Enhancing Reproductive Performance in Mares

Abstract: Reproductive performance in mares can be enhanced by various techniques. Protocols hastening the onset of follicular development help establish pregnancy in mares and ensure that foals are born early in the year. The time spent breeding mares can be reduced by synchronizing estrus and inducing ovulation. After successful fertilization of the oocyte, the developing embryo can survive in the uterus only if postbreeding endometritis, if present, is treated.

Inducing Early Estrous and Ovulatory Cycles

The goal of any breeding operation is to maximize reproductive efficiency and minimize the cost of producing live offspring. The reproductive performance of broodmares can be assessed by determining pregnancy and foaling rates for a season or, preferably, for each cycle. Mean pregnancy rates per cycle are 50% to 60% under current management conditions. However, the rates vary widely depending on stallion and mare fertility. To improve these rates, equine practitioners can use various techniques to induce early estrous and ovulatory cycles to increase the number of breeding cycles during a season. In addition, techniques to synchronize estrus and induce ovulation can be used to make reproductive services time efficient and cost-effective for clients. This article discusses these techniques as well as the treatment of postbreeding endometritis in susceptible mares to ensure that pregnancy is established and maintained to term.

Inducing Early Estrous and Ovulatory Cycles

Mares are seasonal breeders that regularly come into estrus from approximately April through September, and foals are born in late spring and early summer, when food is most readily available and temperatures are moderate. These environmental conditions give foals the best chance to survive and flourish. Most breeding registries have arbitrarily decided that a horse's birthday is January 1. As a result, induction of estrous cycles in anestrous mares and induction of ovulation in mares transitioning from anestrus to estrus are used to allow breeding of mares as early as possible in the year. While the implementation of an extended artificial light period (10 to 100 lux for 14.5 to 16 hours for at least 60 to 90 days) hastens the onset of follicular development by about 2 months, this approach does not eliminate the transition period from anestrus to estrus. During this transition period, one to three waves of follicles grow but fail to ovulate. Regression of these waves of follicles results in irregular, prolonged, and anovulatory estrous cycles.

The transition period can be shortened by administration of hormones such as progesterone, the synthetic progesterin althrenogest, and equine follicle-stimulating hormone (FSH). These treatments also induce ovulation earlier in the year. Instead...
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FREE CE of receiving daily oral or parenteral progestogens (e.g., altrenogest, progesterone), mares can be treated with a single dose of long-acting progestosterone. Mares not responding to increased light exposure for longer than 50 days and having ovarian follicles <15 mm in diameter may be treated with long-acting progesterone (600 mg). This therapy has been shown to induce ovulation within 2 weeks in nearly 60% of treated mares, whereas ovulation occurs in <10% of untreated mares exposed to artificial light. Al though ano vula ti on durin g the trans i -tion period is due to a shortage of luteinizing hormone (LH), it is likely that FSH is also low in these mares. Consequently, mares in transition can be given equine FSH (12.5 mg IM q12h) until at least half of the follicles are >35 mm in diameter. After administration of equine FSH for 3 to 6 days, mares generally develop pre-ovulatory follicles. As a result, ovulation can be induced up to 6 weeks earlier in mares receiving equine FSH than in untreated mares.

Domperidone (1.1 mg/kg PO q24h), a dopamine antagonist, increases secretion of prolactin, a hormone with an important role in the transition from anestrus to estrus. Another dopamine antagonist, sulpiride, has a similar effect on prolactin secretion. It has been proposed that the increase in prolactin secretion induced by domperidone leads to an increase in LH receptor expression on the ovaries. Consequently, the ovaries are more sensitive to endogenous LH, and regular ovarian activity is indirectly induced after domperidone administration. The response to domperidone or sulpiride is improved if either drug is used after a 2-week photoperiod treatment in late transitional mares with follicles >25 mm in diameter. However, it is important to recognize that low ambient temperatures have a negative effect on domperidone-induced follicular activity. Therefore, in locations with very cold winters (e.g., Colorado), only mares kept indoors ovulate in response to domperidone administration.

To summarize, exposure to artificial light can be combined with administration of a progestogen (FIGURE 1) or domperidone (FIGURE 2) to induce estrous cycles and ovulation early in the season.

