Reproduction—Clinical Cases

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1. Introduction
Mare infertility can be frustrating because time and money restrictions are frequently imposed on the veterinarian. The approach of “she won’t get pregnant unless she is bred” (repeatedly) may not always be true, because surgical correction of an anatomical defect, a period of sexual rest to resolve uterine inflammation, or better semen quality may be what is needed for the mare to conceive. Information on these issues can often be gleaned from the mare’s reproductive history. In some cases, a physical and reproductive examination (perineal-conformation examination, rectal and ultrasonographic exam, vaginal and cervical evaluation) is all that is needed to make a diagnosis. In other cases, the cause of the infertility might not be determined with a single examination. Reproduction is a dynamic process, and subtle abnormalities may only be identified during specific times of the estrous cycle.

Infertility cases that the author has seen will be presented. Guidelines for conducting a breeding-soundness exam, treatment protocols, and management of specific problems are provided for reference.

2. Basic Reproductive Examination
The basic reproductive examination for an infertile mare includes obtaining a history, conducting a physical examination, evaluating the perineal conformation, performing a rectal/ultrasonographic exam of the reproductive tract, performing a vaginal speculum examination, and performing a digital examination of the cervix. Ancillary aids include uterine cytology and culture, endometrial biopsy, and endoscopic examination of the reproductive tract. The latter diagnostics are chosen on a case-by-case basis. The importance of obtaining the mare’s performance and reproductive history (as completely as possible) can not be overstated. Often times, the repeatability of a specific clinical finding gives clues to the primary cause of the infertility. If the case is a referral, discussing previous diagnostics and treatments with the referring veterinarian and obtaining his or her insight provides valuable information on a possible direction to pursue. It is imperative that all findings are recorded. They may provide clues as to what to do next if the mare returns a second time, because she did not become pregnant after the first examination. A one-page check-off sheet that includes space after each category (history, perineal conformation, physical exam, etc.) to write one’s findings will ensure that a complete and thorough examination is performed each time.

Before examination of the reproductive tract, the general physical condition of the mare and farm management should be appraised. Systemic problems, poor body condition, laminitis, or pain may adversely affect fertility. Mares with laminitis...
may accumulate fluid within the uterine lumen because they have limited mobility. Abnormal estrous-cycle length or lack of estrous cycles during the mare's natural cyclic season may be associated with pain, systemic endocrine abnormalities such as Cushing's disease, or uterine infection. Feeding or management changes, dental care, or parasite control may be indicated. Race mares that are brought from the track to the breeding farm in late winter may not adapt to the group dynamics of a broodmare band because they were stalled separately during their racing career. Placing them in a small paddock with another mare may improve their ability to cope. Mares housed in northern climates require more calories in January, February, and March, because they expend more energy in the cold. Mares that lose weight in the early spring tend not to cycle properly even when placed under ≥14.5 h of artificial light in December.

Most uterine infections are caused by bacteria or yeast ascending through the vagina. Conformational characteristics that correlate with high fertility include a long sloping hip, a sacral iliac joint 4 cm dorsal to the pelvic floor. If it is 10° off of the vertical plane. Mares that pool fluid after breeding often are flat over their croup with a tail setting 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 integrity of the vulvar lips, the angulation of the vulva, and the location of the dorsal commissure of the vulva in relation to the pelvis need to be evaluated. The vulvar lips should meet evenly and appear full and firm. They function as a seal against external contamination of the uterus. The vulvar lips should lie vertically with a cranial to caudal slope of ≥10° from the vertical plane. The dorsal commissure of the vulva should be ≥4 cm dorsal to the pelvic floor. If it is >4 cm, the vulva is pre-disposed to cranial-ventral rotation, which can lead to pneumovagina and contamination.

The lips of the vulva should be parted to determine the integrity of the vestibulo-vaginal sphincter. An intact sphincter is present in a mare when the labia can be spread slightly without air entering the cranial vagina. By parting the labia, the color and moisture of the vestibular walls can be assessed. Estrus produces a glistening pink to slightly red mucosa. Anestrus generally is reflected by a pale, dry mucosa; dark red or muddy color suggests inflammation. A white, tacky mucosa indicates progesterone dominance.

The perineal body may be defective in older, pluriparous mares. The defect most likely occurs from repeated foalings and poor reproductive conformation, and it results in a sunken anus and lack of tissue between the rectum and the vagina. The integrity of the perineal body can be assessed by placing one finger into the rectum (usually the second finger) and the thumb into the vestibule. A vestibuloplasty or "deep caslick's" is helpful in decreasing contamination of the anterior reproductive tract, especially if the vestibulovaginal seal is lost.

