Uterine diagnostics: what is the best sample?

Uterine disease, particularly endometritis, is commonly encountered in equine reproduction and has major implications for mare fertility and breeding economics. A thorough reproductive examination, combined with selection of appropriate uterine diagnostics and interpretation of those findings, is essential in the work up of cases of mare infertility. Addition of endometrial cytology and alternative culture methods, such as the low-volume lavage and uterine biopsy, will increase the clinician's diagnostic sensitivity and specificity. The practitioner's approach to the investigation of uterine disease in the field and required techniques are described in this review article.

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Emma Chedgey MVB MRCVS CertAVP(ESM) Lisadell Equine Hospital, Follistown, Navan, Co. Meath, Ireland, C15 E27X. chedgeyemma@gmail.com

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Uterine disease, particularly endometritis, is one of the main causes of infertility in the mare. Effective management of uterine disease requires accurate diagnosis, identification of any infectious agents, and elimination of any predisposing factors. Endometritis cases may be broadly categorised into those which are infectious in origin, such as bacterial or fungal endometritis, or those with a sterile inflammatory process, such as mares that are 'susceptible' to persistent mating-induced endometritis. In reality, many mares experience elements of both, with susceptible mares being at a higher risk of developing infectious endometritis. Selecting appropriate sampling techniques and accurately interpreting their findings are essential in guiding case management.

Presentation

Mares with uterine disease most commonly present as cases of poor fertility, including failure to conceive, pregnancy loss or abortion. Mares with a history of uterine fluid accumulation, abnormal or excessive oedema patterns, or shortened or irregular oestrus cycle lengths are common presentations of uterine disease. Other forms of uterine disease can occur, such as pyometra, postpartum metritis or haemorrhage and, in some cases, can present as acute illness in the mare. This clinical summary focuses on the investigation of uterine causes of poor fertility.

Factors predisposing mares to uterine disease

Aged or multiparous mares are at a higher risk compared with younger maiden mares as they are more likely to have developed failures in uterine defence mechanisms, predisposing them to pneumovagina and contamination of the reproductive tract. Poor perineal or vulvar conformation (*Figure 1*), a loss of the vestibular seal, or cervical abnormalities such as cervical tears, stenosis or fibrosis will predispose mares to uterine disease. A large, pendulous uterus in older mares will inhibit natural uterine clearance mechanisms. Similarly, mares with a history of dystocia, trauma to the reproductive tract, pregnancy loss or abortion are considered higher risk for uterine disease.

The diagnostic approach

When a mare is infertile, a common first step is to investigate any potential uterine disease. A reasonable approach to any problem mare is to determine the answers to the following questions: Does the mare have any factors predisposing her to uterine disease? Is inflammation present in the uterus? If so, are bacterial or fungal infections present?

Transrectal ultrasonography

Any thorough reproductive evaluation will begin with transrectal palpation and ultrasonography of the reproductive tract. It is vital to determine the stage of the oestrus cycle, whether the mare's oedema pattern is appropriate for the stage of her cycle, and whether there is any evidence of uterine free fluid (volume/character) or air in the lumen. Uterine free fluid can range from anechoic to hyperechoic, or mixed echogenicity, and should be graded by the depth in centimetres (*Figure 2*). Air is generally evident as hyperechoic lines or foci in the lumen of the uterus and may cast an acoustic shadow if present in large quantities.

Serial examinations before and after breeding will allow the clinician to determine whether the mare shows any signs of being 'susceptible' to post-breeding endometritis. The interpretation of further diagnostics may be influenced by the timing of sam-

Box 1. Preparation of mares for intra-uterine diagnostic procedures

- Restrain the mare, ideally in stocks, sedate if required.
- Wrap or bag the tail and have an assistant hold it out of the way.
- Wash the mare's perineum and vulva with clean, warm water, if necessary use non-residual soap. Dry with paper towels.
- For mares with poor conformation, clean the inside of the vulva to reduce contaminants.
- Use only sterile equipment and lubricant, turn clean rectal sleeve inside out or use sterile gloves.

pling in relation to the oestrus cycle and should be accurately noted. Many uterine abnormalities such as cysts, uterine masses or foreign bodies such as glass marbles may be palpated or detected on ultrasound.

