Clinical Cases in Equine Reproduction

Patrick M. McCue, DVM, PhD, Diplomate ACT

Exposure of mares to an artificial photoperiod remains the most consistent technique to stimulate follicular development in seasonally anestrous mares. Ovulation failure is a significant cause of reproductive inefficiency in horses. Author’s address: Equine Reproduction Laboratory, Colorado State University, Ft. Collins, CO 80523; e-mail: pmccue@colostate.edu. © 2006 AAEP.

1. Management of the Transition Period
The physiologic breeding season of horses extends from April to October in the Northern Hemisphere. Mares are polyestrous in that they exhibit repeated estrous cycles during the breeding season.1 In the winter months, 80% or more of mares enter a prolonged anovulatory phase referred to as winter anestrus.

The onset of the transition period may be defined as the first significant increase in follicular diameter greater than that noted during deep anestrus. From a practical standpoint, transition begins in horse mares when a follicle first reaches 20–25 mm in diameter. In pony mares, the onset of transition occurs when the diameter of the largest follicle reaches 21 mm in anovulatory mares.2 The term “late transition” may be used when one or more follicles are ≥30 mm in diameter in horse mares.

Mares may exhibit multiple waves of follicular growth and regression during the transition period.2,3 The total number of follicles present on each ovary increases during the transition, and ultrasound examination classically reveals a “grape-like” cluster of small- to medium-sized follicles. The transition period may last for 50–70 days or more prior to the first ovulation of the season. Once a mare has ovulated, she will generally continue to cycle at regular intervals.

Behaviorally, transitional mares may display prolonged or irregular periods of estrus. This is likely caused by an extreme sensitivity to the low levels of estrogens being produced by developing follicles.

The mean calendar date of the first spontaneous ovulation of the year in anestrous mares maintained under ambient light conditions in North America ranges from the middle of March to early May.

Exposure of mares in deep anestrus to a stimulatory artificial photoperiod has been used for decades to advance the first ovulation of the year. Mares maintained under lights still go through a transition period of relatively normal length, but the transition begins earlier in the year. In most instances, the duration from onset of adequate light exposure to ovulation is 60–70 days.4 General recommendations for light treatment are:

1. A fixed-length period of light exposure for 14.5–16 h, allowing 7.5–9 h of darkness. It is not necessary to increase duration of light exposure incrementally.
2. Alternatively, adding 2.5–6 h of additional light exposure after dusk is effective.
Conflicting reports exist as to the efficacy of progesterone or synthetic progestins in advancing the first ovulation of the year. However, a majority of controlled research studies indicate that progesterone therapy (1) will not consistently advance the first ovulation when administered to mares in deep seasonal anestrus or early transition, (2) may synchronize the return to estrus and advance the first ovulation in late transitional mares, and (3) is effective in suppressing the prolonged and often irregular periods of estrus in transitional mares.6–9

Initiation of any treatment to mares in deep winter anestrus is less likely to be successful and is associated with a higher risk of return to anestrus after conclusion of therapy than treatment when mares are in transition. Dosage regimens are listed in Table 1.

2. Ovulation Failure
Failure of ovulation during the breeding season represents a significant cause of reproductive inefficiency in the mare and may be responsible for significant economic loss. The incidence of ovulation failure during the physiologic breeding season has been reported to range from 3.1% to 8.2%.10,11

Post-partum mares may exhibit physiologic ovulation failure. A majority of mares develop follicles and ovulate early in the post-partum period (foal heat), and they continue to cycle if they do not become pregnant at a foal-heat breeding. Alternatively, a foal-heat ovulation may be followed by a variable period of anestrus or anovulation until the mare resumes normal cyclic activity. Finally, some mares may have no significant follicular development or may exhibit moderate to substantial follicular development without ovulation during the post-partum period. Mares in the latter groups may remain anestrus or anovulatory for weeks or months before cyclic ovarian activity is initiated.

