Fungal endometritis in the mare

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Summary

Fungal endometritis is a relatively uncommon condition in mares, accounting for less than 5% of diagnosed endometritides. Moreover, it is generally accepted that fungal infection is opportunistic and can only become established in a chronically disturbed uterine/vaginal environment, pneumovagina, persistent endometritis and repeated intrauterine antibiotic therapy are commonly cited as predisposing factors. However, because the exact conditions that allow fungal colonization of the uterus are obscure, recidivism is common and there are currently no treatments proven to offer a high likelihood of resolution, uterine infection with hyphal fungi or, in particular, yeasts is a considerable therapeutic challenge with a poor prognosis. While both the identity of the causal organism and the duration of infection may affect the response to intrauterine treatment, it is sensible to simultaneously correct any suspected predispositions (e.g. pneumovagina) and to treat against a potential reservoir of infection in the caudal reproductive tract (vagina and clitoral fossa). In the author's experience, intrauterine infusion of 2% acetic acid for 3 days or an anti-fungal such as clotrimazole or nystatin for 5-7 days yields a resolution rate of approximately 20% per treatment cycle. If initial treatment is not successful, a prolonged period of breeding rest to allow the re-establishment of uterine and vaginal environments capable of withstanding fungal overgrowth can be surprisingly effective.

Keywords: mare, endometritis, fungi, yeast, reproduction

Endometritis mycotica bei der Stute

Mykotische Endometritiden sind bei der Stute relativ selten und werden bei weniger als 5% der diagnostizierten Endometritiden festgestellt. Allgemein gilt, dass Pilzinfektionen opportunistische Infektionen sind, die sich nur bei einem chronisch gestörten vaginalen/uterinen Milieu, Pneumovagina, persistierender Endometritis und nach wiederholter intrauteriner Antibiose entwickeln. Die exakten Bedingungen für die Manifestation einer uterinen Pilzinfektion sind jedoch unklar. Rezidive sind häufig, und es sind keine Therapiekonzepte bekannt, die einen sicheren Erfolg versprechen. Uterine Infektionen mit hyphenbildenden Pilzen, speziell Hefen, stellen ein erhebliches therapeutisches Problem dar und haben eine schlechte Prognose. Zum einen beeinflussen die Ätiologie und die Dauer der Erkrankung den therapeutischen Erfolg, und zum anderen ist die gleichzeitige Behandlung der prädisponierenden Probleme (z.B. Pneumovagina) und die Beseitigung des potenziellen Erregerreservoirs im kaudalen Abschnitt des Reproduktionstraktes (Vagina, Fossa clitoridis) von besonderer Bedeutung. Die eigenen Erfahrungen zeigen, dass intrauterine Spülungen mit 2% iger Essigsäure über 3 Tage, oder Fungizide wie Clotrimazol oder Nystatin über 5-7 Tage, eine Erfolgsquote von ca. 20% je Behandlungszyklus bringen. Wenn die Erstbehandlung nicht erfolgreich ist, kann ein längeres Aussetzen aus dem Zuchtprogramm für eine Rückkehr zur physiologischen Keimflora des uterinen und vaginalen Milieus die Widerstandkraft gegen Pilzinfektionen erstaunlich effektiv verbessern.

Schlüsselwörter: Stute, Endometritis, Pilze, Hefen, Reproduktion

Introduction

Uterine infection with fungi is an uncommon problem in the mare, with a reported incidence of just 1-5% of confirmed endometritides (for review see *Dascanio* et al. 2001). When it does occur, the fungal and yeast pathogens most commonly implicated are Aspergillus spp and Candida spp, respectively, although a wide range of other organisms has been reported (*Pugh* et al. 1986, *Dascanio* et al. 2001). The most common source of infectious organisms is thought to be the skin or faeces. However, fungi have also been cultured from the urethra and/or semen of stallions (*Malmgren* et al. 1998) and, while there are no conclusive reports of fungal endometritis as a result of mating with an infected stallion, it is important to consider the possibility of the stallion as an asymptomatic 'carrier'. Nevertheless, fungal colonization of the uterus is almost certainly an opportunistic condition, relying on significant disturbance and compromise of the normal vaginal and/or uterine defence mechanisms, or a more generalized immuno-suppression/compromise. This is presumably why fungal endometritis is commonly associated with a history of uncorrected pneumovagina (*Zafracas* 1975) or recurrent persistent post-breeding endometritis and, in particular, intrauterine antibiotic therapy