Rectal/Ultrasound Examination of the Reproductive Tract
Repeated ultrasonographic evaluation of the reproductive tract during the estrus cycle is routine in many reproductive practices. Correlations between uterine edema, cervical dilation, and follicular dynamics can be made, and inconsistencies such as excessive edema early in estrus (lack of cervical dilation or endometritis) or free intra-luminal fluid (severe uterine-drainage problem or endometritis) can be identified. With the advent of ultrasonography, veterinarians can more closely time breeding with ovulation, can determine ovulation within a matter of hours, and can identify pathologies such as anovulatory follicles, granulosa cell tumors, intraluminal fluid, and uterine cysts. However, ultrasonography does not take the place of good manual skills. The tone and consistency of the uterus and follicles and the length and width of the cervix is identified by a veterinarian skilled in rectal palpation. Inconsistencies such as a turgid, firm uterus during estrus or a narrow, long cervix in a mare on the day of ovulation indicate problems such as endometritis and post-breeding endometritis, respectively. A grading system for uterine edema, fluid accumulation, and follicular dynamics should be developed in each practice. By doing so, veterinarians can follow up on each other’s cases, if needed.

Reviewing the ultrasonographic findings over a number of cycles in an infertile mare can provide valuable information on possible causes of the infertility.

Vaginal-Speculum and Digital-Cervix Examination
Vaginal-speculum examination is an integral part of the breeding-soundness examination. Findings include, but are not limited to, determining the integrity of the vestibulo-vaginal sphincter, identifying the presence of abnormal fluids in the vaginal vault such as pus or urine, and finding a discrepancy between cervical relaxation and stage of estrous cycle. Mares with endometritis or maiden mares may have a closed cervix that is located high on the vaginal wall during estrus. Adhesions of the external os of the cervix to the vaginal fornix may be identified, especially if the examination is conducted during diestrus. The cervix will be covered below. It must be examined visually and manually in infertile mares, because it is frequently compromised and the cause of the problem.

3. Ancillary Aids
Many detailed texts are available on the proper techniques of obtaining and interpreting uterine cytology, culture, and biopsies. 1-3 In addition, the correlation between uterine cytology, uterine culture, and 28-day pregnancies rates can be reviewed
in last year’s proceedings.\textsuperscript{4} We recommend that both a uterine cytology and culture be taken in all mares that are infertile, because neither diagnostic technique alone identifies all mares with endometritis. Obtaining a uterine swab from the uterine body during estrus may not be the most accurate method for evaluating the bacterial status of the uterus, because the swab only comes in contact with a small area of endometrium. Performing a small-volume uterine lavage seems to identify a higher number of subclinical infections, most likely because the fluid infused into the uterus comes in contact with the majority of the endometrium.\textsuperscript{3} We now use the technique in all mares presented for infertility. Mares with potential problems are identified at stall side, because efflux collected from these mares commonly is cloudy or has strains of particulate matter. The technique is simple. Sixty milliliters of saline is infused into the uterus through a sterile Bivona catheter\textsuperscript{b} that has been placed aseptically into the uterus. The veterinarian then manipulates the uterus rectally to move the fluid from horn to horn. The efflux is drained in a sterile 50-ml conical tube that an assistant is holding. Either the pellet at the bottom of the tube is cultured after it is allowed to settle for 1–2 h or the fluid is centrifuged for 10 min and the pellet cultured.

Endometrial biopsies provide valuable information on the integrity of the endometrium; however, endometrial biopsies have lost favor as a diagnostic technique, because the histological interpretation provided rarely changes the management strategy. The problem, as seen by this author, is in the classification system used, which is 28 yr old.\textsuperscript{5} Additional histological lesions such as aneogenesis, lymphatic lacunae, severe edema, mitotic figures in endometrial glands, and mucus on the endometrial surface need to be incorporated into the classification. The importance of these lesions in reproductive function has only come to the forefront in the last 5–10 yr and therefore, is not addressed by the current classification system used in the United States. Endoscopy is a helpful tool in identifying mares with uterine adhesions, foreign material in the uterine lumen, endometrial cysts, fetal remnants, and fungal plaques. It is reserved for select cases.