A majority of mares that do not cycle after giving birth are mares that foal early in the year. Consequently, it may be difficult to distinguish between

### Table 1. Medications and Dosage Regimens That Have Been Used in the Management of Transitional Mares

<table>
<thead>
<tr>
<th>Medication</th>
<th>Brand Name</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altrenergest</td>
<td>Regumate®</td>
<td>0.044 mg/kg, PO, SID</td>
</tr>
<tr>
<td>Buserelin (GnRH agonist)</td>
<td>Compounded</td>
<td>10 to 100 µg, IM or SC, BID</td>
</tr>
<tr>
<td>Deslorelin (GnRH agonist)</td>
<td>Compounded</td>
<td>1.5 mg, IM, once</td>
</tr>
<tr>
<td>Deslorelin (GnRH agonist)</td>
<td>Compounded</td>
<td>125 µg, IM, BID</td>
</tr>
<tr>
<td>Domperidone</td>
<td>Equidone®</td>
<td>1.1 mg/kg, PO, SID</td>
</tr>
<tr>
<td>Equine FSH</td>
<td>eFSH</td>
<td>6.25 to 12.5 mg, IM, BID</td>
</tr>
<tr>
<td>GnRH (native)</td>
<td>LHRH</td>
<td>a) 2 to 50 µg/hr, SC, pulsatile or continuous infusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) 50 to 500 µg, IM, BID</td>
</tr>
<tr>
<td>Goserelin (GnRH agonist)</td>
<td>Zoladex®</td>
<td>One-third to one-half of a 3.6 mg implant, SC, once</td>
</tr>
<tr>
<td>hCG</td>
<td>Chorulin®</td>
<td>2,500 units, IV, once</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Generic or Compounded</td>
<td>150 mg, IM, SID</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>Compounded</td>
<td>[−] sulpiride 0.5 mg/kg, IM, SID or BID</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[+] sulpiride 1.0 mg/kg, IM, SID or BID</td>
</tr>
</tbody>
</table>

The symbols [−] and [+] refer to stereoisomers of sulpiride.

3. Light treatment should be initiated during the first week of December if it is desirable to begin breeding mares in February.

4. Minimum light intensity of 10 foot-candles should be used (100 lux = ~10 foot-candles). A 100- to 200-W incandescent bulb in a 12 × 12-ft box stall should be sufficient.

A number of hormone therapies have been used in attempts to stimulate follicular development and advance the first ovulation of the year in seasonally anestrous mares. These include gonadotropin-releasing hormone (GnRH) agonists, gonadotropins, progestins, and dopamine antagonists.

Administration of low doses of native GnRH and GnRH agonists (i.e., buserelin and deslorelin) and equine follicle-stimulating hormone (eFSH) have been used successfully to induce follicular development in transitional mares. Higher doses of GnRH agonists (i.e., deslorelin) and human chorionic gonadotropin will induce ovulation of large follicles (>35 mm in diameter) in late transition.

Dopamine antagonists, such as domperidone and sulpiride, may not be effective under all management conditions. Success rate is likely to be higher in transitional mares and mares maintained indoors under a stimulatory artificial photoperiod. The most current proposed mechanism6 by which dopamine antagonists may stimulate follicular development is as follows:

1. Dopamine antagonist treatment results in increased plasma prolactin levels.
2. Prolactin acts directly on the ovary to stimulate expression of gonadotropin receptors.
3. Pituitary gonadotropin (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]) secretion is not altered by dopamine antagonist therapy, but treated mares are more sensitive to endogenous gonadotropins because of increased numbers of gonadotropin receptors.
post-partum anestrus caused by a short ambient photoperiod and anestrus caused by the effects of lactation. In general, failure to ovulate post-partum is more likely to be attributed to seasonal affects than lactation affects.12,13 However, some mares do not develop follicles in the post-partum period or become anestrus following a foal-heat ovulation, and they will exhibit rapid follicular development and estrus as soon as the foal is weaned.14,15,16 The incidence of lactation-associated anestrus in mares has been reported to be 21–74%.13 In contrast, other investigators have reported that suckling had no effect on post-partum ovarian activity.15

Poor body condition in late gestation and the early post-partum period may also contribute to poor reproductive performance. The effects of inadequate nutrition and poor body condition may be manifested in delayed return to reproductive cyclicity post-partum, reduced pregnancy rates, and increased embryo loss rates.14 The phenomenon of “lactational anestrus” may, in fact, represent the combined effects of season, body condition, and lactation.14 Maintenance of late-term pregnant mares due to foal between January and March (Northern Hemisphere) under a stimulatory artificial photoperiod for the last 2–3 mo of pregnancy may be beneficial. Pregnant mares housed under lights have been reported to foal 21–74%. In contrast, other investigators have reported that suckling had no effect on post-partum ovarian activity.15

