Breeding the Problem Mare by Artificial Insemination

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1. Introduction
Horse breeding is the procedure by which an adequate number of sperm are deposited into the uterus of a “healthy” mare at the right time. Although semen deposition is performed in all breeds by natural cover (NC), it is often, and with increasing frequency, done artificially. Although the main difference between natural breeding and artificial insemination (AI) is the delivery method of semen into the uterus, many other factors determine the success or failure of the breeding. Therefore, natural breeding, if properly done, should not be considered a cheap alternative to AI. Many horse breeders are of the opinion that if the mare does not become pregnant by AI, turning her out with a stallion to be pasture bred or giving her a live cover will maximize the chances of establishing a pregnancy. In my opinion, a mare that fails to become pregnant by AI with good semen quality and does so by NC without human intervention, is an example of poor or inadequate breeding management by the personnel performing the AI. Our ability to differentiate between physiologic and pathologic conditions and to establish appropriate therapies is a hallmark of those of us that practice reproductive medicine and should be the basis for proper reproductive management.

Breeding management includes (1) diagnostic procedures to determine the soundness for breeding of both male and female, (2) necessary therapies before and after breeding, and (3) determination of the optimal time for insemination. This paper will discuss some of the managerial procedures and address a few of the common problems that veterinarians encounter when breeding mares by artificial insemination.

2. Stallion Diagnostic Procedures
Before making a decision of what stallion will be used to breed a particular mare, it is important to determine whether the semen will be fresh, cooled, or frozen. If cooled semen is chosen, collection and shipping schedules, as well as sperm longevity and stallion fertility need to be determined. If the mare is to be bred with frozen semen, an attempt must be made to inquire regarding the quality of the semen post-thaw and the history of the fertility of the stallion with frozen semen. It is also important to try to determine where and who processed the semen, because it is safer to go with laboratories that have ample experience and a good reputation freezing stallion sperm. Mare owners, however, will spend weeks, months, or even years trying to determine what stallion best suits their mare with little or no
regard for semen quality. A logical approach to breeding a mare with cooled shipped or frozen semen is to always assume that the semen will be of mediocre to poor quality. Because of the processing of the semen, some of the heterogeneity of the raw ejaculate is lost, and this relatively physiologically homogeneous sample, coupled with the reduction in sperm numbers, makes it mandatory that the semen be deposited close to the time of ovulation. It must be noted that there is a great range in the fertility of stallions with cooled and frozen semen as reported by Metcalf and Samper et al. Quality of frozen semen has been improving steadily over the last few years and is more widely used now. Because on many occasions the semen for a particular mare will be shipped from far away, the veterinarian will have little or no control of the quality of the semen that she or he has to inseminate a particular mare. Therefore, the breeding management before and after the insemination becomes the most important tools that a veterinarian has to establish a pregnancy in a given mare.

3. Mare Diagnostic Procedures

Age and reproductive history are probably the most important pieces of information that a veterinarian must have available when examining a mare. We must understand that mare owners’ expectations are to have the mare pregnant at the first cycle regardless of the mare’s age or reproductive history. These expectations are even higher when the mare has had a long and successful performance career, is 10 yr or older, and is still a maiden. The older maiden mare problem can be considered a syndrome because the majority of these mares tend to accumulate uterine fluid during estrus, have wide spread glandular dilation, often have anovulatory hemorrhagic follicles, and have a cervix that fails to relax either because of fibrotic changes or sometimes adhesions.

Mares in their mid-teens start to have a reduction in their fertility potential and therefore must be considered potential candidates for repeat breeding.

So, how should we define a problem breeder? I consider that a mare is a problem breeder when she has been bred in two consecutive cycles with good-quality semen and at the appropriate time. Signs that a mare is a candidate for potential problems could include one or all of the following: (1) irregular interovulatory intervals (too long or too short), (2) presence of free fluid before and/or after insemination, (3) a significant increase in the amount of uterine edema after insemination, or (4) presence or persistence of marked endometrial edema post-ovulation.