(*Pugh* et al. 1986, *Troedsson* 1997, *Dascanio* et al. 2001). Prolonged local antibiotic therapy is believed to disrupt the defence mechanisms that normally prevent fungi or yeasts becoming established (*Zafracas* 1975, *Chengappa* et al. 1984), although it is not clear how intrauterine administration predisposes to fungal infection since there is no normal uterine flora to disrupt. It is most probable that the primary problem occurs at the level of the caudal reproductive tract where antibiotics 'leaked' from the uterus disturb the 'commensal' flora such that fungal overgrowth is facilitated by reduced competition, an altered vaginal pH and, possibly, the elimination of bacteria capable of secreting anti-fungal substances. Indeed, it is believed that the fungi or yeasts that colonize the uterus are usually derived from a reservoir in the vagina and external genitalia (*Dascanio* et al. 2001), and are carried iatrogenically into the uterus during insemination or intrauterine treatment. Other factors thought to predispose to fungal endometritis include systemic immune deficiency or endocrine disorders (*Dascanio* et al. 2001), and the presence of a necrotic focus, e.g. following dystocia or placental retention (*Hurtgen* and *Cummings* 1982). Table 1 lists a number of factors that were associated with fungal endometritis in 152 mares submitted to Utrecht University for investigation and treatment during 1987-2001.

While the response of fungal endometritis to treatment is generally poor, it may in part depend on the identity of the causal organism and the duration of infection. For example, it is thought that Candida albicans can penetrate deeper into the endometrium and/or grow intracellularly, where it will be more resistant to clearance by either the immune system or

Table 1 Factors associated with fungal endometritis in 152 mares presented to Utrecht University for investigation and treatment during 1987-2001. Where insufficient information was available, figures are expressed as percentages of the mares for which conclusions could be made.
Begleitfaktoren einer mykotischen Endometritis bei 152 Stuten, die in der Universität Utrecht in den Jahren 1987-2001 zur Untersuchung und Behandlung vorgestellt wurden. In den Fällen mit fehlenden Informationen beziehen sich die Prozentzahlen auf die Stuten, von denen Angaben vorliegen.

Mare status (n=128)	
Barren	74%
Maiden	16%
Foaling	9%
Previous antibiotic treatment	52/60 (87%)
Dystocia / retained fetal membranes	28 (18%)
Abortion	10 (7%)
Early embryonic loss	19 (13%)
Pneumovagina / urovagina	58/147 (39%)
Copious intrauterine fluid	48 (33%)
Systemic endocrine disease	2 (1.4%)

antimicrobials administered via the intrauterine route. Both intracellular colonization and an untreated reservoir of infection in the caudal reproductive tract have been proposed to contribute to the high rate of treatment failure and/or recrudescence (*Dascanio* et al. 2001).

Clinical signs and diagnosis

Mares suffering from fungal endometritis typically have a prolonged history of difficulty in becoming pregnant, for example, 95/128 (74%) mares in which fungal endometritis was diagnosed at Utrecht University during 1987-2001 were barren at the beginning of the breeding season (Table 1). In accordance with previous surveys (e.g. *Pugh* et al. 1986), the majority of mares for which information was available also had a history of recent intrauterine antibiotic therapy (52/60: 87%) and had clinical signs of pneumo- or urovagina (58/152: 35%). Other factors commonly encountered in the history of mares examined in Utrecht include dystocia or retention of the fetal membranes (18%), early embryonic death (13%) and abortion (7%). Mares suffering from fungal endometritis usually display obvious clinical signs of uterine inflammation such as (copious) uterine fluid (33% of the mares in our survey) ranging from thick, grey and purulent, to yellow and watery-mucoid in consistency. *Freeman* et al. (1986) similarly reported that many affected mares displayed a greyish vulval discharge.

