Infertility in the Mare

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INTRODUCTION
Infertility in the mare results in a significant loss of dollars in the horse industry every year. It is defined as the absence of the ability to conceive. There are many causes of infertility that are recognized, including infectious, inflammatory, a faulty uterine immune system, trauma and scarring, hormonal, twinning, neoplasia, and congenital abnormalities.

CAUSES OF INFERTILITY
Infectious endometritis is probably the number one cause of infertility in the mare, and contributes significantly to fetal death. A large variety of organisms have been cultured in both the mare and stallion, but much disagreement exists as to the clinical significance of these positive cultures. A large number of bacteria normally enter the uterus at breeding when the cervix is relaxed. If these are not eliminated by about five days post-ovulation when the embryo descends to the uterus, infertility may result. Mares can conceive while infected, but many will abort, resorb, or produce infected foals. Pyometra occasionally occurs, but does not show systemic manifestations as in the canine.

The mare has a highly efficient defense mechanism that clears the uterus of bacterial contamination, so inflammation is a normal response in fertile mares. This mechanism involves phagocytosis by neutrophils, primarily, which respond to a massive invasion of the uterine lumen by debris and bacteria within 6 hours. Opsonins from the circulation are vital for efficient phagocytosis, and complement is a component of opsonization. The uterine defense system, in addition to the leukocyte function, includes ovarian hormones, non-cellular bactericidal factors, and immune responses. Local synthesis of antibodies, mainly IgA, occurs in the secretory epithelium, and a transport mechanism moves the polymeric Ig across into the uterus, cervix, and vagina. Six of the ten known equine immunoglobulins have been found in uterine flushings. Some investigators report that these are in higher concentrations in fertile mares, while others dispute these findings. When bacteria penetrate the mucosal barrier, the higher protein level stimulates the defense mechanism. Mares with a decreased resistance to infection undergo rapid complement inactivation, possibly of enzymatic origin. A failure of the uterine defense mechanism leads to a prolonged inflammation and an endometritis.

Ovarian hormones greatly influence the inflammatory and immune responses of the uterus. These defense systems have the greatest ability at estrus when the vascular system is the most permeable. Progesterone inhibits the immune response. Many mares become anestrus after embryonic loss, delaying the next estrus for a significant period of time. Infrequent, irregular estrus due to embryonic loss and decreased ovarian activity is common in the least productive mares. Embryonic losses occur because of nutritional deficiencies, endometrial disease, stress, twinning, paternal influences, chromosomal aberrations, failure of maternal recognition of pregnancy, and several other causes. Mares may have lower conception rates when bred on the foal heat. The highest percent of pregnancy loss occurs at days 15–20, when maternal recognition occurs, or at days 30–35, just prior to when the endometrial cups form.
There are a variety of anatomical and congenital conditions in mares that contribute to infertility. Pneumovaginitis, and subsequently pneumo-uterus, result from poor conformation which allows an inadequate vulvo-vestibular closure and urine pooling. Occasionally, maiden mares have a persistant hymen. Chromosomal abnormalities may be related to bilaterally small, smooth, soft or firm, ovaries with no palpable structures. Pathologic cystic follicles, like those in cattle, have not been documented in mares. Cervical lesions prevent conceptions in some mares, and are frequently a result of trauma from breeding or foaling.

Ovarian neoplasm can cause infertility. Three recognized categories of tumors are sex cord-stromal tumors (granulosa cell tumors), primary epithelial tumors (cystadenomas), and germ cell tumors (teratomas and dysgerminoma). Of these, the granulosa cell tumors are the most common. The embryologic origin is uncertain. They produce hormones and cause anestrus, intermittent or frequent estrus, nymphomania, stallion-like behavior or masculine physical characteristics in mares because of the increased testosterone concentration.

The serous cystadenoma is rare. Mares will cycle normally, and can become pregnant due to the normal function of the opposite ovary. The multiple cysts eventually destroy the affected ovary. They do not secrete hormone.

Teratomas are relatively rare and are non-hormone secreting. They can be quite large and contain bone, cartilage, hair, teeth and nerves. They are usually unilateral and the opposite ovary will cycle and pregnancy is possible.

Dysgerminomas are rare but are the one malignant form of ovarian neoplasms. They metastasize to the abdomen and thorax and can cause colic or rapid weight loss.

Endometrial cysts develop from obstructed lymphatics in the reproductive tract. These cysts, if large enough, make the endometrial lining unsuitable for implantation.

Infertility can be recognized by anestrus behavior during the breeding season in unbred mares, continuous or intermittent estrus, aggressive behavior, uterine discharges, or a history of unproductiveness.