Interovulatory Intervals

Irregular cycles are characterized by either prolonged or short luteal phases. Normal mares ovulate at 19- to 22-day intervals once they have entered the regular ovulatory season. The uterus when not pregnant will release prostaglandin at or around day 16 post-ovulation. However, bacteria, microorganisms, or foreign material (i.e., urine) can prevent, delay, or stimulate uterine prostaglandin production, therefore altering the normal interovulatory interval. Therefore, the examination for pregnancy at day 14 or 15 becomes an important part of the routine screening for uterine health and normalcy. A mare that is in obvious estrus with a large follicle and obvious presence of uterine edema at day 14 or 15 post-ovulation should be considered abnormal. Additionally, a mare that, with a known ovulation date, does not have a dominant follicle or uterine edema by days 18–19 should also be considered abnormal. Therefore, veterinarians are encouraged to record ovulation dates and examine the mares at these critical dates because this could be the first and most effective indication of uterine health.

However, there are certain circumstances both physiologic and pathologic that can alter the interovulatory interval. Two examples of situations can be (1) the use of prostaglandin to shorten the normal 15-day luteal phase or (3) the development of anovulatory hemorrhagic follicles. Prostaglandin-Induced Estrus

The use of prostaglandin or its analogs is perhaps the most common reproductive managerial tool used by veterinarians and farm managers. However, it is perhaps also the most abused and sometimes misused hormone in brood mare practice. Reduction of fertility, delay of heat, increase in twin rates, and many other problems have been attributed to the use of prostaglandins. Careful case selection and an accurate knowledge of the day of the cycle will be very valuable to maximize the use of prostaglandins.

Mares have two and sometimes three follicular waves during the 21-day estrous cycle. A primary follicular wave is characterized by the recruitment of several follicles that eventually results in the development of a dominant ovulatory follicle. This is compared to a second wave, where the number of recruited follicles is less and does not necessarily result in ovulation due to the progesterone dominance. Therefore, at any stage of the cycle, there are growing and regressing (atretic) follicles. To distinguish between growing and atretic follicles of the ovulatory wave, more than one ultrasound examination is necessary, especially because atretic follicles of a proceeding minor or major wave may linger and confuse the identity of newly emerging follicles. The mechanism associated with follicle selection, although not completely understood, seems to involve a developmental advantage of one follicle over another such that the future dominant follicle has an increased capacity for estradiol production, sensitivity to follicle-stimulating hormone (FSH), and specificity to respond to LH through the induction of granulosa cell LH receptors, whereas subordinate follicles seem to be insensitive to low systemic concentrations of FSH and have fewer numbers of granulosa.
cell LH receptors. Hence, subordinate follicles regress because of an insufficient ability to respond to gonadotropins. During the dominance phase (estrus), there is a continuous suppression of FSH caused by the combined effect of inhibin and estradiol. The increase in circulating concentrations of both hormones primarily originates from the developing dominant or pre-ovulatory follicle. Apart from its FSH-suppressive effects, high concentrations of estradiol, in the absence of progesterone, seem to exert a stimulatory effect on circulating concentrations of LH.\textsuperscript{10,11} High concentrations of LH during the latter part of estrus are necessary for continued growth of the dominant follicle, as well as for signaling and preparing the follicle and its contents for ovulation. However, in the presence of progesterone during the luteal phase, there is a constant emergence of minor follicular waves. Depending on the mare, the follicles of the minor waves can reach pre-ovulatory size before starting the atretic process and later on regressing.

Random prostaglandin treatment therefore has the potential of finding mares with large growing follicles, large regressing follicles, or a population of both growing and regressing medium size follicles. Treatment of mares with a large growing follicle in most instances results in a very short treatment to ovulation interval (24–96 h),\textsuperscript{12} sometimes with no obvious signs of estrus when exposed to a teaser stallion. On the other hand, mares with a large regressing follicle treated with prostaglandin will often have a delayed return to estrus and a long treatment to ovulation interval, because of the length of time that it takes for a new follicle to emerge and reach pre-ovulatory size (8–12 days). Mares that have a mixed population of follicles will display signs of heat within 3–4 days and ovulate between 5 and 9 days post-treatment. Therefore, to maximize the beneficial effects of prostaglandin use, mares should be monitored to determine last ovulation date and the follicular population present at the time of treatment. This becomes very important when semen has to be ordered a few days in advance. Failure to monitor the mares will in many cases result in undetected ovulations that will invariably delay the breeding of the mare.

