

Unexpected findings in equine uterine small volume lavage

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CASE PRESENTATION

Dreamy was a 24-year-old mare with a long history of infertility. Several attempts at artificial insemination (AI) were performed over the last few years but the mare never got pregnant. She was diagnosed with uterus pendulous and incompetent vaginal-vestibular sphincter and the latter was surgically corrected with Caslick's operation (vulvoplasty). She was also treated, after appropriate uterine culture, with multiple uterine infusions with saline and buffered gentamycin but no improvement was noted. A chemical curettage with kerosene was attempted, to promote regeneration of the endometrium, but again the next AI was unsuccessful.

Given that Dreamy was a valuable mare, the vet resorted to embryo transfer (ET). When the mare was inseminated, the endometrium reacted to semen, therefore it was flushed, with saline for three days. Eight days post ovulation, an ET flush was attempted but, in that occasion, a whitish opaque fluid was recovered, and no embryo was yielded. The mare was treated with multiple small doses of prostaglandin IM to fully open the cervix and to allow repeated flushing with 10% Lugol's solution (i.e. aqueous iodine solution) and saline to help cleaning the uterine lumen, with little success. The white fluid kept discharging for almost a week after the procedure (see figure 1). Transrectal ultrasound revealed an enlarged uterine lumen filled with fluid, debris, and multiple cysts (see figure 2). A small volume uterine lavage was performed and sent to the laboratory for examination (see figure 3).

