

Tutorial Article

Equine fungal endometritis

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Introduction

Fungi are heterotrophic, aerobic or facultatively anaerobic, nonmotile, organisms (Tortora *et al.* 1998b). Fungal cell walls are composed primarily of chitin, a polysaccharide that is also the main structural component of insects and crustaceans (Tortora *et al.* 1998a). **The term fungal is a general one referring to both yeast and moulds.** Fungi are widely distributed in soil, animal excreta, in the vegetative parts of plants, and in substances that contain sugars (Hensyl and Oldham 1982). **Yeast** are round to oblong single cell organisms, while **moulds** are long, branched, filamentous structures (Tortora *et al.* 1998b). Some fungi are dimorphic and exist in both yeast and mold states under certain growth conditions (Tortora *et al.* 1998b). Fungi have been reported in both forms in the equine reproductive tract (Pugh *et al.* 1986). In one of the authors' (JJD) experience, fungi are generally found in a yeast form on cytological examinations (Figs 1-3). **Fungal elements that cause reproductive disease are generally opportunistic, requiring some type of predisposing condition to establish infection.**

An excellent web site for review of fungi is entitled Medical Mycology and is located at the URL <http://fungusweb.utmb.edu/mycology/index.html>. Information regarding the collection and transport of specimens, culture techniques, as well as, fungal nomenclature and imagery are available for browsing.

Aetiology

The most common source of fungi causing reproductive disease in the mare is probably from skin or faecal origin. There are a variety of fungi that have been identified from uterine cultures with *Candida* spp. and *Aspergillus* spp. isolated as the most common yeast and mould, respectively. Although early reports primarily identified *Candida albicans* as the fungal pathogen infecting the mare's reproductive tract, more recent literature and information from our laboratory (Blacksburg) demonstrates that many other organisms may be responsible for fungal endometritis (Table 1) (Doyle 1969; Zafracas 1975; Abou-Gabal *et al.* 1977; Hurtgen and Cummings 1982; Blue 1983; Chengappa *et al.* 1984; Freeman *et al.* 1986; Pugh *et al.* 1986; Elvinger and Roberts 1995, 1996; Petrites-Murphy *et al.* 1996).

The percent of positive fungal samples calculated from the total number of reproductive cultures submitted to laboratories ranges from 2-3% (Elvinger and Roberts 1995, 1996), whereas the percent of positive cervical or uterine fungal samples calculated from the total number of mares diagnosed with endometritis ranges from 1-5% (Collins 1964; Bain 1966; Zafracas 1975; Pugh *et al.* 1986) (Table 2). These numbers may include repeat cultures on the same mare; consequently, the values may be slightly elevated.

The **primary reservoir** for infectious agents that colonise the uterus is the caudal reproductive tract, including vagina and external genitalia, although contamination from faecal matter (pneumovagina, poor perineal conformation, etc.) or **iatrogenic** means (after uterine culture/cytology/biopsy or artificial insemination) is also possible. The use of antibiotics is commonly incriminated as predisposing to fungal infections; however, reports also occur whereby no intra-uterine therapy was undertaken (Zafracas 1975). The prolonged use of antibiotics is believed to disrupt normal biological barriers allowing yeast to overgrow (Farely and Mullaney 1964; Doyle 1969; Zafracas 1975; Chengappa *et al.* 1984; Carter and Chengappa 1995).

A change in vaginal flora may also remove organisms that secrete anti-fungal substances, change the competition for available nutrients, interfere with