The presence of the large diestrus follicles is perhaps the most common factor responsible for untimely breeding of mares mostly by individuals with out experience. It is often that mares bred by AI are not in heat and only have a large size follicle with no other signs of estrus. The presence of a tight cervix, the acute onset of endometrial edema after breeding, and the presence of fluid in the uterine lumen after insemination is often detected in these mares. This problem is seldom encountered in mares bred by NC because the mares will not be receptive to the stallion when not in heat.

Development of Anovulatory Follicles

Around 12 h before ovulation, most dominant follicles become less turgid and change shape to non-spherical, and mares become more sensitive to touch on the ovary with the pre-ovulatory follicle. In addition to the drop in follicular pressure, there is also a separation of the follicular wall and the presence of echogenic material in the follicular fluid.\textsuperscript{10,11} Color-flow Doppler ultrasonography will permit direct, real-time assessment of the degree of vascularization of the ovulatory follicle in the mare which can be observed with power doppler around the follicular wall as an increase in blood flow.\textsuperscript{13}

Ovulation is the rupture of the follicular wall, resulting in a decrease in size of the antrum. In ~50% of ovulations, evacuation of follicular fluid from the dominant follicle is an abrupt process ranging from 5 to 90 s, with ~15% of the initial fluid remaining in the antrum. In the other 50%, release of follicular fluid is a slow and gradual process taking 6–7 min to evacuate all but 4–17% of the initial volume. Complete loss of detectable fluid from the antrum does not always happen. Ovulation may be confirmed by reexamining several minutes later and by observing a progressive loss of fluid in the follicle. Recognizing ovulation may be more difficult in older mares because of atypical ovulations.\textsuperscript{11} Highly irregular, semi-evacuated, and numerous echogenic particles are common characteristics of the ovulating follicle observed more often in old mares.\textsuperscript{6}

The corpus luteum (CL) formed after ovulation may appear as a uniformly white structure on ultrasonography (about 1/3rd to 1/2 of corpora lutea) or it may contain fluid within the antral cavity (1/2 to 2/3rds). A hemorrhagic CL is one that after oocyte release bleeds abundantly into the antral cavity and is not a pathologic condition. Its formation appears to be a random event and it may occur during successive estrous cycles in mares. The ratio of luteal tissue to nonluteal tissue (fluid within the antral cavity) of a newly formed corpus luteum is minimal during early diestrus and maximal during mid-diestrus. The length of an interovulatory interval is similar whether the corpus luteum develops with or without an intraluteal cavity.\textsuperscript{14}

Failure to ovulate properly resulting in anovulatory follicles occurs between 8% and 10% of estrous cycles during the physiologic breeding season.\textsuperscript{11} Anovulatory hemorrhagic follicles may be large (5–15 cm in diameter), persist for 1–7 weeks, and can result in a prolonged period of behavioral anestrus and a long interovulatory interval.\textsuperscript{15} Specific causes of ovulation failure in the mare are not known but have been suggested to be insufficient pituitary gonadotropin stimulation to induce ovulation,\textsuperscript{14} abnormal estrogen production from the follicle itself, or hemorrhage into the lumen of the pre-ovulatory follicle. In a recent study by Gastal et al.,\textsuperscript{16} comparing endocrine profiles of mares that did and did not develop anovulatory follicles, the only significant difference detected in mares with anovulatory follicles was a higher level of estrogen in the follicular fluid, but no differences in FSH or LH.
The incidence of anovulatory follicles increases with age. Mares 16–20 yr old were noted to form anovulatory follicles during 13.1% of estrous cycles during the physiologic breeding season, with almost one half of the mares having a recurrent anovulatory follicle during the same breeding season.