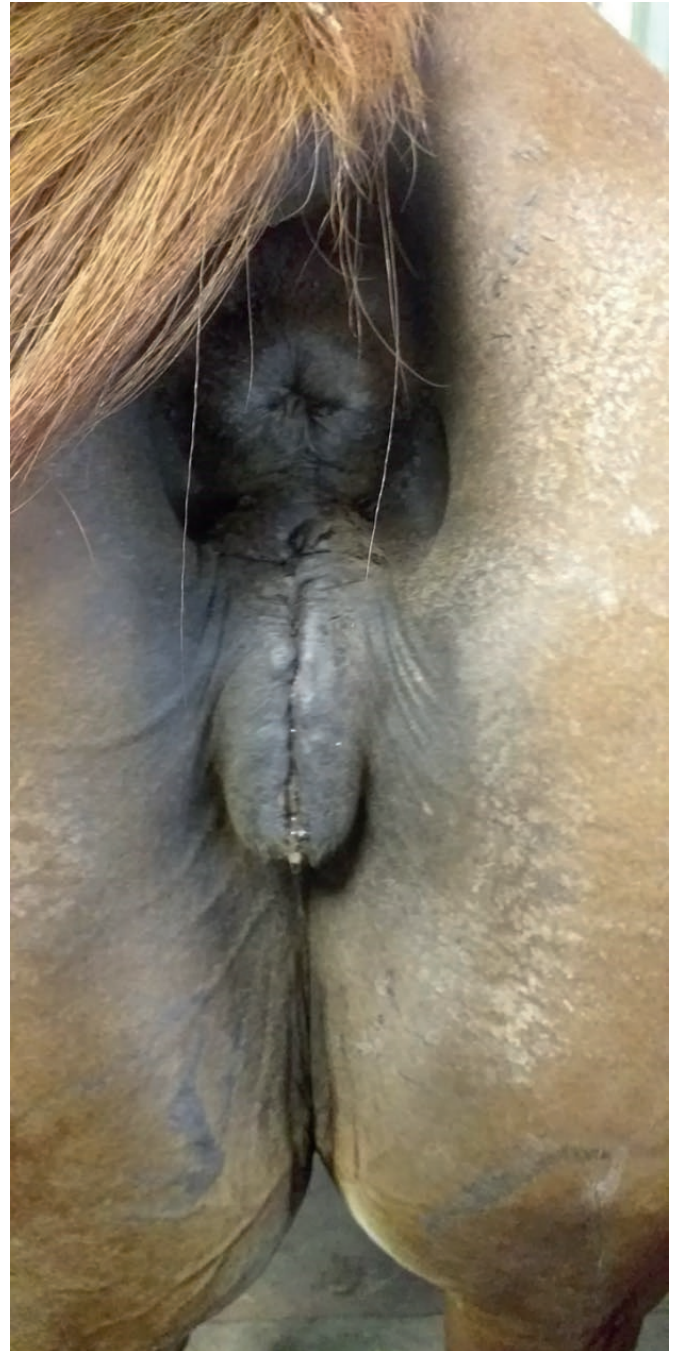


Figure 1

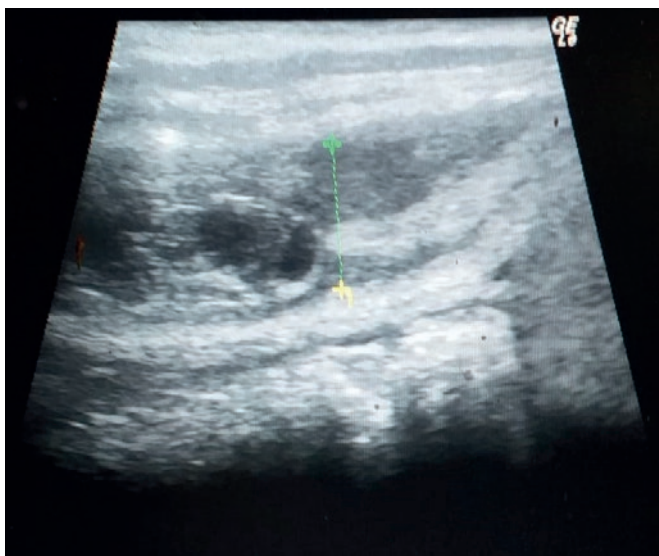


Figure 2: Enlarged uterine lumen with abundant fluid, debris and cystic lesions

WHAT IS YOUR DIAGNOSIS?

The examination of cytology smears revealed increased numbers of degenerate neutrophils, occasionally containing intracellular bacteria, along with high numbers of fungal hyphae. The latter had a thin double wall with

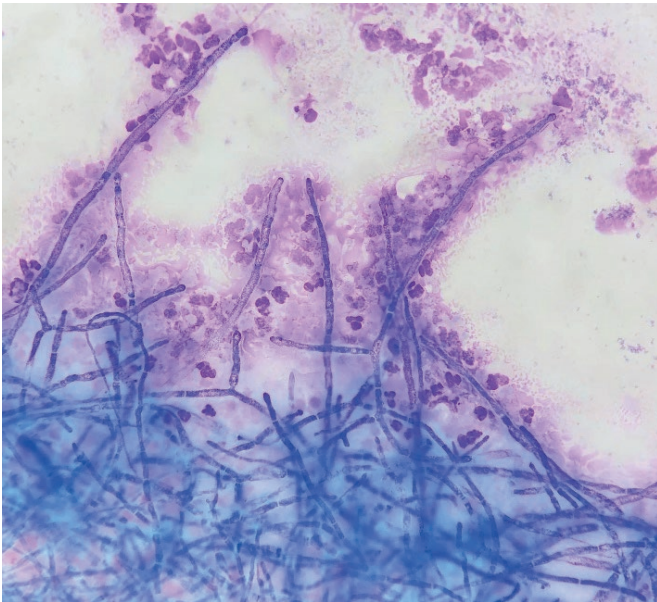


Figure 3: Cytopsin preparation obtained from the uterine lavage. Wright-Giemsa, 50x field

multiple, septate branches. Fluid culture results indicated growth of *Aspergillus* spp. The final diagnosis was fungal endometritis.

