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When to refer an infertile mare to a theriogenologist

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ABSTRACT

Most equine infertility cases can be solved with a methodical, thorough physical and reproductive examination and appropriate diagnostic laboratory aids. Repeated examinations may be needed in some cases to identify subtle anatomical abnormalities or irregularities between hormonal and physiological relationships of the reproductive tract. For pregnancy to occur, hormonal signaling must be exquisitely synchronized with physical changes of the reproductive tract and deposition of fertile semen in the uterus. Asynchrony of these events, infection, inflammation, previous trauma to the reproductive tract or ''stress'' can interfere with conception or maintenance of pregnancy. Infertile mares are presented for three common problems: (1) accumulation of intrauterine fluid during or immediately after estrus; (2) long standing infection and/or chronic inflammation; or (3) irregular or no estrous cycles. By defining the problem, diagnostics can be chosen to determine the cause. Treatment protocols should be designed around the diagnosis and antibiotics, ecbolics or steroids should not be used indiscriminately. In all cases of mare infertility, semen quality needs to be determined to be satisfactory as a subfertile stallion bred to a subfertile mare greatly decreases the likelihood of pregnancy.

Keywords: Infertility; Endometritis; Intrauterine fluid accumulation; Anovulatory follicles; Mare

1. Introduction

Referral of a difficult infertility case to an equine theriogenologist depends on many factors, including one's experience and training, one's availability to conduct repeated examinations or treatments, and one's relationship with the client. Cases that are commonly referred include mares that do not clear an infection after repeated treatments, mares that do not become pregnant after repeated breedings (without an obvious cause for the infertility), mares with abnormal cycles, and mares that repeatedly produce anovulatory follicles. Anatomical defects such as cervical fibrosis are an important cause of infertility and can be difficult to diagnose; in that regard, a veterinarian may repeatedly treat a resistant bacterial or yeast infections, without realizing that the primary cause of the infertility is a fibrotic cervix. An inability to become pregnant after repeated breedings may be mare-dependent, stallion-dependent or both. Poor semen quality contributes to a mare's infertility; therefore, semen end points, including total number of sperm and progressive motility should be recorded for each dose of fresh, cooled or frozen semen. First-cycle pregnancy rates of stallions should be requested before obtaining semen for a subfertile mare, as low fertility in both the mare and stallion compounds the likelihood of breeding failure. Anovulatory follicles are frustrating to manage, as they occur repeatedly in aged mares. Although their cause is not definitively known, they have been associated with uterine infections, laminitis, insulin resistance, and Cushing's Disease. A complete, thorough physical

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examination, in addition to the reproductive examination is needed in these cases (and the cause may still not be identified).

Distance or time constraints in an ambulatory practice may inhibit one's ability to properly synchronize ovulation with insemination or irrigate a uterus within 12 h of breeding. These restraints can adversely affect pregnancy rates of subfertile mares, especially if mares are bred with frozen or cooled semen with poor longevity, or if the mare has a severe uterine drainage problem. Some cases are best referred because the client is unwilling to follow recommendations, whereas others (mare and owner) cause the veterinarian and farm manager great frustration. None of us are able to solve all infertility problems, nor can we successfully communicate with all clients. Consulting with or referring a case to another specialist is almost always productive and may be greatly appreciated by the client. Those of us with a referral service are obligated to learn proper communication skills and use them daily. It is imperative that we always keep the referring veterinarian informed and not ''steal'' their clients, or we will loose their trust and our referral base. This manuscript discusses examination techniques, diagnostic aids and treatment strategies for diagnosing and treating infertile mares.

