PMNs in 10 high-power fields (HPF)\textsuperscript{24} or...
counting 100 to 300 cells on a slide – a study showed only counting 100 cells overestimated or underestimated the prevalence of subclinical endometritis in cows.

The threshold for an acute endometritis, based on the presence of PMNs, varies between studies, with 0.5% to 5% PMNs reported. Kozdrowski et al suggested 1% as the threshold for acute inflammation when using the cytobrush for cytology, compared to 2% PMNs in swab samples. Cocchia et al compared different sampling techniques for endometrial cytology and showed cytobrushes yielded a higher cell count, compared to swab and low volume lavage.

Bacteria, rods and cocci can be visualised under oil immersion and high magnification (×1000). Yeast spores may vary in size, colour and shape and branching hyphae may be seen if a lot of thick debris is not present. The debris contains mucus, biofilm, degenerated PMNs, epithelial cells and inflammatory byproducts, and is associated with cytological specimens from LVL. The centrifugation may disrupt cell walls and thereby increase the amount of debris.

Histology

The endometrial biopsy is fixed in formalin, embedded in paraffin and cut in 3µm to 4µm sections, deparaffinated, rehydrated and stained with haematoxylin and eosin, and evaluated according to Kenney.

The infiltration of PMNs in stratum compactum, stratum spongiosum and in the luminal epithelium is graded as none, mild, moderate or strong. PMNs per HPF can also be counted. Inconsistent criteria for acute endometritis diagnosis make it difficult to compare different studies. Some researchers consider 1PMN/5HPF as positive, whereas other studies have used a higher threshold for a positive diagnosis since the presence of a few neutrophils in the uterus during oestrus does not necessarily indicate inflammation.

The presence of PMNs in endometrial tissue is considered the best standard other methods can be compared to. Studies have shown swabs and cytobrushes have the lowest sensitivity – 0.34 and 0.5, respectively. Nielsen has reported high sensitivity for bacterial culture from the biopsy (0.8), but other studies demonstrated lower sensitivity (0.63 and 0.5).

A cytobrush or a swab only touches a very small surface of the uterus, and the biopsy is comparable in size, so local inflammatory foci may remain undetected – this may explain the higher sensitivity of the LVL compared to swab and biopsy for both culture and cytology. The sensitivity of the different sampling methods increases if bacterial culture and cytology are done in conjunction.

Positive bacterial culture, together with PMN detection, has traditionally been considered as a positive endometritis diagnosis. E coli infections are associated with negative cytology, and it has been suggested some E coli strains have a low chemotactic potential. If PMNs are present, but
no pathogens isolated, it could be a sterile inflammation or failure of the diagnostic test to isolate the pathogen, and an LVL should be considered as a diagnostic test.

Uterine sampling is problematic because of the risk of instrument contamination with potential pathogens when passing them through the caudal genital tract. Double-guarded techniques, coupled with aseptic procedures are, therefore, essential.

Positive growth of pathogens should be supported by the presence of PMNs on a cytological specimen, with mare’s history and the results of the gynaecological examination in mind.

References