### DIAGNOSING INFERTILITY

A diagnosis of infertility and its underlying etiology is based on a complete and careful evaluation that may require several clinical and laboratory procedures. Uterine cultures, uterine cytology, endometrial biopsies, serology, endoscopy, ultrasound, and rectal palpation are common techniques available to the practitioner.

A general physical appraisal should be the first procedure when evaluating the reproductive status of a mare. The overall condition of the mare in terms of her body fat can have a direct effect on her reproductive efficiency. Mares are most fertile when they are lean and gaining weight. Any masculinity should be noted.

The reproductive exam should begin with a visual appraisal of the vulva, vagina and cervix. Conformational and traumatic defects are common. Recto-vaginal tears, pneumovagina (windsucking), cervical lacerations, muscle separation, abscesses, diverticulae, and abnormal dilatation of the cervix can be found by visual inspection with a speculum or digitally. A rectal exam is the most important step in reproductive evaluations. The uterus can be palpated to determine its tone, size, and texture. The ventral wall at the junction of the horns is the most frequent location for grossly palpable uterine pathology. Atrophy of myometrium, mucosal atrophy, endome-

### TABLE 1. Differential Diagnosis of Ovarian Enlargement

<table>
<thead>
<tr>
<th>Granulosa Cell Tumor</th>
<th>Percent Follicular Structures (Functional)</th>
<th>Ovarian Hematoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Breeding Age</td>
<td>Usually Breeding Age</td>
</tr>
<tr>
<td>Site</td>
<td>Unilateral</td>
<td>Us. Unilateral</td>
</tr>
<tr>
<td>Season</td>
<td>All Year</td>
<td>Breeding Season</td>
</tr>
<tr>
<td>Estrous Cycle</td>
<td>Anestrus, Irregular</td>
<td>Anestrus,</td>
</tr>
<tr>
<td>Character</td>
<td>Cycles, Nymphomena, Invertece</td>
<td>Nymphomena,</td>
</tr>
<tr>
<td>Behavior</td>
<td>Normal to Abnormal—Vultristic, Viscious</td>
<td>—</td>
</tr>
<tr>
<td>Ovarian Character</td>
<td>Varying size, Solid, cystic, Indistinct</td>
<td>Large cystic struct.</td>
</tr>
<tr>
<td></td>
<td>Fossa, Serous Fluid</td>
<td>Fossa palpable Cyst fluid serous.</td>
</tr>
<tr>
<td>Response to Hormones</td>
<td>0 Fair</td>
<td>0</td>
</tr>
<tr>
<td>Treatment</td>
<td>Hemi-ovariectomy</td>
<td>Hemi-ovariectomy</td>
</tr>
<tr>
<td></td>
<td>Ovaricentestis LB/HEG, Progesterone.</td>
<td>Ovaricentestis</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Good Wk-months</td>
<td>Good Days to weeks</td>
</tr>
</tbody>
</table>

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trial cysts, and lymphatic lacunae can be detected, as can atrophy of endometrial folds. Oviducts cannot be consistently palpated. The ovaries should be examined for hypo- or hyperplasia. The normal size is 6-7.5 cm in length, but vary greatly in size due to the season. Their consistency, and a comparison with the opposite ovary are important to note. An enlargement of one ovary can indicate one of several disease conditions. Differential diagnoses to consider include tumors, hematoma, or an enlarged lobulated ovary of older mares developing at the beginning of the transition period. Granulosa cell tumors produce hormones that can be detected in the serum and may be used for differentiation (See Table 1).

Ovarian hypoplasia is caused by several conditions. Seasonal anestrus causes a regression of ovarian size, as does severe malnutrition, stress, or obesity. Chromosomal abnormalities are seen occasionally and 63,X0 (Turner's Syndrome) is the most common. Reproductive endocrinopathies can cause hypoplasia, and can be detected by injecting exogenous GnRH. If the mare shows normal estrus behavior, forms a corpus luteum, and returns to estrus 15 days after the last day of the previous estrus, it is reasonable to assume that the endocrine system is functioning adequately.