2. Physical and breeding soundness examination

The more thorough and methodical the reproductive examination the more likely the cause of the subfertility will be identified. A reproductive examination for an aged, infertile mare should include the mare's history, a physical examination, evaluation of perineal conformation, transrectal palpation and ultrasonographic examination of the reproductive tract, vaginal speculum examination, and digital examination of the cervix [1]. Ancillary diagnostic aids that may be performed include uterine cytology and culture, endometrial biopsy, and endoscopic examination of the uterus. The latter diagnostics are chosen on a case-by-case basis. The importance of obtaining accurate and comprehensive information regarding the mare's performance and reproductive history cannot be overstated. Frequently, repetition of a specific clinical finding give clues to the primary cause of the infertility. Some breeds are inherently more ''infertile'' than others, which may be related to genetic predispositions and conformation. Becoming familiar with breed generalities is often helpful when creating a differential diagnostic list. For example, Thoroughbred mares have a high incidence of aspiration of air due to poor perineal conformation, Saddlebred mares have a high incidence of fungal infections secondary to cervical incompetence, whereas Standardbred mares can have anatomical defects but are extremely fertile and require less intensive management. Discussing previous diagnostics and treatments with the theriogenologist to whom the case is referred may provide insight into the cause and provides valuable information to the specialist regarding additional avenues to pursue. All findings should be recorded and those records reviewed if the mare does not become pregnant. In that regard, completing a reproductive examination sheet on every infertile mare ensures that a methodical examination is conducted and that the information is subsequently available. Examination sheets can be tailored to one's specific needs, but they should include sections for recording history, perineal conformation, and findings on physical and reproductive examinations; the latter includes information regarding transrectal palpation and ultrasonography, and vaginal and cervical examinations. The size and number of follicles, CL, endometrial cysts, and the amount of uterine edema and/or intra-uterine fluid should be noted. Cervical length and width, and the location of the uterus in relationship to the pelvis during estrus, should all be recorded.

The general physical condition of the mare should be appraised. Systemic problems, poor body condition, laminitis, or pain, may adversely affect fertility. Lame or painful mares may accumulate intrauterine fluid due to limited mobility. Estrous cycles that are abnormal in length, or the absence of cyclicity during the physiologic breeding season may be associated with uterine infection, pain, ovarian tumors, or a systemic endocrine abnormality, such as Cushing's Disease or insulin resistance. Farm management should be assessed, as group dynamics may adversely affect body condition of young or old mares, especially if mares are group fed. Mares housed in cold climates require more energy in the winter months (December to March in the northern hemisphere) as they expend more calories. Mares that lose weight in the winter or early spring are predisposed not to cycle properly, even when placed under $\geq$14.5 h of artificial light in December.

Since most uterine infections are due to bacteria or yeast ascending through the vagina, the anatomy of the pelvic region and perineum must be evaluated critically. Mares that pool fluid after breeding (especially thoroughbred mares) often are long and weak in their loin, flat over their croup, with a high tail setting that is level with the sacral iliac joint, and a sunken anus. The perineum is best evaluated during estrus, when relaxation and elongation of the vulvar lips are greatest. The transrectal examination provides information regarding

when a mare may ovulate, stage of cycle, pregnancy status, presence of cervical, uterine or ovarian pathology, and presences of inconsistency between cervical findings, uterine tone, and follicular development. When evaluating the reproductive tract via transrectal palpation, the length and width of the cervix (inches or cm) and the location of the uterus in relationship to the pelvis should be recorded. Mares with cervical fibrosis will have an elongated, narrow cervix during estrus (length >5–6 cm, width 1–2 cm), whereas mares with a torn cervical muscle will have a short (<3 or 4 cm), wide (>4–5 cm) cervix that is not well defined, even during diestrus. A uterus located more than 15 cm below the pelvis may interfere with uterine clearance of inflammatory debris. Uterine tone (thick, doughy, edematous, or flaccid), is associated with changes in the estrous cycle, seasonality, and pathological processes. Size, shape and turgidity of follicles on the ovary are used to predict ovulation, and to identify abnormal structures, such as suspected anovulatory follicles or tumors.

Repeated ultrasonographic evaluations of the reproductive tract during the estrous cycle may be needed to identify the cause of infertility, as the tract changes dynamically during the cycle, and subtle abnormalities may be noted only during a specific portion of the cycle. Inconsistencies in the relationships among ovarian follicular growth, ovulation, uterine edema pattern, and cervical relaxation, may provide clues regarding the cause of infertility. Mares with excessive uterine edema on the first or second day of estrus, or mares that accumulate a small amount of fluid cranial to the cervix may not dilate their cervix properly or may have chronic inflammation, whereas mares that retain uterine edema after ovulation may have lymphatic lacunae, endometritis, angeosis or all three abnormalities [2]. A vaginal speculum examination should always be performed in infertile mares, as the integrity of the vestibulo-vaginal sphincter, the presence of fluid such as pus or urine in the vaginal vault, or discrepancies between cervical relaxation and stage of estrous cycle and the color and moisture of the vaginal mucosa are identified visually. The external os of the cervix may be adhered to the vaginal fornix, or the cervix may be inflamed, closed and located off the vaginal floor during estrus in a mare with endometritis.