Uterine diagnostics

Most uterine diagnostic techniques are preferably performed with the mare in early oestrus. During this time, sampling is more easily performed as the cervix is relaxed and the risks associated with iatrogenic contamination of the uterus are lower in the presence of oestrogen, when uterine clearance is most effective. All techniques should be performed under conditions of strict hygiene to ensure accurate results and prevent contamination of the uterus from the perineum or caudal reproductive tract (*Box 1*).

Uterine culture and sensitivity

In an era of responsible antimicrobial usage, accurate diagnosis of bacterial endometritis is imperative before administration of any treatments. Culture allows the identification of the etiological organism and allows for sensitivity testing to be performed in order to guide antimicrobial therapy if required.

Collection of an endometrial swab will allow for culture and sensitivity testing in cases of suspected infectious endometritis. In general, samples should be collected when the mare is in oestrus with a relaxed cervix to ensure a representative sample is obtained, although mares with excessive uterine fluid or discharge can be sampled at any stage of the cycle. It is commonplace for mares to be swabbed post-partum on the foaling heat (day 7–10) and many breeders organisations will request a clean swab as part of the stud's pre-breeding screening requirements.

While very useful as a screening test, bacterial culture from a cotton swab has been shown to have a relatively low sensitivity of 34% (Nielsen, 2005), so other diagnostics should be considered in known problem mares or those with clinical signs of endometritis.

Collection

For routine screening of mares, a trans-cervical swab can be easily taken through a sterile speculum using a standard culture swab, artificial insemination pipette and pen torch (*Figure 3*). This technique is quick, inexpensive and the equipment needed is readily available (*Figure 4*). The cervix can be visualised concurrently for any pathology and to assist with staging of the mares cycle.

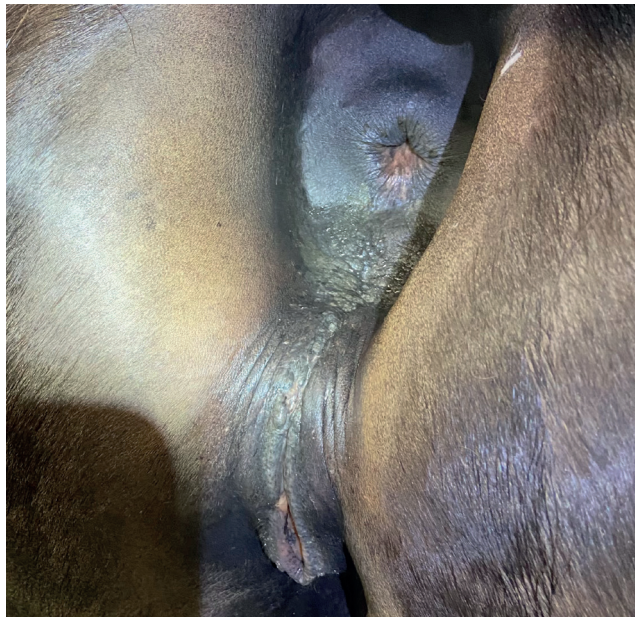

Figure 1. Poor perineal conformation will predispose mares to uterine disease.

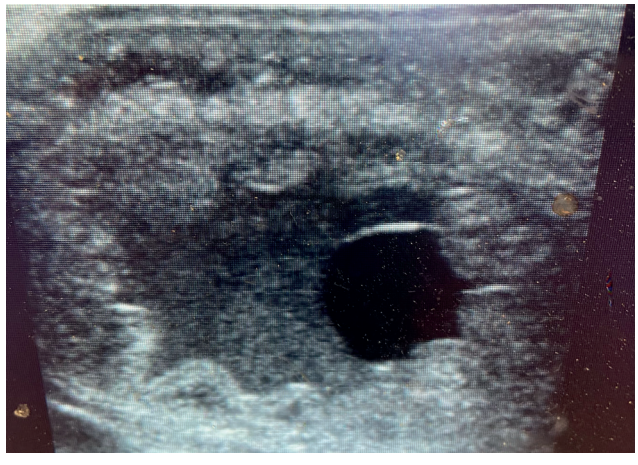

Figure 2. 16 day pregnancy suspended within uterine free fluid in a mare with endometritis.