Before any contaminating intra-uterine procedures are done, a uterine culture should be collected for identification and antibiotic sensitivity of uterine organisms. It is no longer applicable to culture the cervix. The significance of positive cultures is not always clear. Healthy mares harbor bacteria in their reproductive tracts, and these organisms make the recognition of disease difficult. Up to 80% of mares culture positive 72 hours after breeding and from one to thirty days after foaling. Generally, only mares with a questionable breeding record should have a culture performed. The sample should be taken from the uterus during mid-estrus. Cytology should be done to evaluate the uterine response to any bacterial contamination. Bacteria associated with a significant inflammatory reaction (neutrophilia) indicate a positive culture. Sometimes positive cultures are not associated with significant histopathologic evidence of endometritis. A positive bacterial culture without inflammation is not significant, and antibiotics are not needed. A single positive culture is insignificant unless there is evidence of infection visually or biopsy indicates active inflammation. Discharges should be noted, although only contagious equine metritis can be diagnosed by the type of discharge (grey). Pure cultures on successive days are more meaningful. Significant bacterial infections include colonies of Streptococcus spp., E. coli, Klebsiella spp., Staphylococcus spp., Proteus vulgaris, Pseudomonas aeruginosa, Corynebacterium pyogenes, Shigella equi, Bacillus subtilis, Acinetobacter spp., Citrobacter spp., and Enterobacter spp. Aspergillus spp. and Candida spp. are the primary fungal and yeast organisms encountered. The incidence of endometritis increases with age and affected mares have an increasing difficulty in carrying a pregnancy to term.

Endometrial biopsies are used to evaluate inflammatory changes in the endometrium, but do not necessarily identify the etiology. The correlation between finding bacteria and inflammatory changes is not high, usually because of a failure to recover bacteria. With an increase in endometrial fibrosis, the foaling percentage drops because of a high percentage of embryonic death (3-4 times normal). Four categories have been established as indicators of periglandular fibrosis in biopsy samples. Expected foaling percentages are related to the severity of periglandular fibrosis (See Table 2). Seasonal changes influence the quantitative assessment of fibrosis, and the changes may not be uniform throughout the uterus. Biopsies are indicated when:

- mares have abnormalities of the genital tract.
- pyometra or mucometra is present.
- barren mares are bred 3 or more times with good sperm at 48 hours pre-ovulation.
- there is evidence of early embryonic or fetal death.
- non-pregnant mares are anestrus during the breeding season.

<p>| TABLE 2. The Relationship Between Foaling Rates and Endometrial Degeneration |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Neely Categories</th>
<th>Doig Categories</th>
<th>Degree of Periglandular Fibrosis</th>
<th>Percentage of Glandular Degeneration</th>
<th>Expected Foaling Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I high A</td>
<td>B</td>
<td>Absent</td>
<td>&lt;10%</td>
<td>70-90%</td>
</tr>
<tr>
<td>II high B</td>
<td>C</td>
<td>Mild (&lt;2 layers)</td>
<td>10-35%</td>
<td>50-70%</td>
</tr>
<tr>
<td>II low C</td>
<td>D</td>
<td>Moderate (2-4 layers)</td>
<td>35-60%</td>
<td>10-50%</td>
</tr>
<tr>
<td>III D</td>
<td>E</td>
<td>Severe (&gt;4 layers)</td>
<td>&gt;60%</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>

Iowa State University Veterinarian
—genital surgery is required.
—examining for a fertility evaluation.
—mares are barren due to unknown causes. 18,20

The results are used to predict the future reproductive performance of the mare.7

An internal exam of the reproductive tract is accomplished by endoscopy using a fiberoptic scope. This instrument is useful in visualizing various abnormalities such as transluminal adhesions, atrophy or endometrial folds, lymphatic cysts and foreign bodies.7 Many conditions are visualized that cannot be detected by palpation. Small flat embedded fibroses, synechiae, mucus membranes, septa, polyps, sacculations, ulcerations, and cysts fall into this category.29,31 Endoscopy allows the clinician to observe the entire uterine cavity, including the horns, and thus increases the accuracy of diagnosis.30 Biopsies should be done concurrently. Endoscopy is indicated in the following situations.

1. When evacuating abnormal discharges from postpartum mares.
2. In infertile mares with a history of abortion or resorption.
3. In severe purulent endometritis.
4. When there is uterine pathology.
5. During tubal cannulation.
6. When there is a suspected foreign body.
7. For direct cultures.
8. For direct biopsy.29

Ultrasound, as a tool in the diagnosis of reproductive conditions, is a fairly new development. The interpretation of readings requires that one's knowledge of anatomy be adapted to cross-sectional readouts. Ultrasonic anatomy of the uterus is influenced by the stage of the estrous cycle and is dependent on prevailing circulating levels of ovarian steroids.14 Ultrasound is most frequently used for early pregnancy diagnosis, and is highly accurate at 15 days, although best at 20–24 days.34 False negatives are rare and no false positives are observed.23 It is useful for the detection of twins, false pregnancies, and open mares.34

Serology can be used for pregnancy detection by detecting PMSG (EgCG). It appears in the blood on day 35–40, peaks between days 65–75, and disappears around day 120.22 Two tests are commercially available for the practitioner, D-Tec* MP and MIP-Test®.35,36

Adequate diagnosis and treatment of infertility in the mare is based on a sound knowledge of the seasonal variations and estrous cycle in the mare, and its effects on the physical changes at the gross, histological, and endocrine levels.