The cervical examination is often overlooked, although cervical abnormalities are a common cause of chronic infertility. Incomplete dilation of the cervical canal during estrus results in fluid accumulation both before and after breeding in old, maiden mares. Cervical trauma at foaling may result in a cervical tear, cervical fibrosis or adhesions between the external os and vaginal fornix, or within the canal itself. Affected mares present with the complaint of repeated bacterial isolation from culture swabs or intrauterine fluid accumulation, despite repeated treatment with intrauterine antibiotics, or they have a history of early embryonic death or abortion. If a cervical tear or adhesion is suspected during estrus, a digital examination of the cervix should be conducted when the mare is under the influence of progesterone. Fibrosis of the cervical canal is best identified during estrus.

2.1. Laboratory diagnostics

Diagnostics most commonly used to determine the cause of infertility in an aged barren mare are uterine cytology and culture. In some cases, endometrial biopsy and/or uterine endoscopy are also performed. Uterine culture samples are usually obtained by a guarded culture swab, whereas cytological specimens are obtained by passing a second swab into the uterus, collecting cells on the cap of a guarded swab, or with a cytology brush. Recent work from Denmark [3] has shown that culture swab results may not always be accurate, as only 45% of mares with bacteria isolated from the surface of an endometrial biopsy had bacteria isolated from a uterine culture swab. These data have stimulated interest in current culture techniques. Riddle et al. evaluated relationships between uterine culture, cytology and pregnancy rates in Thoroughbred mares in central Kentucky [4]; twice as many mares were identified as having endometritis by endometrial cytology than by culture swab, with the degree of inflammation more important in diagnosing infertility than the mere presence or absence of inflammation (defined as >2 neutrophils). Isolation of micro-organisms was always associated with decreased pregnancy rates, regardless of cytological findings. The uterine inflammatory response, as identified in cytological smears, appeared to differ among micro-organisms, with β-hemolytic *Streptococcus* being associated with a higher number of positive cytologies than coliforms or yeast.

The use of a small-volume uterine flush has recently been reviewed as a method for culturing the uterus of chronically infertile mares [5]. The technique was found to be a rapid, accurate method for identifying mares with chronic endometritis, and was twice as sensitive as swab culture estimated by Neilsen [3], when the same "best standard" (neutrophils in the endometrium) was used. When micro-organisms were recovered, endometritis was confirmed by recovery of a cloudy or mucoid efflux, an increase in pH, cytological findings of debris,

bacteria or neutrophils, or neutrophils on endometrial biopsy (if taken). *Escherichia coli* was most commonly isolated and it appeared to differ in pathogenicity from β-hemolytic *Streptococcus*.

Histological evaluation of an endometrial biopsy has lost favor with some veterinarians, as it is thought not to provide enough practical management data. However, the endometrial biopsy provides valuable information regarding whether a treatment reduced inflammation, if the mare has uterine degeneration that adversely affects uterine clearance or blood flow, and if additional therapy is required. The findings of a mucoid layer overlying the epithelium, changes in epithelial size and shape, degree of endometrial edema as it relates to stage of cycle, angeosis and/or lymphatic lacunae, periglandular fibrosis, mitotic figures within the glandular epithelium, or cystic glandular degeneration, are associated with specific pathological processes and their presence dictates treatment strategies [6]. Mares with excessive endometrial edema may have cervical incompetence, defects in uterine drainage or chronic inflammation. Angeosis, fraying and tearing of the elastin tunics in the arterioles, occurs in conjunction with lymphatic lacunae and indicates that blood flow to the endometrium is abnormal and uterine drainage may be impaired [7]. Cystic glandular degeneration or mitotic figures in glandular epithelium may be associated with chronic inflammation or endometrial irritation. Endometrial biopsies should be evaluated by pathologists interested and experienced in equine reproductive pathology, or by equine theriogenologists trained in pathology [8].

Uterine endoscopy is usually limited to mares with possible focal infections, uterine adhesions, or foreign bodies. The procedure can be performed during diestrus or in early estrus. The uterine lumen may be filled with either air or saline. Visibility is best with air insufflation, however, air can be irritating to the endometrium. Upon completion of the examination, the infused air should be removed through a catheter or pump, the uterus lavaged with saline, and in some cases antibiotics infused (for example, if the mare has a history of endometritis). These procedures should provide the information needed to make a diagnosis; the next issue is how to best treat the mare.