4. Cervix

Proper cervical function is needed for a successful pregnancy. Trauma such as a cervical tear, fibrotic changes to the cervical body, or adhesions to the vaginal fornix renders many mares infertile. Afflicted mares frequently accumulate intra-uterine fluid and have microorganisms isolated from their uterus over subsequent cycles, even after repeated treatment with uterine lavage, intra-uterine antibiotic, or antymycotic drugs. Some mares clear the infection during winter anestrus only to have the infection flair in the spring when the mare starts to exhibit estrous behavior. Cervical defects or adhesions between 4 and 8 o’clock are the most difficult to identify, because vaginal exams are most commonly conducted during estrus when the cervix is lying on the vaginal floor. They tend to be associated with uterine infections, because the cervix is pulled open ventrally during diestrus. This allows bacteria that reside in the anterior vagina to gain access into the cervical canal and possibly seed the uterus. If a cervical defect is suspected during estrus, the cervix should be reexamined when the mare is under the influence of progesterone. In addition to a vaginal-speculum examination of the cervix, a digital exam of the external os and the entire length of the cervical canal should be conducted. Fibrotic lesions associated with a previous foaling injury may be identified during rectal palpation of the cervix and reproductive tract. The cervical canal may be bent laterally to the right or left, which is likely the result of tearing and subsequent scarring of the muscle on one side of the cervical canal during foaling. Mares with this type of defect have a history of delivering a large foal, needing assistance during the foaling, or having a dystocia or abortion in late gestation.

Whether or not lesions will require surgical intervention is determined by clinical history (repeated uterine infections or pregnancy loss) and the clinical experience of the veterinarian. Cervical tears that affect more than one-third of the length of the canal usually need to be repaired surgically. Treatment for cervical adhesions (adhesion of the external os to the vaginal fornix) must be determined on an individual basis, because their removal may result in additional adhesion formation. Cervical tears that are not identified for ≥1 yr after they occur require a longer convalescence between repair and successful breeding than tears that are repaired within months of their occurrence. Long-standing cervical lesions are commonly associated with chronic bacterial or fungal endometritis. The infection may flair after surgical repair, because air and blood may enter the uterus during the procedure. The blood is a medium for growth of microorganisms, and the air irritates the uterine lining. We recommend ≥60 days of sexual rest after repair of a cervical lesion that is more than 1 yr old, but mares with a cervical lesion that is <90 days old may be bred successfully in as little time as 3 wk after repair. Cervixes that have torn and are repaired surgically may lose their pliability. They may not open completely, which results in a physical drainage problem. The uterus should be treated after breeding with uterine lavage, ecobolics, and possibly, intra-uterine antibiotics. Mares mated by natural service should be reenforced with semen after the horse dismounts. A surgically repaired cervix will likely tear again when the mare foals; therefore, a cesarean section is recommended.

Incomplete dilation of the cervical canal during estrus is associated with chronic endometritis, fluid retention in the uterine body after breeding, and
lack of pregnancy after repeated breeding by natural service. Mares with this type of cervical incompetence may have fibrosis of the cervical canal, an elongated cervix during estrus with a narrow canal diameter, or a tight internal cervical os in the presence of a soft, edematous, open external os. Fibrotic cervixes are not pliable or soft when examined digitally during estrus. They have a meaty texture, and the canal is narrow and, in some cases, seems elongated. Defects may be identified on rectal palpation during diestrus, because the cervix may have a 20–45° bend in the canal. The reproductive history may include delivery of a large foal with or without assistance. Repeated embryo-transfer attempts over a number of years may also result in cervical fibrosis. There is minimal historical data on damaged cervixes; the tissue is difficult to cut for histological assessment, because it is thick and tough.

Mares with cervical fibrosis may develop uterine infections that require prolonged intra-uterine and systemic treatment, especially if the lesion is >1 yr old and the mare had been bred repeatedly before the defect was diagnosed. After the infection is cleared, sexual rest for ≥30 days is recommended so that the chronic uterine inflammation can resolve. Aggressive post-breeding management may be required to assist with physical uterine clearance. We recommend uterine lavage between 4 and 8 h after breeding and repeated use of ecbolies and intra-uterine antibiotics in the first 48 h after breeding. Systemic antibiotics around the time of breeding and a single dose of dexamethasone (10 mg, IV) 24 h after breeding to reduce uterine inflammation have also been used. The number of cases with cervical fibrosis and chronic endometritis that we have identified has been low; however, those treated aggressively have carried a foal to term. Some of these mares tend to be “every other year” mares, because cervical involution is prolonged after foaling.