**THERAPY FOR INFERTILITY**

The choice of therapy for uterine disease depends on the diagnosed etiology, the uterine size and tone, the character of the endometrium, the duration of the problem, economics, and how available the animal is for treatment. Uterine disease is categorized into a non-infectious inflammation, pyometra, metritis, and endometritis.26 Irrigation and infusion of the uterus is used extensively since it assists in controlling infection, debriding necrotic tissue, regulating estrous cycles, and inducing estrus.15 Pressure infusion can be used to introduce antibiotics, correct a pneumo-uterus associated with prolonged estrus, increase uterine tone, induce estrus, and is often used with chronically affected mares.28

Non-infectious inflammation is generally treated by physical or chemical curettage, especially in animals with normal estrus/ovaries, and with early abortion.56,33

Pyometra therapy must initiate estrous. To accomplish this, the uterus is flushed with a dilute antiseptic (1% Lugol's) by infusing and siphoning 500–2000 mls at a time. This stimulates the release of luteolytic factor and CL regression. Prostaglandin can also be used exogenously. The uterus decreases in size and increases in tone. Two to four treatments are usually required. Once the uterus has returned to a more normal size, antibiotics are infused.26 Cervical adhesions are often a complication of pyometra, and even with rigorous, conscientious therapy, the prognosis remains poor for future reproductive capabilities.

Metritis therapy is similar to treatment for pyometra except that prostaglandins are used to evacuate the uterus and shorten the cycle. Antibiotics and/or antiseptics are then used.26 Alternative therapy includes estrogens plus oxytocin in place of the prostaglandins.

Endometritis therapy is aimed at eliminating any predisposing factors, employing local and systemic antibiotics, and utilizing chemical antiseptics, plasma or colostrum, and hormones.20,24 Chemical curettage is employed in non-infected endometritis.26 Treatment of infectious endometritis is aimed at a specific
spectrum of bacteria or fungi according to the culture sensitivity (C/S) results. In the absence of C/S, a broad spectrum antibiotic should be used. Systemic dosage rates and frequencies are used as guidelines for infusing the medication. The antibiotic is diluted in at least 120 mls of water or saline, and repeated daily for 3–5 days, up to 14 days. An indwelling catheter is helpful when prolonged therapy is indicated. Intra-uterine infusions of the mare's own plasma (collected at estrus) help to bring about a clinical improvement of endometritis, due to serum-derived opsonins (complement) that enhance the phagocytic rate of bacteria. Colostrum can also be used for intra-uterine therapy. Colostral contact with the uterus favorably influences the hormonal control of the estrous cycle. Mare colostrum contains 6g/dl of IgG, which can compensate for a deficiency of IgA at the uterine secretory surface. The effects of colostral treatment persist through one estrous cycle to early pregnancy or even longer. It has been recommended that 120 ml of mare's colostrum in 380 mls of saline be infused during mating estrus. However, the uterus size should be determined by rectal palpation to avoid over-infusion of the uterus and to insure an adequate dosage. An undistended maiden mare's uterus has a capacity of about 35 mls, while an older mare may hold 60–150 mls. The mare should be cultured 30 days after treatment.

When considering therapy, it is important to remember that a genital infection is rarely localized only to the uterine cavity, but usually affects other associated tissues such as myometrium, serosa, vagina, cervix, oviducts, etc. The pharmacological agents used in the therapy of uterine disease are antibiotics, and antimycotics (See Table 3). A popular antiseptic is Lugol's (1–2% of stock) and is effective against Hemophilus equigenitalis. Dihydrostreptomycin is not effective against any organisms found in the uterus. Nitrofurazone is effective towards some uterine pathogens, as is amikacin. When a fungal infection is suspected, antibiotic treatment should be stopped, and any other underlying causes corrected (e.g. pneumovagina). Antimycotic agents such as iodine or nystatin may result in successful treatment.