3. Management of mares with inadequate uterine drainage

Intrauterine fluid is a clinical sign, not a diagnosis. Inadequate uterine drainage has many causes, including impaired cervical function, postmating-induced endometritis, a dependent uterus, impaired lymphatic drainage, angeosis, or bacterial or yeast infections [7,9–14]. The location of fluid accumulation within the uterine lumen, uterine body near the cervix, or uterine horns, provides a clue as to the cause. Mares with primary cervical incompetence will retain fluid just cranial to the cervix. They tend to be maiden mares, usually >10 years of age, or mares with a history of a traumatic foaling. Mares susceptible to mating-induced endometritis tend to be pluriparous and >12 years of age; they accumulate fluid in the uterine horns and body as a result of impaired myometrial contractions, poor lymphatic drainage, excessive glandular secretions, angeosis, a large pendulous uterus, and possibly abnormalities in hormonal and neurological signaling. Some mares with postmating endometritis may have an incompetent cervix during estrus due to cervical adhesions or fibrosis. Fluid retention for >18 h postbreeding results in decreased pregnancy rates [15] and if mares are repeatedly bred, they may develop bacterial or yeast infections. Once infected, mares will require antibiotic treatment, and breeding opportunities may be missed. Susceptibility to endometritis can occur gradually, as it can be related to degenerative uterine changes, or it may occur acutely (e.g. after a traumatic foaling).

Unfortunately, identifying mares susceptible to postmating endometritis is difficult because many mares show no signs of inflammation before mating, only to accumulate fluid after mating. The most useful diagnostic techniques for identifying the cause of fluid retention are transrectal palpation and ultrasonographic examination of the reproductive tract and evaluation of the mare's conformation. The amount of intrauterine fluid that is significant before breeding is not clear. Small volumes of intrauterine fluid during estrus may not adversely affect pregnancy rates; in contrast, quantities exceeding 2 cm in height (detected ultrasonographically) have been associated with decreased pregnancy rates [16]. The presence of intra-luminal fluid ≥ 18 h after breeding or retention of uterine edema after ovulation is diagnostic of defective uterine clearance. Therefore, an ultrasonographic examination of the reproductive tract should be conducted in all suspect mares after breeding, to determine if additional therapy is needed.

Treatment for postmating endometritis is aimed at improving physical clearance of inflammatory byproducts associated with insemination, and reducing the duration of the inflammatory response. Uterine irrigation in combination with an ecbolic drug, oxytocin (10–20 IU given IV or IM) or cloprostenol (250 μg IM) given between 4 and 6 h postbreeding, has increased pregnancy rates in highly susceptible, barren mares [17–22]. Uterine irrigation is most commonly per-

formed postbreeding if mares have intrauterine fluid >2 cm in height (detected with ultrasonography). If fluid accumulations are smaller, or there is residual endometrial edema after ovulation, only oxytocin may be needed. Oxytocin induces high-amplitude uterine contractions for approximately 30 min. Cloprostenol, a prostaglandin analog, also induces uterine contractions, however, uterine clearance is considerably slower, with contractions lasting for approximately 5 h [20,23]. Administration of cloprostenol has been advocated for lymphatic stasis, due to its prolonged effect on uterine contractility. However, it should not be given >12 h after ovulation, as it may induce premature luteal regression [24–26].

The length and severity of the inflammatory response may be diminished by corticosteroid treatment. A single injection of dexamethasone at breeding (50 mg IV) or 24 h after breeding (10 mg IV) decreased uterine fluid accumulation and increased pregnancy rates in mares with abnormal reproductive features, including pneumovagina, pendulous uterus, or cervical incompetence (Bucca S, Personal communication 2008; LeBlanc M, Unpublished observations 2008). Oral administration of prednisolone (0.1 mg/kg) given every 12 h for 4 days, beginning 48 h before breeding, decreased neutrophil numbers in uterine fluids of barren mares, decreased uterine fluid, increased fluid clarity, and increased pregnancy rates [27]. The intrauterine infusion of broad spectrum antibiotics after breeding is controversial. Clinical studies and field experience have demonstrated the effectiveness of a single postmating antibiotic treatment in combating endometritis [18]. However, its effectiveness may be associated with subclinical infections not identified by culture swab or dilution of inflammatory byproducts.