An inability of the cervix to dilate properly during estrus is a common cause of infertility in old, maiden mares (>10 yr of age) and in some young, maiden mares (Thoroughbred mares). Saddlebred and Morgan mares seem to be afflicted more commonly than Quarter Horse or Arabian mares (personal observation). Most breeds of mares are bred artificially with cooled or frozen semen. Therefore, an adequate amount of semen can be deposited in the uterus, because it is not difficult to pass an artificial-insemination pipette through the cervical canal; however, these mares tend to retain intra-uterine fluid, and if not treated promptly after breeding for fluid retention, they can have decreased embryo-recovery rates, poor quality embryos recovered, or decreased 14-day pregnancy rates. If mares are bred by natural service, there is the additional problem of decreased semen deposition within the uterus.

Prostaglandins F2α (cloprostenol), prostaglandin E1 or E2, and estradiol 17β have been used in an attempt to dilate the cervix during estrus. There are no scientific reports on the ability of any of these hormones to dilate the cervixes of mares during estrus. Estradiol 17β has been used for >40 yr as a means of improving estrous behavior of young Thoroughbred mares that are mated naturally and dilating their cervixes. A dose of 10 mg given 1 and 2 days before breeding has been advocated. Clinicians have noted that estrous behavior is improved after treatment and that the cervix is more edematous and open on vaginal-speculum examination. Repeated doses of cloprostenol (250 μg, IM) before breeding have also been advocated. Cloprostenol is given every 12 h beginning on day 2 of estrus, and treatment is continued until 12 h before breeding. Treatment with cloprostenol is resumed between 8 and 12 h after breeding in an attempt to clear the uterus of fluids associated with breeding. It should be discontinued 12–24 h after ovulation. The author does not routinely use repeated doses of cloprostenol in mares with cervical incompetence, because repeated use (250 μg) has been associated with diarrhea.

Intracervical PGE2 or PGE1 have been used in mares for cervical ripening before induction of abortion or parturition. Although successful in inducing abortion, their use has been associated with complications such as retained dead fetuses, retained placentas, and a partially prolapsed uterus after repeated use. Intracervical PGE2 or PGE1, either in tablet form or as a compounded cream, have been used to dilate the cervix during estrus. Local application of tablets include either 2 mg of prostaglandin E2 (mixed in 2–4 ml of lubricating jelly and deposited in the cervical canal) or 200 μg of misoprostol (PGE1 analog) tablets (softened in a small volume of sterile saline or lubricating jelly and inserted into the external cervical os) one time daily. We have been using a compounded PGE1 cream (misoprostol; 2000 μg in 3 g of cream) for the last three seasons in mares whose cervixes do not dilate properly during estrus. A small amount of cream is rubbed on the external os and within the cervical canal preferable 2–3 h before breeding. Clinical impression is that the cervix remains dilated for ~8 h after treatment. It is possible that cervical dilation is prolonged by the presence of semen within the uterus, because the cervical response can be dramatic in some maiden Thoroughbred mares naturally mated. Repeated use of the cream may induce cervicitis, and therefore, only one treatment during estrus is recommended. Mares with an incompetent cervix during estrus should be treated for post-mating-induced endometritis and fluid retention. The use of hormones to dilate the cervix of mares with fibrotic changes does not seem to be successful.
5. Treatment of Mares With Post–Mating-Induced Endometritis

Oxytocin has been used as the primary treatment for clearance of inflammatory debris after breeding and intra-uterine fluid accumulations since 1992. It is associated with strong uterine contractions that last for 20–30 min when a dose of 10–25 IU (IV) is used. It is frequently administered after the uterus is lavaged with saline or Lactated Ringer’s between 4 and 8 h after breeding. Treatment with oxytocin may be continued over the next 24–48 h with doses given every 6–8 h. There is a clinical impression that it does not always clear the uterus of inflammatory debris. This lack of clearing may be caused by inappropriate uterine contractions, lack of propagation of uterine contractions, or continuous formation of fluid because of inflammation.

The use of prostaglandins after insemination has also been investigated, because uterine contractions are controlled by the synergistic action of oxytocin and prostaglandin. Three prostaglandins have been evaluated for their ability to clear the uterus of a radiocolloid. Only one, cloprostenol, was associated with uterine clearance of radiocolloid in both reproductively normal mares and mares with a delay in uterine clearance. Administration of cloprostenol was associated with a longer time for expulsion of radiocolloid (4–5 h) compared with oxytocin (15–30 min). We use it in conjunction with oxytocin in mares with lymphatic lacunae and/or cervical incompetence. Its use after ovulation is controversial.