Pregnant mares housed under lights have been reported to foal ~10 days earlier than mares not maintained under lights.18 They are also more likely to have a foal-heat ovulation, have continued estrous cycles,13 and ovulate earlier in the post-partum period.19

Ovulation failure occasionally occurs during the physiologic breeding season. Anovulatory follicles may be quite large (5–15 cm in diameter), persist for up to 2 mo, and result in abnormal estrous behavior and prolonged interovulatory intervals.20 Specific causes of ovulation failure in the mare are not known; however, it may be caused by pituitary gonadotropin stimulation insufficient to induce ovulation,21 insufficient estrogen production from the follicle itself,22 or hemorrhage into the lumen of the pre-ovulatory follicle.14 Mares may develop anovulatory follicles without prior exposure to exogenous hormones.12

Anovulatory follicles were reported in a recent study to occur in ~8.2% of equine estrous cycles.11 The incidence of anovulatory follicles increases with age. Mares 16–20 yrs old were noted to form anovulatory follicles during 13.1% of estrous cycles during the physiologic breeding season. A high percentage (43.5%) of mares that developed anovulatory follicles experienced subsequent estrous cycles with anovulatory follicle formation during the same breeding season. The interval between actual ovolutions for mares that developed anovulatory follicles was noted to be 38.5 days.

It is often difficult to determine beforehand if a dominant follicle in an estrual mare will fail to ovulate. Formation of an anovulatory follicle is usually preceded by development of normal endo-

metrial folds or edema. Initial growth patterns of follicles destined to become anovulatory are usually within normal limits, and the first indication of a problem is typically detection of echogenic particles within the follicular fluid during ultrasonographic examination.

Anovulatory follicles may contain blood and have, consequently, also been called hemorrhagic anovulatory follicles. Hemorrhage can be detected ultrasonically as scattered free-floating echogenic spots within the follicular fluid. The follicular fluid may form a gelatinous, hemorrhagic mass within the follicular lumen. Ultrasonographically, hemorrhagic follicles may contain echogenic fibrous bands or strands traversing the follicular lumen. A progression from echogenic particles and strands to complete infiltration of the follicular lumen with echogenic material is commonly observed. In other instances, the only ultrasonographic sign observed during development of an anovulatory follicle is a thickening of the follicular wall.

A majority of anovulatory follicles eventually become luteinized (85.7%), while some remain as follicular (non-luteal) structures (14.3%). Progesterone levels may be used to determine the luteal status of anovulatory follicles.11 Mares with anovulatory follicles containing a highly echogenic lumen invariably have elevated progesterone levels. Administration of prostaglandins will result in the destruction of the luteal cells in mares with luteinized anovulatory follicles, a rapid decline in serum progesterone levels, and a return to estrus. Prostaglandin treatment has no apparent effect on non-luteinized anovulatory follicles. Fortunately, a majority of non-luteinized anovulatory follicles will spontaneously regress in 1–4 wks. Administration of human chorionic gonadotropin or the GnRH agonist deslorelin is generally not effective in inducing ovulation or luteinization of a follicular-type anovulatory follicle.

3. Granulosa Cell Tumor

The most common ovarian tumor in the mare is the granulosa cell tumor (GCT).23 GCTs are almost always unilateral, slow growing, and benign. An examination of the affected ovary by transrectal ultrasonography often reveals a multicystic or honeycombed structure, but the tumor may also present as a solid mass or as a single large cyst.24 The contralateral ovary is usually small and inactive, although mares with a GCT on one ovary and a functional contralateral ovary have been reported.25 Behavioral abnormalities such as prolonged anestrus, aggressive or stallion-like behavior, and persistent estrus or nymphomania may be expressed in affected mares.

GCTs are hormonally active, and clinical diagnostic assays for the detection of a GCT include the measurement of inhibin, testosterone, and progesterone.26,27 Inhibin is elevated in ~90% of the mares with a GCT.26 Serum concentrations of mo-
The use of eFSH involves initiating treatment 5–7 days after ovulation and administering progesterone the day after treatment was started to induce luteolysis. Mares were typically treated with 12.5 mg eFSH twice daily for 7–8 days before a cohort of follicles >35 mm in diameter were present. Treatment with eFSH was then discontinued, and human chorionic gonadotropin (hCG) was given to induce ovulation.