TABLE 1: Types of fungi isolated from the reproductive tract of mares

<i>Actinomyces</i> spp.	<i>Hansenula anomala</i>
<i>Aspergillus fumigatus</i>	<i>Hansenula polymorpha</i>
<i>Aspergillus</i> spp.	<i>Monosporium apiospermum</i>
<i>Aureobasidium pullulans</i>	<i>Monosporium</i> spp.
<i>Candida</i> spp.	<i>Mucor</i> spp.
<i>Candida albicans</i>	<i>Nocardia</i> spp.
<i>Candida guilliermondii</i>	<i>Paecilomyces</i> spp.
<i>Candida krusei</i>	<i>Penicillium</i> spp.
<i>Candida lusitanae</i>	<i>Rhodotorula glutinis</i>
<i>Candida parapsilosis</i>	<i>Rhodotorula minuta</i>
<i>Candida pseudotropicalis</i>	<i>Rhodotorula rubra</i>
<i>Candida rugosa</i>	<i>Candida tropicalis</i>
<i>Rhodotorula</i> spp.	<i>Scedosporium apiospermum</i>
<i>Candida stellatoidea</i>	<i>Saccharomyces cerevisiae</i>
<i>Candida zeylanoides</i>	<i>Torulopsis candida</i>
<i>Coccidioides immitis</i>	<i>Cryptococcus neoformans</i>
<i>Trichosporon beigeli</i>	<i>Trichosporon cutaneum</i>
<i>Fusarium</i> spp.	<i>Trichosporon</i> spp.

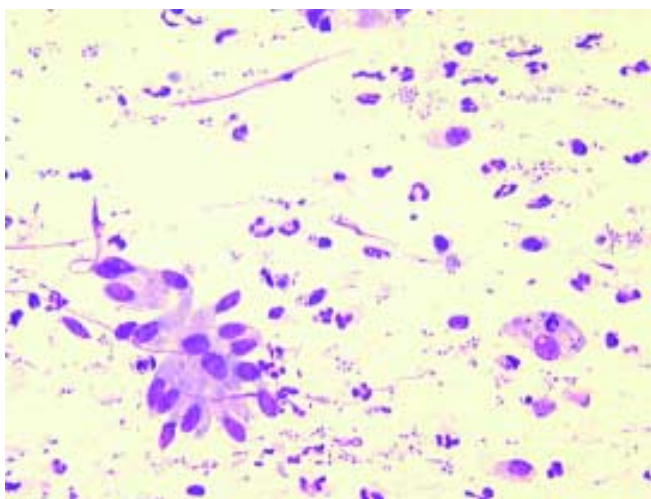


Fig 1: Cytological preparation demonstrating the presence of yeast forms (*Candida albicans*), a group of endometrial cells and an increased number of neutrophils, some of which have phagocytised yeast. Stained with Diff-Quik. Magnification ~100x.

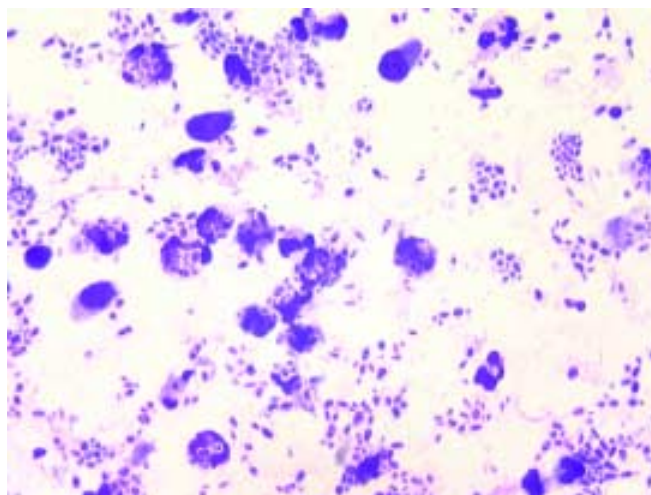


Fig 2: Cytological preparation demonstrating neutrophils, many with phagocytised yeast (*Candida albicans*). Stained with Diff-Quik. Magnification ~400x.

vitamin synthesis by normal microflora, and result in a change in vaginal pH (Carter and Chengappa 1995). Unfortunately, the actual mechanism resulting in fungal endometritis has not been proven and, in most cases, it is based on clinical impression. It may be possible that **intrauterine antibiotics**, when cleared from the uterus by increased myometrial tone or passive leakage, may affect the vaginal flora leading to vaginal colonisation with fungi. Since the uterus is not normally colonised throughout the oestrous cycle, the role of intrauterine antibiotics may not be as important as the repeated invasion of the reproductive tract, potentially placing commensal organisms from the caudal reproductive tract into the cranial reproductive tract. Mares that have been