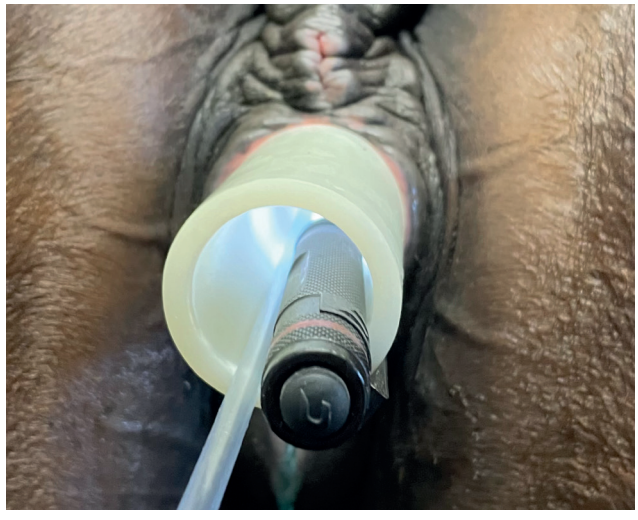

Figure 3. Obtaining a trans-cervical swab through a sterile speculum.

Table 1. Classification of endometritis based on numbers of neutrophils identified per high-power field on uterine cytology samples.

Number of neutrophils/hpf	Degree of inflammation
0–rare	Normal
1–2	Mild
3–5	Moderate
5+	Severe

Figure 4. Equipment to take a cervical swab through a sterile speculum.

Figure 5: Discoloured uterine lavage fluid from a mare with endometritis.

Figure 6. a) Flocculant fluid from uterine lavage, b) urine present in uterine lavage fluid.

The swab should be carefully passed through the relaxed oestrus cervix, taking care not to allow contamination in the vagina or vestibule, and rotated along the endometrium. The swab is then carefully removed through the speculum to avoid contamination and placed in standard transport medium.

Double-guarded endometrial swabs are commercially available and are generally considered superior to the aforementioned technique, as the risk of contamination by the caudal reproductive tract is reduced. The guarded device is guided within the operator's hand to the cervix and the inner swab is advanced into the uterine body, where it is gently rolled on the endometrium before being pulled back into the guard and the whole unit withdrawn.

The swabs, once collected, should be inoculated onto blood (+/- macconkey or chromogenic) agar and incubated under aerobic conditions at 37ºC for 24 or 48 hours. Colony size, morphology, presence of haemolysis or pigmentation can guide identification, with further confirmation using specific growth media, gram stain and additional testing as required. *Streptococcus equi* var. *zooepidemicus* and *Escherichia coli* are the most frequently isolated pathogens from the mare's uterus. If fungal infection is suspected, growth on Sabouraud dextrose agar is indicated. A pure growth of one (or two) known pathogenic organisms is considered significant, while mixed growths and small numbers of colonies should be interpreted with caution, as they may be reflective of sample contamination.

Cytology

Collection of endometrial cytology samples has been well reviewed (Ferris et al, 2015). In general, sampling sensitivity and specificity improve where bacteriology and cytology are used in combination. A positive bacterial culture in the absence of polymorphonuclear cells on uterine cytology is more likely to be a result of contamination. A negative culture result in the presence of moderate to high levels of polymorphonuclear cells on cytology suggests subclinical infectious endometritis caused by a metabolically inactive or hard to grow organism, one which is located only focally on the endometrium or a sterile inflammatory process, and may prompt further investigation. Additionally, cytological samples are inexpensive to process and results are rapidly available (in the same day where appropriate laboratory facilities are available). This may assist in clinical decision-making and may also allow the clinician to determine which samples to submit for bacteriology – reserving this more costly technique for cases with cytological evidence of inflammation (*Table 1*).