TREATMENT AND FOLLOW-UP

Dreamy was well in herself and no major changes were noted in her behaviour. In fact, in horses endometritis is not a life-threatening condition and the major concern is related to infertility and consequent economical losses. She was retired from reproduction activity and the owner elected palliative treatment. Uterine lavages were performed daily with 10% Lugol's solution for 4 days. It is recommended to carry out uterine flushes during oestrus, when the uterus is open and it can respond to oxytocin, which is given to promote complete recovery of the discharge. The lavages are performed until the effluent fluid appears clear with no evidence of floccular material.

DISCUSSION

Fungal endometritis is a relatively rare condition affecting the mare. Common sources of infection are genitalia skin or faecal material; however, the stallion can also be an asymptomatic carrier. Pathogens mostly responsible for fungal uterine infection are *Candida* spp and *Aspergillus* spp and the infection is most often opportunistic, associated with underlying anatomic (conformation of vulva and perineum) or with metabolic predisposing conditions (such as PPID or EMS). Mares affected by fungal endometritis generally have a long history of infertility, abortion, early embryonic death, or dystocia. A definitive diagnosis of fungal endometritis is based on endometrial cytology, culture, and/ or quantitative polymerase chain reaction (qPCR).

Samples for endometrial cytology can be obtained using a double-guarded uterine swab, cytobrush or following centrifugation of the effluent fluid from a low volume

uterine lavage. The latter technique is likely to produce a more representative sample of the entire uterus when compared with a standard swab technique and it is often the preferred choice of clinicians working in the field, as it does not require particular tools. Low volume uterine lavage is carried out by injecting a low volume (60 to 150 ml) of phosphate-buffered saline (PBS) or lactated Ringer's solution or physiological saline into the uterus through a sterile insemination catheter. Thus, the uterus is transrectally massaged to distribute the fluid evenly throughout the uterine lumen. Meanwhile, the catheter is moved back and forth to collect as many cells as possible. Finally, the effluent fluid is recovered by gravity in a sterile bag or bottle.

Treatment of mares with fungal endometritis is complex and multimodal and includes correction of anatomical defects (when present), uterine lavages and antifungal therapies, administered systemically and/or by intrauterine infusion. Topical antifungal treatment of the vagina and clitoris is also advisable.

Nonspecific uterine lavage therapies have been shown to be very effective in decreasing fungal load and removing excessive mucus and biofilms, that can represent a perfect medium for fungal growth. Whilst awaiting culture and sensitivity results, suggested therapies include lavages with 20% N-Acetylcysteine solution, 20% DMSO, 3% hydrogen peroxide, Tris-EDTA, 2% acetic acid in combination with 0.9% sterile saline/lactated Ringer's solution.

Based on current literature, intrauterine antifungal treatment can be performed with infusions of polyenes (Amphotericin B, Nystatin) azoles (Clotrimazole, Fluconazole, Miconazole) or Lufenuron. However, Amphotericin B is irritating when infused into the uterus, whereas azoles, which are fungistatic drugs, need to be used for long time to be effective. The use of Lufenuron, which is supposed to inhibit fungal growth by interfering with chitin biosynthesis, is quite controversial. A single intrauterine administration of 540 mg Lufenuron (Program), has been reported to be successful in treating fungal endometritis. However, in other studies, Lufenuron failed to demonstrate *in vitro* antifungal activity, therefore its use it is not recommended.

Fluconazole can be also administered orally, and it is well tolerated by horses. This type of therapy decreases the risk of iatrogenic infections associated with uterine lavage procedures and might be helpful in invasive forms of uterine infections. However, oral administration of fluconazole is recommended only when sensitivity results are available, as many fungi appear to be resistant to this drug.

Finally, concurrent bacterial infections are often present, therefore treatment of bacterial endometritis is also required.

Unfortunately, fungal endometritis carries a guarded prognosis regarding fertility as treatment failure and recurrent infections are common, due to inadequate selection of antifungal drug, dose or duration of therapeutic regimen, or failure to eradicate reservoirs of infection.