Prediction of the formation of an anovulatory follicle is difficult. In the authors' opinion, the first clinical indication of the possible development is the failure to respond to an ovulatory inducing agent within 48–96 h. The cycle when anovulatory follicle will form is usually preceded by normal development of endometrial edema. Initial growth patterns of follicles destined to become anovulatory are normal, and the first indication of a problem is ultrasonographic detection of echogenic particles within the follicular fluid or a strand within the antrum. Anovulatory follicles may contain blood and have consequently also been called hemorrhagic anovulatory follicles. Scattered, free-floating echogenic spots within the follicular fluid detected during ultrasound examination of a dominant follicle may be a result of hemorrhage or the shedding of granulose cells from the follicular wall. Hemorrhagic follicular fluid may form a gelatinous mass within the follicular lumen. Ultrasonographically, hemorrhagic follicles may contain echogenic fibrous bands or strands traversing the follicular lumen. Follicular wall thickening can sometimes be observed as a sign of development of an anovulatory follicle.

More than 80% of anovulatory follicles eventually become luteinized, although some remain as follicular (non-luteal) structures (15–20%). Progesterone levels may be used to determine the luteal status of anovulatory follicles, with anovulatory follicles containing a highly echogenic lumen invariably have elevated progesterone levels. Prostaglandin treatment will result in the destruction of the luteal cells in mares with luteinized anovulatory follicles and a return to estrus. Prostaglandin treatment has no apparent effect on non-luteinized anovulatory follicles, but in general, these structures are endocrinologically inactive and often do not interfere with subsequent cycles.

The majority of non-luteinized anovulatory follicles will spontaneously regress in 1–4 wk. Induction of luteinization by administration of human chorionic gonadotropin (hCG) or the gonadotropin-releasing hormone (GnRH) agonist deslorelin is generally ineffective.

Pregnancy obviously will not occur if the follicle becomes hemorrhagic or luteinized without ovulating. In many instances, mares are bred before it is recognized that a dominant follicle is destined to become anovulatory. In addition, it may be difficult to discern between a partially luteinized anovulatory follicle and a corpus hemorrhagicum or early corpus luteum in mares that are not examined frequently (i.e., daily) by ultrasonography.

The potential of color-flow Doppler for evaluating the vascular dynamics associated with equine reproduction will be an important tool in determining the vascular patterns of primary corpora lutea and that of anovulatory hemorrhagic follicles. The differential changes in vascular perfusion of a dominant-sized follicle can be quantitated by the number of colored pixels in an image so that follicle status (anovulatory or ovulatory) can be assessed and time to ovulation predicted.

Presence of Free Fluid
Although free uterine fluid is considered by some as a primary pathological condition, in my opinion, it should be seen as a clinical sign that could indicate a primary pathology elsewhere in the reproductive tract. Uterine fluid accumulation is detected by ultrasonographic evaluation. Ability of the veterinarian to detect the fluid can be influenced by the resolution of the ultrasound unit, the amount and echogenicity of the fluid, and the location of the fluid within the uterine lumen. In addition, mares with very heavy edema will have an increased surface area in the uterus, resulting in more areas for free fluid to dissipate. The pressure exerted by the veterinarian on the uterus with the ultrasound transducer while examining the mare can also result in the dissipation and inability of free uterine fluid to be detected. There are several conditions or circumstances that could lead to fluid accumulation. Some of these include (1) inability of the uterus to contract because of age or uterine position, (2) cervical tightness or fibrosis, and (3) poor perineal conformation or cervical incompetence leading to urine accumulation.

Older pluriparous mares tend to have a uterus that becomes more dependent in the abdomen because supporting ligaments stretch from repeated pregnancies. Uterine degeneration results in decreased uterine contractions and the more ventral position of the uterus in the abdomen interfere with physical clearance. Afflicted mares tend to retain uterine fluid. This results in low grade inflammation that may escalate after breeding. Mares with cervical incompetence may also retain uterine fluid. The cervix may fail to relax in maiden mares or the cervix may become fibrotic due to it being traumatized from a previous foaling, from aging, from prolonged, chronic endometritis, or from infusion of caustic substances into the vagina or uterus. As previously discussed, mares with a diestral follicle may be inadvertently bred. These mares will invariably have a tight cervix due to high circulating progesterone concentrations. When bred, these mares will have a sudden increase in uterine edema and will accumulate uterine fluid.