4. Identification and management of mares with chronic endometritis

Long-standing infections due to gram-negative bacteria or yeast are frustrating for veterinarians to treat; although owners expect rapid recovery, chronic infections often recur, but with a different organism isolated. Chronic gram negative or yeast infections are most common in old, pluriparous mares predisposed to self-contamination. Chronic endometritis also occurs in mares with cervical fibrosis, cervical tears, cervical incompetence (old maiden mare syndrome), mares with vestibule-vaginal reflux, or in mares with a pendulous uterus. Endocrine dysfunction such as equine pituitary disorder, or insulin resistance, frequent intrauterine antimicrobial therapy for endometritis, or prolonged progesterone administration, may predispose mares to fungal infections [28]. Organisms most often isolated from mares with uterine infection include *Streptococcus zooepidemicus*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and various yeast species such as *Candida*, or *Aspergillus*.

Diagnosis is made from history, reproductive examination, vaginal examination, ultrasonographic evaluation of the reproductive tract, uterine cytology, culture and in some cases, endometrial biopsy or endoscopy. Mares may or may not accumulate intrauterine fluid. Treatment depends on the organism recovered, the length of time the mare has been infected, and whether the organism produces a biofilm. Biofilms are cross-linked carbohydrate bonds; they provide a protective environment for bacteria or yeast to grow [29] and interfere with antibiotic penetration. Potent producers of biofilms include *Pseudomonas* spp., *E. coli*, *Klebsiella*, and yeast [29,30]. Mares with persistent gram negative or fungal

Table 1
Antibiotics used for intrauterine infusion in the treatment of endometritis in mares

Drug	Dose per infusion	Comments
Amikacin	2 g[a]	Buffer with bicarbonate or large volume of saline (200 mL); excellent gram negative coverage
Ampicillin	2 g[b]	Use only the soluble product; susceptible gram-positive and *E. coli*
Ceftiofur sodium	1 g	Resistant to many beta-lactamases; broad spectrum; save for resistant organisms
Gentamicin	1–2 g[a]	Buffer with bicarbonate or large volume of saline (200 mL); some *S. zooepidemicus*; *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Serratia* spp., *P. aeruginosa*, and *S. aureus*
Penicillin (potassium)	5×10^6 U	*S. zooepidemicus*
Neomycin	4 g	Gram-negative organisms; some *E. coli* and some *Klebsiella* spp.)
Ticarcillin	6 g	Anti-pseudomonal penicillin; gram-positive organisms; infuse with ≥200 mL of saline
Ticarcillin-clavulanic acid	3–6 g	Beta-lactamase inhibitor confers greater activity against *Enterobacter; S. aureus*, *B. fragilis;* infuse with ≥200 mL of saline

[a] Buffered with equal volume of 7.5% bicarbonate and diluted in saline.
[b] Use at high dilutions (can be irritating).

Table 2
Antibiotics for systemic treatment of endometritis in mares

Drug	Dosage	Route	Interval	Comments
Amikacin sulfate	10 mg/kg	IV or IM	24 h	Excellent gram negative coverage
Ampicillin sodium	25 mg/kg	IV or IM	12–24 h	Susceptible gram positive organisms and *E. coli*
Ceftiofur	2.5 mg/kg	IM	12–24 h	Broad spectrum gram positive and some gram negative organisms
Gentamicin	6.6 mg/kg	IV	24 h	Slow IV infusion; *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Serratia* spp., *P. aeruginosa*, *S. aureus*
Enrofloxacin[a]	5.5 mg/kg 7.5 mg/kg	IV PO	24 h	Slow IV infusion; gram negative infections caused by susceptible bacteria resistant to alternative, 1st-choice drugs; do not infuse IU
Penicillin G (potassium)	25000 IU/kg	IV	6 h	Synergistic with aminoglycosides; do not store mixed in syringe for more than 12 h; do not mix in syringe with gentamicin; *S. zooepidemicus*
Penicillin (procaine)	25000 IU/kg	IM	12 h	As for above
Trimethoprim-sulfonamide	30 mg/kg (combined)	PO	12 h	*S.aureus*, *E. coli*, *Klebsiella* spp., *Proteus*; some *Nocardia* spp.
Metronidazole	25 mg/kg	PO	12 h	*Bacteroides fragilis*[b] metritis