Definitive diagnosis of fungal endometritis is usually based on a combination of aerobic culture and cytological examination of uterine secretions recovered using a guarded swab or by (small volume) lavage. If fungal endometritis is to be considered, it is important to alert the laboratory since additional culture on a bacteria-inhibiting Sabaraud's agar will reduce

the likelihood of missing fungi or yeasts due to bacterial overgrowth. In addition, mycological culture should be continued for at least 5 days before declaring a sample negative, because fungal growth can be very slow (*Freeman* et al. 1986). Cytological examination will typically, but not always, reveal signs of an ongoing inflammatory reaction (e.g. frequent neutrophils), and may also reveal obvious yeasts or, less commonly, elongated fungal hyphae. Yeasts are oval to spherical in shape and around 3-5 mm in length, and many have a surrounding capsule with a low affinity for dyes which, therefore, remains clear after staining (*Dascanio* et al. 2001). Candida albicans may be particularly prone to misdiagnosis because cultured colonies resemble bacteria while, on superficial examination, a gram or Diff-Quick stained group of yeasts can be mistaken for staphylococci.

Endometrial biopsies have also been advocated in the diagnosis of fungal endometritis, particularly where cytology is inconclusive, or to confirm that treatment of invasive yeasts has been successful (*Dascanio* et al. 2001). Endometrial biopsy may be especially useful for indicating whether treatment is likely to result in a satisfactory outcome, fungal elements deeper in the endometrium or extensive endometrial fibrosis, whether a predisposing cause or a result of the fungal infection, are associated with poor subsequent fertility (*Hurtgen* and *Cummings* 1982).

Treatment and prognosis

The prognosis for mares with a fungal endometritis is generally poor, either because the organisms resist elimination or because they subsequently return. The major reasons for treatment failure are proposed to include the resistance of invasive forms to intrauterine therapy, failure to remove predisposing factors or recontamination from a reservoir in the caudal reproductive tract. On the other hand, some cases of fungal endometritis recover spontaneously or without specific therapy, presumably because the disturbances that allowed fungal colonization have been corrected (e.g. removal of a necrotic focus). For this reason, initial treatment should concentrate on addressing any suspected predispositions (e.g. poor vulval closure), ensuring that the mare is in oestrus (when local immunity is maximal), and a combination of large volume uterine lavage and uterotonic drugs (e.g. oxytocin) to reduce the amounts of fungal organisms and inflammatory debris in the uterus.

Not surprisingly, given the generally poor response to therapy, numerous treatment regimes have been tried, using either specific anti-fungal agents or uterine irritants. Disappointingly, few have been subjected to controlled clinical trials. Nonspecific intrauterine treatments described anecdotally to be effective in some instances include 0.05% polividone-iodine solution and 2% acetic acid (*Zafracas* 1975). However, intrauterine polividone-iodine infusion should be used with caution since there are reports of it resulting in severe endometrial damage, including fibrosis and adhesion formation (for review see *Perkins* 1999). Similarly, while the author commonly uses 250 ml infusions of 2% acetic acid for the treatment of uterine yeast infections, the acetic acid is washed out within 2-3 minutes using large volumes of lactated Ringer's solution because it can cause severe uterine irritation characterized by serous exudation and, on occasions, sloughing of endometrial tissue. While this does not sound healthy, it is

not inconceivable that, in the case of invasive yeast forms, the irritation assists penetration to, or removal of, the organisms. Of 33 fungal endometritis cases treated during 49 cycles in Utrecht with 1-3 consecutive days of intrauterine acetic acid, 11 (33% of mares: 22% of treatment cycles) were subsequently found to be free of fungal or yeast infection.