### TABLE 3. Guidelines for the Administration of Intrauterine Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin K+</td>
<td>5 x 10^9 U</td>
<td>Very effective for Strpt. Economical. Commonly used.</td>
</tr>
<tr>
<td>Gentamicin SO4</td>
<td>500–1000mg</td>
<td>Very effective, non-irritating when diluted.</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1–3 g</td>
<td>Irritating if not diluted.</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>2–5 g</td>
<td>Used for persistant Pseudomonas. Slightly irritating.</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>1–3 g</td>
<td>Used for Pseudomonas. Do not use for Klebsiella.</td>
</tr>
<tr>
<td>Amikacin SO4</td>
<td>2 g</td>
<td>Used for persistant Pseudomonas, Klebsiella, and persistant gram negative organisms.</td>
</tr>
<tr>
<td>Kanamycin SO4</td>
<td>1 g</td>
<td>Toxic to sperm.</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>1 x 10^9 U</td>
<td>Used for Pseudomonas.</td>
</tr>
<tr>
<td>Neomycin SO4</td>
<td>3–4 g</td>
<td>Used for sensitive E. coli.</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2–3 g</td>
<td>Irritating, especially orally.</td>
</tr>
<tr>
<td>Nitrofurazones</td>
<td>50–60ml</td>
<td>Questionable effectiveness.</td>
</tr>
<tr>
<td>Povidone-I (1–2% of stock)</td>
<td>250ml</td>
<td>Good irrigation for nonspecific inflammation, and for yeast. Irritating if &gt;10%.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>250ml</td>
<td>Irrigation for nonspecific inflammation. Irritating if &gt;5% or in suspension form.</td>
</tr>
<tr>
<td>Nystatin</td>
<td>500.000 U</td>
<td>For yeast. Dilute in 100–250ml water.</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>200mg</td>
<td>For fungi. Dilute in 100–250ml water.</td>
</tr>
<tr>
<td>Dinehydrolactone (5% of stock)</td>
<td>50–100ml</td>
<td>Penetrating agent to carry drugs. Effectiveness and safety unknown.</td>
</tr>
<tr>
<td>EDTA TRIS (1.3g NaEDTA + 6.05g TRIS/L water, to pH 8 glacial acetic acid)</td>
<td>250 ml, 3 hours later use FU antibiotic.</td>
<td>Theory: EDTA binds Ca++ in bacterial cell walls, making more permeable to antibiotic.</td>
</tr>
</tbody>
</table>

Absorption. It is active against Strep. spp. which is involved in 50–65% of infections. Chloramphenicol is bacteriostatic against Staph. spp., Strep. spp., E. coli, Proteus spp., and Klebsiella spp. It is rapidly absorbed after intrauterine infusion, blood levels peak in less than 1 hour, and it lasts 12 hours. Oxytetracycline is useful in only a limited number of infections, and it is poorly absorbed from the uterus to the bloodstream. Gentamycin is an expensive drug, but causes no uterine inflammation and is effective against Staph. spp., Pseudomonas spp., Klebsiella spp., Proteus spp., E. coli, Step. spp. and Hemophilus equigenitalis (CEM). Ampicillin is similar in action to penicillin. Intravenous administration (7–10 mg/kg) is effective for treatment of B-hemolyzing streptococci. Dihydrostreptomycin is not effective against any organisms found in the uterus. Nitrofurazone is effective towards some uterine pathogens, as is amikacin.
(500,000 IU, 4–5 days), amphotericin B, and clotrimazole (400–600 mg every third day for 3–4 treatments) may be the best treatments available. Clotrimazole is a human drug used as a suppository.17 Hormones can be used in combination with antibiotics or alone to treat several disease conditions. A sensitive antibiotic + estradiol in solution (e.g. ethinyl estradiol + nitrofurazone) stimulates the expulsion of exudate from the uterus.10 Prostaglandins cause a longer follicular phase. Prostaglandin F2α, 10–15 mg, once subcutaneously, is used, when a mare is having a prolonged luteal function, to cause ovulation. The mare will not always respond to teasing, even if ovulation occurs.7 This same drug can be used to shorten the estrous cycle. Commonly it is given 5–6 days after the ovulation of the first foal heat, after which the mare will have a new heat in 2–5 days. This is useful for late foalers whose conception rate on the foal heat is low.12

The causes of infertility in the mare are many and varied, and result in considerable economic loss for the breeder. The more common factors contributing to reproductive losses, and methods available to the practitioner for their diagnosis and treatment have been outlined here.

Several disadvantages result from uterine antibiotic therapy. The estrous cycle may be lengthened or shortened. Fungal infections may develop. A low dose or the incorrect antibiotic may cause resistance. Systemic effects can result from absorption. Some antibiotics are irritating and can cause endometrial damage. A post-breeding infusion may decrease the foaling rate.26

CONCLUSIONS

The causes of infertility in the mare are many and varied, and result in considerable economic loss for the breeder. The more common factors contributing to reproductive losses, and methods available to the practitioner for their diagnosis and treatment have been outlined here.

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