Dilute and give slowly IV.
[a] Do not use in pregnant mares or in young growing horses (risk of arthropathy).
[b] *Clostridium difficile* and *C. perfringens* diarrhea and death have been reported after use of metronidazole in western USA.

infections may not always be identified with routine culture swabs, as the infection may be focal and not diffuse throughout the endometrium. Therefore, a small-volume uterine flush may be needed to isolate the offending micro-organism. Efflux fluid collected from mares with infection is cloudy or clear, with strands of particulate matter. Treatment is directed at removal of the biofilm, if present, followed by intrauterine infusion or systemic administration of antibiotics. If an organism is a known biofilm producer, we recommend irrigating the uterus with a DMSO/saline solution (50–100 mL of DMSO/L saline) for 2–3 days in an attempt to remove the biofilm. Intrauterine antibiotic treatment is then started on the third or fourth day of uterine irrigation. Intrauterine antibiotics should be infused daily for 4–5 days. The antibiotic chosen depends on the sensitivity pattern of the organism recovered (Tables 1 and 2). Mares may also require systemic antibiotic treatment. In some cases, prednisolone (0.1 mg/kg) is administered for 5–7 days to decrease the inflammatory response after the mare has been treated with the appropriate antibiotics and no uterine organisms are isolated after treatment. Our clinical impression is that addition of DMSO to irrigation fluids assists in clearing mucus. Mares irrigated with the solution will commonly have mucoid strains in the efflux collected on the third or fourth day of lavage therapy. Some veterinary clinicians prefer to infuse kerosene and not DMSO in these mares. Both DMSO and kerosene are solvents and their solvent properties may assist in mucus breakdown. The reproductive tract should be examined by transrectal palpation and ultrasonography on the 4th of fifth day of treatment, to determine where the mare is in her estrous cycle and if there is intrauterine fluid or persistent or excessive uterine edema. Mares with long-standing chronic infections should receive 30 days of sexual rest to enable the inflammatory response to subside. The uterus should be reevaluated prior to breeding, to determine if treatment was successful or if a yeast infection developed secondary to the prolonged antibiotic therapy.

Yeast infections are most common in mares with a breach in an anatomical barrier that protects against uterine contamination (e.g. sunken perineal body, severely damaged vestibule-vaginal fold, over stretched vagina resulting in urine reflux, and cervical tears or adhesions). They may also occur after repeated intrauterine antibiotic therapy, and in mares with a cervix that does not open properly [28]. Treatment is directed at correction of anatomical defects, in addition to intrauterine and possibly systemic antimycotic therapy. Treatment success is difficult if an owner elects not to surgically repair the anatomical defect, or if the mare's cervix is fibrotic. We take a conservative approach to treatment. The first time yeast or fungi is isolated, the uterus is irrigated with a disinfectant solution such as betadine/saline flush solution (25 mL of provodone iodine in 1 L of saline) for 5–7 days. The mare is then given one diestrus period to recover. If the yeast is isolated again, the organism is identified and its sensitivity pattern is determined (samples are sent to the Cornell Microbiology Laboratory, Ithaca, NY, USA). The uterus is irrigated again for 5–7 d with either a

Table 3
Systemic and topical antifungal agents used for treatment of endometritis in mares

Drug	Dosage	Route	Interval (h)	Spectrum
Systemic				
Amphotericin B	0.3–0.9 mg/kg	IV[a]	24–48	Broad spectrum
Ketoconazole	20 mg/kg (in 0.2 N HCl)	NGT[b]	12	Yeast[c]
Fluconazole	Loading dose 14 mg/kg 5 mg/kg	PO, IV	24	Yeast
Itraconazole	5 mg/kg	PO[d], IV	12–24	Broad spectrum[e]
Topical				
Clotrimazole	400–700 mg	IU	24 h × 7 days	Broad spectrum
Miconazole	500–700 mg	IU	24 h × 7 days	Broad spectrum
Nystatin	$0.5–2.5 \times 10^6$ units[f]	IU	24 h × 7 days	Yeast
Amphotericin B	100–200 mg	IU	24 h × 7 days	Broad spectrum
Fluconazole	100 mg	IU	24 h × 7 days	Yeast