Sample collection

Samples may be collected with a plain cotton swab using either an unguarded or guarded technique as described for culture above. The swab is gently rolled onto a microscope slide to collect cellular material and allowed to air dry. Alternatively, samples can be collected using a sterile cytobrush, which leads to a higher yield and better preservation of cellular material (Bohn et al, 2014). Once collected, smears can be prepared and analysed with routine cellular stains such as Diff-Quick or gram-stained to help bacterial identification. The presence of uterine epithelial cells indicates that the sample is representative and the presence of polymorpho-

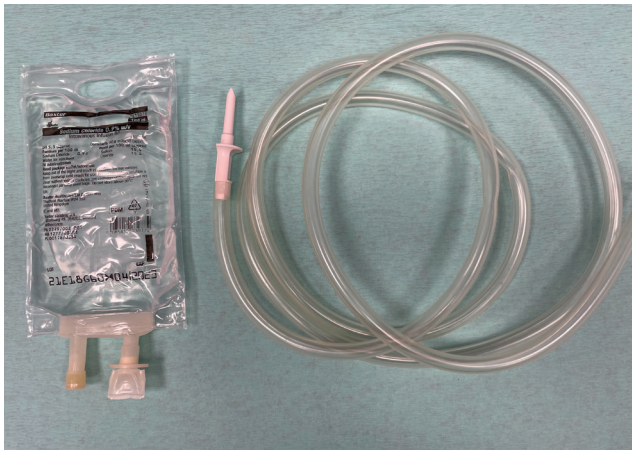

Figure 7. Low-volume lavage catheter and fluids.

nuclear cells, lymphocytes, macrophages, red blood cells, bacteria, yeast, fungi, mucus, urine crystals and debris are all noted.

Low volume lavage

Another technique which may be used, especially if previous culture and cytology sampling has yielded negative results, is the low volume lavage, as described by LeBlanc et al (2007). This technique has been found to have a higher sensitivity (71%) for detection of endometritis by culture than traditional swabs (LeBlanc et al, 2007), presumably because it samples a larger surface area of endometrium than a swab or cytobrush. However, there may be a higher risk of iatrogenic contamination of the uterus during the procedure.

This technique has the advantage of being able to visually inspect the lavage fluid which may appear grossly cloudy, bloody or with presence of urine or mucus (*Figures 5, 6a* and *6b*). An abnormal gross appearance may be up to twice as sensitive as endometrial culture in the detection of endometritis (Diel de Amorim, 2016). While there is no defined cut-off for polymorphonuclear cells in a low volume lavage, normal mares generally exhibit no more than one polymorphonuclear cell per 400x field (Ferris et al, 2015).

Technique

Using a standard mare flushing tube or catheter (*Figure 7*), infuse 60–250ml of sterile saline or lactated Ringers' solution, allow the fluid to contact the endometrium for at least 30 seconds, which may be encouraged by transrectal massage. The use of a balloon catheter is advocated by some authors, but is not essential for this technique. The fluid bag is then lowered to the ground and effluent fluid is collected by gravity flow; further rectal massage or use of 20IU oxytocin intravenously may aid the fluid recovery. The resultant fluid is then centrifuged with cytology and culture of the resultant pellet.

Uterine biopsy

An endometrial biopsy can be used to establish whether uterine disease is present in cases of poor fertility, to determine the type of process involved and to estimate a prognosis for that mare to carry a foal to term.

Table 2: Summary of the Kenney-Doig categories of endometrial biopsy and associated prognoses

Category	Features (if more than one feature present, increase the category)	Foaling rate
I	Essentially normal, inflammation or fibrosis slight and sparse	80–90%
IIa	Mild, scattered inflammation, mild fibrosis, endometrial atrophy in late breeding season	50–80%
IIb	Moderate, scattered inflammation, moderate fibrosis	10–50%
III	Severe, irreversible changes including inflammation and fibrosis	10%

Adapted from Snider et al (2011). Note: history of barrenness for more than 2 years increases the category assigned.