The specific anti-fungal agents are divided into two major classes, the polyenes and the imidazoles. The former include amphotericin B and nystatin and the latter clotrimazole, ketaconazole and fluconazole (*Carter* and *Chengappa* 1995). If available, it is advised to perform a drug sensitivity screen to aid in the selection of an appropriate anti-fungal (*Dascanio* et al. 2001). In general, however, the imidazoles are thought to be more effective candicidals and, because they are thought to work more effectively in an acid environment, acetic acid lavage prior to medication may be desirable (*Dascanio* et al. 2001). Although many anti-fungals are poorly absorbed from the gastrointestinal tract, fluconazole is a notable exception and has therefore been proposed as the drug of choice for systemic therapy, although this therapeutic modality is expensive (*Dascanio* et al. 2001). Suggested drugs and doses for daily intrauterine treatment of fungal or yeast endometritis (*Troedsson* 1997) include, nystatin (0.5-2.5 million units), amphotericin B (100-200mg), fluconazole (100mg) and clotrimazole (500-700mg). While the response to therapy is generally reported to be poor, it has been speculated that, for cost reasons, treatment is often too intermittent or short-lasting. On the other hand, there is little hard information on the success of specific therapies or on the influence of type of organism, duration of infection or duration of therapy. In a clinical trial performed in Utrecht in which 3.75-5 mg of the experimental imidazole, parconazole, was infused daily for 6 or 10 days into the uterus of mares suffering from fungal endometritis, the causal agent was eliminated from 27 of 81 mares treated at 135 cycles (i.e. successful in 33% of mares and 20% of treatment cycles), shorter (2-3 day) treatments were ineffective (*Fontijn* and *Ravenhorst*, unpublished data). Although parconazole was only temporarily available on a test license, recent experience suggests that currently favoured anti-microbials (nystatin and clotrimazole) offer a similar likelihood of resolution (20%).

Hess et al. (2002) reported the resolution of fungal endometritis in 4 mares treated with a single intrauterine administration of 540mg of the benzoylphenyl urea derivative, Lufenuron (Program®, Novartis Animal Health). Lufenuron is primarily used for flea control in dogs and cats and inhibits insect development by interfering with chitin biosynthesis, since fungi also have chitin-rich cell walls, Hess et al. proposed that lufenuron might have inhibited fungal growth. However, further studies are required to establish the efficacy of lufenuron as an intrauterine anti-fungal treatment, especially in the light of recent studies that have failed to demonstrate any in vitro antifungal activity of lufenuron (e.g. against Aspergillus or Fusarium spp: *Scotty* et al. 2005).

An important adjuct to intrauterine treatment is simultaneous topical treatment of the vagina and clitoris (e.g. with clotrimazole cream) to remove any reservoir of organisms that might otherwise lead to re-infection. Two further observations worthy of mention are: (1) clearance of uterine fungal infection has been noted in a handful of mares after a prolonged period of rest following apparently unsuccessful anti-fungal treatment,

we speculate that reducing the number of organisms followed by a period in which the natural balance can be restored in the vagina may increase the likelihood of 'spontaneous' clearance. (2) In our hands, the number of mares that succumb to a post-treatment bacterial endometritis is high (62/148: 41%, predominantly Streptococcus spp) and can mask the continued presence of the fungus. It can also be difficult to decide whether to treat this common sequel with antibiotics, since the latter may have been implicated in the initiation of fungal endometritis. Finally, while some mares certainly become pregnant and produce foals following successful treatment of fungal endometritis (*Zafracas* 1975, *Hess* et al. 2002), there is little hard data on post-treatment fertility.

Conclusions

Fungal endometritis is an uncommon problem for which there is currently no satisfactory treatment. Intrauterine infusion of either 2% acetic acid or specific anti-fungal agents appears to offer a resolution rate of little higher than 20% per treatment cycle. And while correcting any anatomical abnormalities and treating the caudal reproductive tract may reduce the risk of re-infection. Significant advances in therapy may depend on a clearer understanding of the conditions that permit establishment and maintenance of fungal infection in the mare's uterus.

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