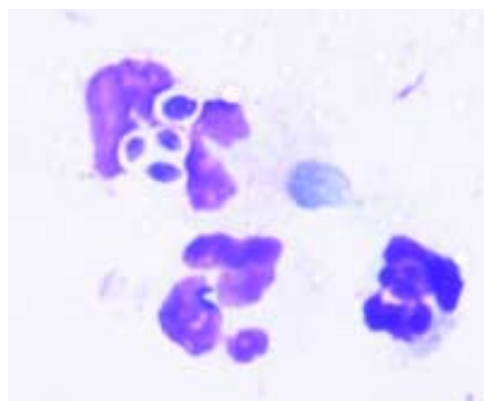


Fig 3: Cytological preparation demonstrating a couple of neutrophils, one of which has phagocytised yeast (*Candida albicans*). The capsule is clearly visible surrounding the yeast form. Stained with Diff-Quik. Magnification ~1000x.

identified as having **poor uterine defence mechanisms** with decreased ability to clear fluid/organisms from their uterus (Katila 1996) may then provide an environment for opportunistic fungi to proliferate and cause endometritis. Pneumovagina has also been incriminated as predisposing mares to fungal infections (Zafracas 1975), supporting the idea of contaminants leading to fungal infections.

Circumstances leading to systemic yeast infections may include **immunosuppressive therapy, cytotoxic drugs, immune deficiencies and endocrine disorders** (Carter and Chengappa 1995). Mares **administered progesterone** are thought to be more at risk for fungal endometritis as neutrophil phagocytic ability may be decreased, uterine muscular activity may be altered and the cervical tone may increase. Other contributing factors to the presence of fungal infections include the presence of a moist environment, exposure to large numbers of fungi, and the presence of a necrotic focus as occurring with trauma, infection or ischaemia (Carter and Chengappa 1995). Necrotic foci can occur with an abortion or with a retained placenta (Hurtgen and Cummings 1982), although infections with fungi at these times do not generally result in the cycle of recurrent infections that may occur with infections in nonpregnant mares. However, there has been a report of the prolonged presence of fungal elements in the clitoral fossa and within the *stratum compactum* and *stratum spongiosum* from a uterine biopsy 14 days postfoaling in a mare with a negative uterine culture (Petrites-Murphy *et al.* 1996). One author (W. B. Ley, unpublished data) has documented a case of fungal endometritis occurring in a 7-year-old mare, 12 days *postpartum*. There has been no proof that fungi produce exotoxins or endotoxins, therefore systemic signs from a localised fungal infection should be minimal. It is thought that cell-mediated is more important than antibody-mediated immunity in the response to fungal infections (Carter and Chengappa 1995). Specifically with *Candida* spp. infections, there has been reported a decrease in cellular

TABLE 2: Number of positive fungal cultures from various reports

Investigator	Total No. uterine cultures	Total No. endometritis cases	No. positive for fungi	% positive for fungi
Pugh (Pugh <i>et al.</i> 1986)	-	89	5	5.6
Zafracas (Zafracas 1975)	-	180 [†]	16	8.88
Bain (Bain 1966)	-	341	7	2.05
Collins (Collins 1964)	-	1015 [†]	12	1.18
Elvinger (Elvinger and Roberts 1995, 1996)	2181*	-	45	2.06
VMRCVM	714	-	22	3.08

*Includes vaginal, cervical and uterine cultures.

[†]Diagnosis made via cervical culture.

VMRCVM = Virginia-Maryland Regional College of Veterinary Medicine.

immunity (Witkin 1987). This is explained by an increase in prostaglandin E₂ production by macrophages in response to *Candida* antigens, thereby decreasing interleukin-2 and therefore T-lymphocyte proliferation and cell-mediated immunity.

Certain fungi have the ability to become invasive to tissues. *Candida albicans* has been demonstrated to grow intracellularly (Garcia-Tamayo *et al.* 1982). The mechanism of colonisation of *Candida albicans* is thought to be by initial adherence to epithelial cells through a manno-protein called adhesin. This also makes the organism more difficult to remove. The spores are then transformed to mycelia that can colonise the epithelial surface. The mycelia also have the ability to penetrate cells and grow intracellularly. This may then make the fungi less available for immune clearance and could result in later emergence resulting in an apparent re-infection.