Culture from uterine biopsy samples has been reported to have a high sensitivity of 82% (Nielsen, 2005) possibly because bacteria can be isolated from the deeper tissues. Detection of polymorphonuclear cells on histopathology of biopsy samples is considered the most accurate in identifying endometritis (Nielsen, 2005).

In addition to identifying the presence and degree of inflammation, histopathology allows the identification of uterine degeneration or endometrosis which is characterised by fibrosis and glandular dilatation and nesting, enabling a more accurate prognostication for the mare's future fertility (*Table 2*). Further information may also be gained, such as the presence of eosinophils which has been linked with inflammation caused by pneumovagina, the presence of bacteria, fungal organisms or abnormal cellular infiltrates (such as in neoplasia).

Technique

Uterine biopsy is easily performed using a standard biopsy tool. The instrument is guarded by hand or through a sterile speculum and introduced in a sterile manner through the cervix and into the uterine body. The operator then places their hand into the previously evacuated rectum and guides a fold of endometrium, ideally at the base of one of the uterine horns, into the opened mouth of the tool which is then closed and pulled caudally until the sample is felt to break away from the endometrium (Ricketts, 1975). The resulting tissue sample can be swabbed for bacterial and fungal culture of the deep tissue layers, smeared onto a glass slide for cytological evaluation and submitted in formalin solution (10%) or Bouin's fixative for histopathology (*Figure 8*). This procedure can be performed at any stage of the oestrus cycle, but early oestrus may be preferred to minimise the effects of any potential iatrogenic contamination during the procedure. The stage of the cycle should be noted in the submission to allow interpretation by the examining pathologist.

Further investigation

In most cases, good clinical evaluation, combined with the diagnostic methods described above, will be effective in establishing

Box 2. Suggested approach to mares with infertility caused by suspected uterine disease

- Consider signalment of mare and collect complete reproductive history
- Physical examination including assessment of body condition and perineal conformation
- Rectal palpation and ultrasonography to assess the stage of cycle and any abnormalities
- Vaginal and cervical examination via palpation and speculum
- In oestrous: collect endometrial swab +/- cytobrush sample for culture and cytology
- If the results are inconclusive: collect low volume lavage and submit samples for culture and cytology
- In longstanding infertility cases or those where the above diagnostics inconclusive: collect uterine biopsy for histopathology and submit for culture if previous methods are negative
- If focal or unusual uterine disease is suspected, consider hysteroscopy (during dioestrus)
- If no diagnosis is reached with above investigations, consider non-uterine cause of infertility.

KEY POINTS

- Uterine disease in the mare, particularly endometritis, is a common and important cause of poor fertility, often leading to a failure to conceive or maintain a pregnancy to full term.
- A thorough history, clinical examination and transrectal ultrasonography are essential to guiding investigation of uterine disease.
- Traditional methods of uterine culture, while useful for screening for bacterial infection, have limitations in the investigation of poor fertility.
- Addition of uterine cytology and alternative culture methods, including the low volume lavage or culture of uterine biopsy specimens, will increase the sensitivity and specificity of findings.
- Histopathology of endometrial biopsies provides the most information about the presence of uterine disease and offers an expected prognosis for future fertility and should be utilised in any case of chronic infertility.
- All of the diagnostics described can be performed easily by the clinician in the field, allowing for comprehensive evaluation of the majority of poor breeders.

a diagnosis in the majority of cases of uterine disease. However, further investigation may occasionally be required (*Box 2*).

Hysteroscopy

It may be useful in some cases to directly visualise the uterine lumen to help the identification of focal disease processes or foreign bodies. This may be achieved with relative ease using a modern videoendocope, such as those used for endoscopy of the upper airways. The sterile endoscope is passed through the cervix and the uterus is distended with air for visualisation, the technique is most easily performed with the mare in diestrus as the closed cervix helps maintain distension. Following the procedure, oestrus should be induced using prostaglandin injection to minimise the effects of any inadvertent contamination. EQ

Figure 9. Uterine biopsy specimen after collection.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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