Treatment with multiple doses of cloprostenol in the 48 h after ovulation is associated with decreased circulating-progesterone concentrations in the first 7 days of the estrous cycle. Interestingly, the lower progesterone levels reported in a group of mares treated with cloprostenol in one study did not result in decreased fertility. We routinely use cloprostenol (250 μg, IM) 12 h after breeding in mares with lymphatic lacunae and in mares with decreased cervical drainage because of an incompetent cervix. We administer cloprostenol every 12 h if deemed necessary; however, we discontinue its use 12–24 h after ovulation. Mares that are treated with cloprostenol have ultrasonographic examinations of their reproductive tract every 24 h to identify ovulation.

Neither oxytocin nor cloprostenol seem to successfully clear intra-uterine fluid in all mares. Intra-uterine fluid may only be a clinical sign of a uterine problem and its causes, and therefore, treatment may differ. The author poses a few questions on fluid retention that need to be answered. Is fluid accumulation in an old pluriparous mare on day 2 or 3 of estrus caused by the same pathologies as that of a young maiden mare with a tight cervix after breeding? Are the constituents of the fluid similar in young and old mares? Are there pathological lesions in the neuroendocrine control or the myometrium of the old pluriparous mare that differ from that of the young mare? Because of the lack of success of ecbolics clearing uterine fluid, especially in older pluriparous mares, the author and others have used acupuncture as an adjunct therapy. In Chinese medicine, fluid accumulation in an old mare is considered to be caused by a different problem than that of a young mare. Therefore, the diagnosis and treatment differs. The use of acupuncture for uterine-fluid retention in mares has resulted in visible ultrasonographic and palpable uterine changes 24 h after treatment (unpublished observation). When combined with ecbolics and uterine lavage, successful pregnancies have been established in mares that did not conceive with conventional Western therapies.

6. Lactational Anestrus

This condition occurs in mares occasionally, most frequently in mares that foal in February and March. The problem seems to be related to photoperiod, pain, uterine infection, or a combination of any of the three. Weight loss associated with pain or uterine infection exacerbates periods of anestrus. A thorough physical examination of the mare including evaluation of the musculo-skeletal system should be conducted to determine if systemic problems may be interfering with estrous cycles. In addition to a reproductive examination, a small-volume uterine lavage may be helpful in ruling out subclinical metritis or endometritis.

Most mares that develop lactational anestrus will exhibit normal estrous behavior during foal heat; they ovulate but do not exhibit estrus at the 30-day estrus or do not respond to prostaglandin. On rectal examination, the ovaries are small, firm, and contain small follicles, if any. The uterus is flaccid, and the cervix is open. Plasma progesterone concentration is <1 ng/ml. Various treatment regimens have been used in an attempt to resume estrous cycles; however, response has been variable, possibly because the cause of the condition in each mare is different. Regimens that we have used include lengthening light exposure to 16 h daily, increasing daily caloric intake, and treatment with GnRH, sulpiride, acupuncture, and/or eFSH. Mares that are treated with sulpiride are given 0.5 mg/kg (q 12 h, IM) after a minimum of 10 days of lengthened daylight. In addition to the lights and sulpiride, afflicted mares are fed a processed rice-bran pellet (22–25% fat) in an attempt to increase energy. If a mare responds to treatment, she will do so in 16–20 days. Some mares begin to cycle as soon as 5–6 days after the first treatment. Acupuncture has been used in combination with appropriate pain medication and proper management in mares that have pain associated with laminitis, thrust, or non-specific back, hip, and stifl problems. We have performed acupuncture on mares until they develop a 20–25 mm follicle and then treat with either sulpiride (as stated above) or with eFSH. We have also used eFSH by itself...
when the mare has developed follicles that are ≥20–25 mm in diameter. The eFSH is given at a dose of 6.25 mg (q 12 h until a 35-mm follicle has developed). The mare is then given hCG 36–42 h after the last treatment with eFSH. Although the number of mares that were treated with the eFSH is small (n = 5), all responded by ovulating after hCG was given. It is recommended that after the mare exhibits estrus, a uterine cytology and culture should be taken to determine if there is a uterine infection.

References and Footnotes