Fungi have been cultured from **urethral cultures** (*Mucor* spp.), fresh semen (*Absidia* spp.) and extended semen (*Candida* spp.) from stallions (Malmgren *et al.* 1998). There have been no reports of fungal endometritis as caused by natural coitus or artificial insemination by infected stallions. In man, there is some evidence that the male may harbour fungi that can result in re-infection of their sexual partner (Horowitz *et al.* 1987). Fungi have been isolated from the penis and from seminal fluid in human males, suggesting that infection of the urethra or seminal vesicles may occur. Males, thus infected, remained asymptomatic carriers (Horowitz *et al.* 1987).

Diagnosis

The **history may suggest conditions predisposing mares to fungal infections**, such as the repeated use of intrauterine antibiotics or numerous intrauterine diagnostic and therapeutic procedures. Affected mares often have a history of treatment for recurrent uterine infections with intrauterine antibiotics. On physical examination it is common to find a grey discharge from the vulva that is associated with a fungal endometritis (Freeman *et al.* 1986). The diagnosis of fungal endometritis is most often made with a combination of aerobic culture and cytology of uterine secretions. Culture alone is insufficient to confirm a diagnosis of fungal endometritis. Positive signs of an inflammatory response must be confirmed on cytology or biopsy as contamination of the uterus occurs during oestrus and could lead to a false positive diagnosis (Hinrichs *et al.* 1989).

One of the authors (C. Schweizer) had a mare that did not have inflammatory cells identified on cytology, but was positive on fungal culture. This mare subsequently had positive cytology 2 days later and it was theorised that the initial cytology was negative due to sampling near the time of initial colonisation of the uterus. **A guarded uterine swab or similar methodology** can be used to obtain a sample of uterine fluid/cells for microscopic examination (Dascanio *et al.* 1997). The swab contents are placed on a microscope slide, smeared, air dried and then stained using a modified Wright's stain (Diff-Quik)¹. **This staining method allows the identification of cell types, bacteria and fungi.** Yeast often have a capsule surrounding them which is visible by altering the focal point when viewing the microscope slide (**Fig 3**). This capsule is thought to serve as a protective barrier preventing phagocytosis and possibly to have immunosuppressive properties (Volk 1992). The capsule does not have strong affinity for dyes and thus remains clear after staining (Volk 1992). Yeast are oval to spherical in shape ranging 3–5 µm diameter (Carter and Chengappa 1995). Less commonly, fungi may appear in hyphal form on cytological examination.

Fungi may be cultured on blood agar as part of a routine aerobic culture and, once isolated, sub-cultured on **Sabaroud's agar. Fungal organisms can take longer than bacteria to grow on culture plates**; consequently, if the laboratory is not aware that a search for fungal elements is required, the culture may be deemed negative due to early reporting of culture findings (Freeman *et al.* 1986).

A **biopsy** may also be diagnostic of fungal endometritis when cytology is inconclusive. **Biopsies** may also be used to confirm the successful treatment of fungal endometritis as cytology and culture may be negative with invasive forms. The biopsy specimen should be stained with a silver stain such as **Gomori's methanamine silver stain** (Freeman *et al.* 1986). Fungi appear stained black or grey. Fungal elements may be viewed either adherent to the luminal surface, within endometrial gland lumens or within the endometrium (Hurtgen and

Cummings 1982; Freeman *et al.* 1986; Pugh *et al.* 1986). It has been suggested that invasive fungal elements are probably rare (Freeman *et al.* 1986).

The clitoral fossa and vaginal mucosa should be cultured in addition to the uterine lumen. The possibility exists that a reservoir for fungal elements occurs in mares that may serve as source for recontamination of the uterus even after successful intra-uterine treatment of a fungal endometritis.

Therapy

Fungal infections can be difficult to eliminate. This may be due to invasive forms that are not exposed to luminal therapies, poor uterine clearance mechanism, inadequate immune response or to recontamination from posterior reproductive tract sources. A number of the cultures examined from our laboratory (Blacksburg) reveal **repeat isolation of yeast organisms from single mares despite multiple treatments.**

The first goal of a successful treatment plan should address the conformation of the mare. Correction of any anatomical barriers that may predispose to faecal contamination or uterine inflammation may prevent recurrent infections.