[a] Diluted to 1 mg/mL in 5% dextrose and administered over 1–2 h.
[b] Nasogastric intubation is required to avoid the irritant effect of HCl on the oral cavity and throat.
[c] Yeasts: *Candida* spp.
[d] Bioavailability of the oral suspension is superior to that of the capsules.
[e] Broad spectrum: yeasts, *Aspergillus*, dimorphic fungi.
[f] Must be diluted in sterile water (100–200 mL), as it precipitates in saline.

betadine/saline solution or with a DMSO/saline solution and the mare treated systemically with an anti-mycotic drug, e.g. itraconazole, for 21 days (Table 3). An anti-mycotic drug may also be infused into the uterus during estrus for 5–7 days, and again during the subsequent estrus (Table 3). Some mares with recalcitrant fungal infections may require three treatment periods before the infection is cleared, whereas others clear the infection without treatment. Mares treated with an appropriate anti-mycotic drug sometimes have bacteria such as *Streptococcus zooepidemicus* or *E. coli* isolated from their uterus following treatment. These bacteria may have been present at time of isolation of the yeast, or may have been iatrogenically introduced during uterine manipulations. Yeast is a potent biofilm producer and it facilitates development of microbial microcolonies within infected tissues [31]; therefore, mixed infections are possible. Mares that develop bacterial infections after intrauterine treatment with an antimycotic drug are treated with uterine irrigation containing a disinfectant (e.g. betadine). If antibiotics are given, they are administered systemically.

5. Management of mares that do not have normal estrous cycles

Abnormal estrous cycle length or lack of estrous cycles during the cyclic season may be associated with uterine infection, aging, poor body condition score (<4 out of 9), pain, granulosa cell tumor, anovulatory follicles, or a systemic condition, e.g. Cushing's Disease, insulin resistance, or ovarian senility. Mares that do not exhibit estrous cycles during the 4 months surrounding the summer solstice should have a thorough physical examination, including evaluation of locomotion, and appropriate blood samples drawn if Cushing's Disease or insulin resistance is suspected.

Anovulatory follicles are more common in aged than in young mares (<10 years old) [32]. The cause of their development is unknown, but theories include an inability of the follicle to respond to LH, prostaglandin release from chronic endometritis or pain, and high plasma cortisol or insulin concentrations. Mares that develop an anovulatory follicle will exhibit normal estrous behavior, but estrus will be prolonged and the dominant follicle continues to grow when it is expected to ovulate. On ultrasonographic evaluation, anovulatory follicles develop either hyperechoic particles within the fluid filled lumen as they increase in size, fibrous strains within the lumen, or a thick white rim at the periphery. The mean diameter of anovulatory follicles is approximately 50 mm [32]. Treatment with either hCG or deslorelin (GnRH agonist) is futile, as the follicles will not ovulate. Approximately 65% of anovulatory follicles will eventually produce progesterone, whereas the remaining 35% do not. Follicles that produce progesterone will respond to prostaglandin, with the mare returning to estrus. Unfortunately, many of these mares will subsequently form another anovulatory follicle. As anovulatory follicles have been associated with chronic endometritis, a small-volume flush of the uterus should be obtained from mares that repeatedly form anovulatory follicles, to ensure that there is no endometrial inflammation or infection. Mares with

chronic foot pain, that are obese, that do not shed their coat properly, or have bacteria or yeast repeatedly isolated from uterine culture, should be tested for insulin resistance. Diagnostic tests include dexamethasone suppression, measurement of ACTH, plasma cortisol, and insulin. Some mares with insulin resistance can be managed by decreasing carbohydrates and feeding a diet high in fiber, whereas others require daily administration with pergolide. However, mares treated with pergolide need to be monitored closely, as daily doses >2 mg have been associated with development of anestrus, as pergolide is a dopamine agonist that blocks secretion of GnRH.

6. Summary

Causes for infertility in aged mares are numerous; accurately diagnosing the problem requires a thorough physical and reproductive examination. In some cases, more than one examination is needed to identify the cause. Treatment may span weeks or may include surgical repair of anatomical defects. Barren mares should be evaluated during the late summer or early autumn, so that they can be treated before the transitional period and the uterus or cervix has time to heal. A patient, thorough approach frequently results in a successful pregnancy.

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