A modified eFSH protocol has been developed based on the results of several recent research trials. Mares are examined by ultrasound 5–7 days after ovulation to determine the number and diameters of the largest ovarian follicles. Mares are allowed to spontaneously develop a cohort of follicles during the “common-growth phase” in response to endogenous FSH. Treatment with exogenous eFSH is initiated when the diameter of the largest follicle reaches 23–25 mm. They are administered progesterogens (i.e., 250 µg of cloprostenol sodium) on the second day of eFSH therapy. An individual mare may be treated for ~3–4 days depending on follicular response. Once the follicles are ~32–35 mm in diameter, treatment with eFSH is discontinued; the mare is then allowed to “coast” for 36 h. hCG (2,500 IU, IV) or deslorelin acetate (1.5 mg IM) is administered to induce ovulation(s).

Ultimately, it is likely that eFSH therapy will have to be tailored to the individual needs of the mare. In general, mares treated with eFSH average 3–4 ovulations and 1.5–2.0 embryos collected per cycle. Unfortunately, some mares treated with eFSH develop multiple large follicles that do not ovulate in response to administration of an ovulation-inducing agent. In addition, eFSH treatment is more effective at inducing multiple ovulations in younger mares than in older mares.

### 5. Clinical Uses of N-butylscopolammonium Bromide in Equine Reproduction

The anticholinergic drug N-butylscopolammonium bromide is approved for use in the control of abdominal pain (colic) associated with spasmodic colic, flatulent colic, and simple impactions in horses. Recent clinical trials have supported anecdotal information that administration of N-butylscopolammonium bromide induces rectal relaxation and, therefore, may reduce the potential risk of rectal tears during medical or reproductive examination.

The manufacturer's recommended dose of N-butylscopolammonium bromide for treatment of spasmodic colic is 0.3 mg/kg body weight administered intravenously. This is equivalent to ~7.5 ml of the 20 mg/ml injectable solution in a 500-kg horse or a total dose of 150 mg. The drug has a rapid onset of activity (<1 min) after IV administration and a duration of action of ~15–25 min.

In our clinical reproduction practice, we initially used the 0.3 mg/kg dose and now routinely use a dose of ~0.04–0.08 mg/kg (i.e., a total dose of 20–40 mg in a 500-kg mare) to facilitate rectal examinations and reproductive procedures. Clinical uses in

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibin</td>
<td>0.1–0.7 ng/ml</td>
</tr>
<tr>
<td>Testosterone</td>
<td>20–45 pg/ml</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
</tr>
<tr>
<td>Estrus</td>
<td>&lt;1 ng/ml</td>
</tr>
<tr>
<td>Diestrous</td>
<td>&gt;1 ng/ml</td>
</tr>
</tbody>
</table>

Table 2. Hormone Concentrations in the Normal Nonpregnant Mare

nomeric (free) inhibin alpha-subunit is inversely correlated with tumor size. It is hypothesized that inhibin produced by the GCT is responsible for the inactivity of the contralateral ovary through the suppression of pituitary FSH release. Serum testosterone levels may be elevated if a significant theca cell component is present in the tumor (i.e., a granulosa-theca cell tumor [GCTT]). Testosterone is elevated in ~50–60% of affected mares and is usually associated with stallion-like behavior.

Progesterone concentrations in mares with a GCT are almost always <1 ng/ml, since normal follicular development, ovulation, and corpus luteum formation do not occur.

Therefore, inhibin levels of >0.7 ng/ml, testosterone levels of >50–100 pg/ml, and progesterone levels of <1 ng/ml are suggestive of a GCT in a non-pregnant mare (Table 2).

GCTs are usually surgically removed if the tumor affects follicular development on the contralateral ovary, causes behavioral abnormalities, or is a source of colic. Surgical approaches for tumor removal include colpotomy, flank and ventral midline laparotomy, paramedian laparotomy, and laparoscopy.

Ovulation from the remaining ovary will occur ~6–8 mo after tumor removal. Attempts at inducing follicular development and ovulation in the remaining ovary within 1 mo of tumor removal by the administration of GnRH have not been successful. Attempts at stimulation of follicular development in the contralateral ovary using purified eFSH have not been reported.