Large volume uterine lavage should decrease overall fungal numbers, increase inflammatory cell infiltrates and, possibly, lead to an increase in myometrial tone depending on the osmolarity and temperature of the instilled solution. Additives such as DMSO, acetic acid (vinegar) or dilute betadine have been used in an attempt to combat fungal infections (Abou-Gabal *et al.* 1977; Asbury and Lyle 1993). DMSO, *in vitro*, has been reported at concentrations of 10–20% to decrease growth of *Candida albicans* and at $\geq 30\%$ to inhibit growth (Pottz *et al.* 1967). It appears to be bacteriostatic at lower concentrations (5–10%) and bacteriocidal at higher concentrations. Caution should be used since endometrial cell loss and ulceration occur at higher concentrations with epithelial ulceration occurring with a 25% DMSO intra-uterine infusion (Frazer *et al.* 1988). Conversely, intra-uterine solutions as high as 75% DMSO have been used experimentally with no increase in periglandular fibrosis 21 days after treatment, suggesting that no long term damage results from its use (Frazer *et al.* 1988).

Dilute iodine (0.05%) or 2% vinegar (20 ml of white vinegar in 1000 ml sterile saline) has been reported anecdotally to be effective (Zafracas 1975; Abou-Gabal *et al.* 1977; Asbury and Lyle 1993). Intrauterine iodine should be used with caution as luminal adhesions may form depending on a particular mare's sensitivity. Lowering the pH of intrauterine saline solutions to 3.7 has been demonstrated to cause a significant increase in prostaglandin secretion (Pascocoe *et al.* 1989). The pH of a 2% vinegar solution is about 3.2 depending on the original pH of the physiological saline used, which has a pH range 4.5–7 (average pH 5.5). An increase in prostaglandin release may affect corpus luteal function and lead to an increase in myometrial tone and return to oestrus.

Oxytocin (10–20 u i.m. or i.v.) is often used postlavage to stimulate myometrial activity to aid in the evacuation of uterine contents (LeBlanc *et al.* 1994).

Although treatment of a fungal endometritis may stop with uterine lavage, it often includes the use of a postlavage intrauterine infusion of an anti-fungal medication. **The 2 main categories of anti-fungal agents are the polyene and the imidazole drugs.** Their mode of action is through interfering with the formation of ergosterol in the cell wall of fungal organisms (Horowitz *et al.* 1987). This results in an increased permeability to the cell wall and eventual cell death. The polyene antibiotics include amphotericin B, nystatin, and natamycin (Carter and Chengappa 1995). The other class, the **azole derivatives**, include **clotrimazole, econazole, ketoconazole, fluconazole and itraconazole.** Fluconazole and itraconazole are members of the triazoles with 3 nitrogen molecules, whereas clotrimazole, miconazole and ketoconazole are imidazoles with 2 nitrogen molecules (Carter and Chengappa 1995).

It is felt that **imidazole derivatives are more effective candidicidal agents than polyene agents** (Eschenbach 1986). Many anti-fungal drugs like an acid environment for absorption (Schafer-Korting 1993). This may be another reason to use **dilute acetic acid lavages of the uterus prior to infusion of medications.**

In man, topical anti-fungal agents themselves can have a negative effect on normal vaginal microflora **potentially removing the normal microflora, so that recurrent infections may now be more probable** (Ross *et al.* 1995). While the uterus of the horse is considered not to have a normal microflora, disruption of the vaginal flora from contact with uterine secretions laden with anti-fungal agents could occur. Subsequent contamination of the uterus from vaginal microflora may result in a recurrent infection.

Most of the anti-fungal medications have poor absorption from the gastrointestinal tract. An exception to this is the azole anti-fungal drug fluconazole. Fluconazole has excellent absorption with peripheral fluid compartments having similar concentrations to plasma (Grant and Clissold 1990). Therefore, fluconazole, although relatively expensive, may be considered for the treatment of deep-seated fungal infections whereby topical therapy is unsuccessful and the organism is sensitive.