A common problem observed in some mares with poor perineal conformation or with cervical tears is the reflux of urine into the cranial vagina and the uterus. Vesicovaginal reflux, also known as uro vagina or urine pooling, is the retention of incompletely voided urine in the cranial vagina caused by an exaggerated downward cranial slope of the reproductive tract. Pneumovagina from a defective vulval conformation also predisposes to the condition. Transient urine pooling, which is sometimes found
in post-partum mares, usually resolves after uterine involution has occurred. Clinical signs can include urine dripping from the vulva, urine scalding, and a history of failure to conceive. Diagnosis is easiest using a speculum examination during estrus to detect urine in the cranial vagina. Uterine infection with an accumulation of exudate in the vagina can be confused with the condition. In most cases, urine pooling, if severe enough, will result in urometra. The volume of urine and its echogenicity will determine how easy it could be to diagnose the urometra. Mares that do not evacuate the urine can often retain the crystal sediment that can be determined ultrasonographically as a bright hyperechoic line in the uterine lumen. Chronic cases of urine sediment retention will be observed as 0.5- to 1-cm hyperechoic plaques in the uterine lumen. Severe cases of urine pooling that do not resolve with vulvoplasty or caslick procedure may require a more extensive surgical correction.

Diagnosis and Treatment of Problem Mares

There are probably as many opinions as there are veterinarians in what constitutes an appropriate set of diagnostic procedures and therapeutic options for a subfertile or problem mare. This summary will describe the routine procedures that are performed in my practice and some of the most basic treatment protocols. It is emphasized that, although some blanket therapies are established for certain routine conditions, the daily examination and the clinical signs will dictate changes or adjustments to the treatment protocol on a daily basis.

**Diagnosis**

It is well established that a high percentage of mares that do not become pregnant have some degree of uterine contamination. It must be assumed that the uterine environment is a sterile environment that should be free of inflammatory cells. In a recent study by Riddle et al., it was reported that mares with bacterial uterine growth and/or cytological evidence of inflammation had a reduced pregnancy rate. In my practice, uterine culture and cytological evaluation of the uterus is the first diagnostic procedure that is performed. Case selection for performing a culture and cytological evaluation include (1) mares that ovulate with a high degree of edema, (2) mares that have free uterine fluid that is present throughout the estrus period, (3) mares with short or long luteal phases, (4) mares that have failed to conceive after being bred under good breeding management conditions with a stallion of good fertility, and (5) mares with evidence of uterine or vulvar discharge.

Mares that have a positive culture (isolation of micro-organisms) or a positive cytology (greater than 2 neutrophils/field) when appropriate techniques are used should be treated. Appropriate techniques include but are not limited to taking the swabs when there is uterine edema and/or free uterine fluid, clean aseptic techniques, and proper time of contact between swab and endometrial surface. When swabs are negative and an infection is suspected, a uterine biopsy specimen is submitted for culture or a low-volume uterine flush is performed.

Other than culture and cytology, it must be insured that the cervix is patent and that the uterus is free of foreign material and adhesions.

Treating and Breeding a Problem Mare

To establish an appropriate therapy, it is imperative that certain clinical signs are evaluated: uterine culture and/or cytology, degree of uterine edema, and presence or absence of uterine fluid. If fluid is present, the relative amount, degree of cervical relaxation, and stage of the cycle should be recorded. For example, a mare with heavy uterine edema and no follicles should be considered abnormal, whereas a mare with a large pre-ovulatory size follicle and no edema and a tight cervix could be in normal diestrus.