### 4. Superovulation With eFSH

Induction of multiple ovulations has been an elusive goal in the mare. Superovulation would potentially increase the efficiency and decrease the cost of embryo transfer by increasing embryo collection rates. In addition, increasing the number of ovulations may help to increase conception rates of mares bred to subfertile stallions.

Unfortunately, techniques that have been successful in superovulation of ruminants, such as administration of porcine follicle-stimulating hormone (pFSH) and equine chorionic gonadotropin (eCG), have little effect in the mare.

A purified eFSH product is now available commercially. The original or “standard” protocol for use of eFSH involves initiating treatment 5–7 days after ovulation.
equine reproduction include, but are not limited to, procedures involving manipulations per rectum: (1) palpation of the reproductive tract of mares or stallions, (2) management of twins, (3) embryo transfer, (4) collection of oocytes by transvaginal aspiration, and (5) deep uterine inseminations.

6. Managing Uterine Fluid Post-Breeding

A transient inflammatory response in the endometrium is an inevitable consequence of mating by either natural service or by artificial insemination, and it is caused primarily by the exposure of the uterus to spermatozoa. The inflammatory response is characterized by an influx of neutrophils, immunoglobulins, complement, and other factors into the uterine lumen. Neutrophil numbers are highest 8–12 h post-mating, and the majority of reproducibly “normal” mares are capable of eliminating the inflammation within 24–36 h. The intensity of the inflammatory response may be increased in mares inseminated with frozen-thawed spermatozoa. This may be due, in part, to the limited amount of seminal plasma present in frozen semen.

A persistent mating-induced endometritis (PMIE) may develop in some mares. Mares prone to PMIE are usually older (i.e., >12 yr of age), pluriparous, and may have poor perineal conformation. The primary cause of PMIE is impaired or delayed uterine clearance due to a defect in uterine muscular contractility. Inadequate cervical relaxation, poor perineal conformation, impaired uterine lymphatic drainage, and endometrial vascular degeneration may also contribute to the problem.

Management of mating-induced endometritis is aimed at limiting the severity and duration of the inflammatory response and clearing the uterus of fluid, inflammatory by-products, and bacteria.

Treatment strategies may include:

1. Limiting the number of breedings or inseminations (i.e., one mating, if possible).
2. Using artificial insemination (if permitted by breed regulations) to reduce the amount of bacteria and debris introduced into the uterus.
3. Lavaging the uterine lumen post-mating to eliminate dead spermatozoa, inflammatory cells, fluid, and debris. An initial lavage may be performed 4–8 h after mating without adversely affecting pregnancy rates. A total of 1–4 l (or more) of sterile saline or lactated Ringer’s solution may be required. The lavage procedure is repeated until the uterine effluent is clear. Early treatment is aimed at interrupting and/or limiting the inflammatory response. Delaying lavage treatment until after ovulation may allow time for the inflammatory response to reach peak levels.
4. Administering oxytocin (10–20 IU) intravenously, subcutaneously, or intramuscularly beginning 4–8 hrs after mating to stimulate uterine contractions and promote physical clearance of fluid and inflammatory by-products. Oxytocin does not appear to adversely affect the transport and function of gametes within the oviducts and does not adversely affect fertility. Oxytocin causes uterine contractions for 30–50 mins, and therapy may need to be repeated multiple times per day for several days in mares with a history of fluid retention. Prostaglandins (i.e., cloprostenol, 250 µg, IM) may be administered instead of oxytocin to promote uterine contractions if given before ovulation. Uterine contractions are stimulated for 4–5 hrs after prostaglandin administration. Administration of prostaglandins after ovulation may adversely affect formation and function of the corpus luteum.
5. Infusing antibiotics into the uterine lumen may be indicated in conjunction with uterine lavage and oxytocin treatment if a bacterial infection is suspected. Systemic antibiotics are not routinely administered for uterine infections, but they are occasionally recommended for refractory or recurrent infections.
6. Using the Caslick’s procedure for mares with poor perineal conformation or poor muscular tone to the vulva prevents aspiration of air and fecal material.

References and Footnotes


*Bioniche Animal Health USA, Inc., Athens, GA 30601.