Sometimes the limiting factor in the treatment of fungal infections is the cost of medications resulting in shortened drug course or every other day therapy. Alternatives to commercially available human preparations may allow veterinarians the opportunity to treat a mare for a longer duration using a higher dosage, thereby limiting treatment failures. Compounding pharmacies can prepare inexpensive fungal medications at various concentrations and volumes to make intra-uterine therapy more affordable. **Unfortunately, minimal inhibitory concentrations for intrauterine anti-fungal agents in the horse have not been reported.** Veterinarians should have a drug sensitivity screen performed to pick the most appropriate medication. Not all laboratories will perform them; consequently, if a

fungus is isolated, **a sub-culture can be sent to an appropriate laboratory².**

Some mares with fungal endometritis may spontaneously clear (Hurtgen and Cummings 1982). These mares have been confirmed to have an infection based on both culture and evidence of an inflammatory response.

The difficulty in treating equine fungal endometritis may be attributable to an insufficient treatment period, inadequate dose or inappropriate choice of anti-fungal drug. Most of the drug dosages used to treat fungal endometritis are extrapolated concentrations. **Suggested dosages for daily intra-uterine infusion for antimycotic agents include** (Ley 1994; Troedsson 1997):

- Clotrimazole, 500–700 mg
- Nystatin, 0.5–2.5 million units
- Amphotericin B, 100–200 mg
- Fluconazole, 100 mg

Unfortunately, the cost of anti-fungal medication often decreases the duration of therapy or results in every other day treatment regimes. The cost also may affect the route of administration preventing the use of systemic therapy. In addition, the possible intracellular presence of fungi, such as *Candida* spp., may preclude exposure of the organism to intrauterine drugs.

Factors leading to treatment success may include the timely identification and treatment of delayed uterine clearance, poor perineal conformation, or fungal colonisation of the stallion or of the more distal reproductive tract of the mare. Another reason for the continued presence of a fungal infection may be an inadequate cellular immune response, preventing clearance of the organism.

Prognosis

The prognosis for mares with fungal endometritis is usually guarded to poor (Zafracas 1975; Freeman *et al.* 1986). The **uterine biopsy results** for many of these mares indicates significant fibrosis. It is unknown whether the fibrosis was caused by the fungal infection or by a previous infection and age related changes. In man, *Candida albicans* may cause vaginal tissue damage through the release of proteases (Granger 1992). It has been suggested that when fungal elements are seen within the endometrium that future fertility is lowered (Hurtgen and Cummings 1982). This may be due to increased tissue damage from the inflammatory response to invasive endometrial fungi. Of 16 mares treated in one report, 6 of 13 treated mares conceived whereas 1 of the remaining 3 untreated mares conceived either during that breeding season or the subsequent breeding season (Zafracas 1975). Treatments consisted of iodine infusions or treatment with nystatin.

Summary

- **Once an intrauterine fungal infection is diagnosed and confirmed on either cytology or biopsy, a drug**

sensitivity pattern should be obtained.

- **Any anatomical barrier defects** should be identified and corrected. Vulvoplasty (Caslick procedure) should be performed on mares positive for fungal endometritis to diminish the possibility of faecal contamination of the reproductive tract.
- **The vagina and clitoral fossa should be cultured** to identify potential sites for re-colonisation of the uterus.
- **Therapy should continue for a minimum of 7–10 days.**
- **Re-evaluation by culture and cytological examination of the uterus, vagina and clitoral fossa should be performed at the next oestrus.**
- **A uterine biopsy stained with Gomori's methanamine silver stain** or a similar stain may also provide evidence of resilient uterine infections when the culture and cytology are normal.
- **Minimal contamination breeding techniques** (Kenny 1975) should be instituted and the mare bred near ovulation to limit the number of intrauterine invasions.
- **All instruments that are to enter the uterus should have a double guard** or sterile plastic sheath to avoid placement of vaginal organisms within the uterus.
- **Cultures of the stallion's penis, prepuce and semen should be considered when multiple mares are identified with fungal infections or when natural coitus has occurred with infected mares.**

Manufacturers' addresses

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