Treatment for Bacterial Uterine Infections

It should be emphasized that in my practice, there is no standard or blanket therapies, and all mares are treated based on clinical signs. To have an efficient use of time and minimize the treatment to breeding interval, it is also critical that the day of the previous ovulation is accurately recorded.

A mare that has a bacterial growth will be infused daily for 3–5 days with an appropriate antibiotic in a volume not to exceed 50 ml. On mares being infused, a daily rectal ultrasonographic exam is performed to establish the degree of uterine fluid accumulation. If fluid accumulation is detected either before the first infusion or during the treatment period, a uterine lavage with lactated Ringers is performed. All efforts are made to recover the uterine lavage fluid. In an effort to maintain the uterus free of fluid. Mares that are being infused with intruterine antibiotics are treated q 12 h or q 4 h with carbetocin or oxytocin, respectively. This is particularly important in those mares that fail to relax or open the cervix completely. Every effort is made to treat mares during the follicular phase of the cycle so that cervical relaxation, white blood cell influx, and uterine contractility aids in uterine evacuation. Antibiotics are given systemically to mares when the bacteria recovered is resistant to antibiotics that can be safely infused into the uterus, (i.e. enrofloxacin). Systemic antibiotics may also be used in mares with poor perineal conformation that require a constant vulvar seal maintained by the vulvoplasty (Caslick), and in mares with a pendulous, dependent uterus that does not drain fluid readily.

The degree of edema is followed on a daily basis, and those mares that ovulate with a high degree of edema or whose edema persists for >24 h post-ovulation are considered abnormal. If very expensive frozen semen is going to be used in these mares,
a biopsy sample is taken to determine the degree of uterine inflammation.

Mares treated for endometritis are usually bred on the prostaglandin-induced cycle after the treatment period without obtaining a second uterine culture. However, these mares are examined 4–12 hrs after insemination and uterine lavage and ebolic agents are used after breeding.

Mares considered problems are only inseminated once, hopefully with good-quality semen. If using fresh semen, mares are bred within 48 h before ovulation, with cooled semen within 24 h after ovulation, and with frozen semen within 4 h after ovulation. Frozen semen is deposited at the tip of the uterine horn by rectally guided insemination. Cool semen of mediocre quality is centrifuged, and the mare is inseminated with 2–2.5 ml of semen also deposited by deep horn insemination. To insure ovulation, mares are given an ovulatory-inducing agent. All mares are evaluated between 4 and 8 h after insemination, and the two main parameters observed are amount of fluid accumulation and degree of edema. If free fluid is detected, a uterine lavage is performed with warm Ringers lactate until the efflux is clean. Uterine lavage is performed regardless of the ovulation status. Waiting for the mare to ovulate may start a uterine inflammatory process that could be difficult to control, particularly if the mare is bred 24–48 h before ovulation. If no fluid is detected but the degree of uterine edema has increased, the mare is treated with 20 mg of IM dexamethasone, and an ebolic agent such as oxytocin; carbethocin is used for no more than 3 consecutive days. Mares are examined on a daily basis thereafter to establish day of ovulation, as well as uterine contents and edema. A caslick’s procedure is performed on most problem mares because not all mares need to have a sloped vulva to aspirate air because contaminated with fecal material. Many mares with a small ano-genital distance (anus to dorsal vulvar commissure) are easily contaminated.

4. Conclusion

Mare owners and veterinarians must realize that breeding a problem mare is a challenging procedure that requires dedication and knowledge of several areas of veterinary medicine and strict adhesion to basic and sound medical principles. The constant and persistent use of sound techniques and clinical practices will result in the establishment of pregnancy in many of these difficult mares. However, poor practices or not enough dedication will result in frustration for both owners and veterinarians.

The practice of reproductive medicine is not limited to rectal palpations and inseminations. It is important that we as professionals use our knowledge of the areas of veterinary medicine including but not limited to physiology, pathology, and pharmacology to have success with these problem mares. By establishing blanket therapies that can be performed by individuals without medical knowledge will trivialize our procedures and our profession. This will lead to the relegation of these practices to individuals with technical training but no medical skills or knowledge.

References