Although equine FSH can also be used in the transitional period, it is expensive ($300 to $600 for 3 to 6 days of treatment). Therefore, treatment of transitional mares using equine FSH is only justified in combination with embryo collection to take advantage of the resultant multiple ovulations.

**Critical Point**

Exposure to artificial light can be combined with administration of a progestogen or domperidone to induce estrous cycles and ovulation early in the season.

![FIGURE 1](image-url)

Enhancing Reproductive Performance in Mares

![Inducing early estrous and ovulatory cycles (option 1).](image-url)

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**Estrus Synchronization and Induction of Ovulation**

Controlling the time of estrus and ovulation not only helps veterinarians save valuable time but also improves the cost efficiency of reproductive services. It is also very useful for mare owners who lack a stallion or experience with teasing.

The most widely used drugs to control a mare’s cycle are prostaglandins (PGs), most notably PGF₂α. In mares, the corpus luteum is responsive to a single injection of PGF₂α from day 5 to 12 after ovulation. As a result, approximately 33% of all cycling mares respond to administration of PGF₂α. The interval from treatment with PGF₂α to ovulation is typically 9 to 11 days. However, depending on the stage of follicular development on the ovaries, ovulation can occur between 2 and 15 days after treatment. Administration of PGF₂α may appear to fail to induce estrus when a large follicle with a diameter >35 mm is on the ovaries at the time of treatment. These large follicles may develop in mares with two follicular waves in a cycle. A large diestrous follicle may
lead to a sudden onset of estrus, and ovulation can be missed. In these cases, luteolysis and ovulation occur almost in synchrony. PG analogues may also cause LH release sufficient to induce ovulation within 24 to 48 hours, thus even before luteolysis is complete.9

Because many other tissues are sensitive to PGF$_{2\alpha}$, common adverse effects of this treatment include sweating, diarrhea, pelvic muscle spasms, and increased heart rate. To reduce these effects, the dose can be reduced 10-fold to 0.5 mg in two injections given 24 hours apart.10 This approach has proven to be safe and effective in clinical practice.

As an alternative to the use of PGF$_{2\alpha}$, progestogens can be administered to induce artificial luteal phases that control a mare’s estrous cycle. These luteal phases can be achieved with either repeated daily injections of progesterone or oral administration of altrenogest. This approach is most effective if used for 15 days, allowing sufficient time for luteal regression to occur naturally. If progestogens are administered for only 10 days, the mare should receive a PGF$_{2\alpha}$ injection on the last day of treatment to induce lysis of any remaining luteal tissue. Mares treated in this manner usually begin to show estrus in 3 to 5 days, and ovulation usually occurs 9 to 11 days after the end of treatment. However, progestogens not only fail to suppress FSH secretion and follicular growth but also are not particularly effective in preventing ovulation during treatment.11 Consequently, ovulation can occur at any time within approximately 2 weeks after treatment or even during treatment. Therefore, neither progestogens nor PGs alone are very effective for precise synchronization of estrus between mares.

Control of a mare’s estrous cycle can be improved further by coadministration of estradiol and progesterone or other progestogens.12 This protocol can be instituted at any stage of a mare’s estrous cycle if the mare has passed through the transition phase and has regular heat cycles. The treatment is based on the moderate suppressive effect of estradiol on FSH secretion, which controls follicle growth, and of progesterone on estrus. Progesterone and estradiol-17β are injected daily for 10 consecutive days. PG is injected on the 10th day of treatment, and ovulation is induced when a follicle that is ≥35 mm in diameter appears on an ovary. Approximately 75% to 80% of mares ovulate 9 to 10 days after the PG injection (FIGURE 3).

Induction of ovulation in follicles that are ≥35 mm in diameter decreases the number of inseminations or matings per cycle by increasing the number of ovulations taking place within 48 hours. Two products are currently used to hasten ovulation in cycling mares: human chorionic gonadotropin (hCG; 1500 to 2500 IU) and the gonadotropin-releasing hormone (GnRH) analogue deslorelin. hCG binds to equine LH receptors in gonadal tissue, and the LH-like bioactivity of hCG causes maturation and ovulation of the dominant follicle in estrous mares. In contrast, deslorelin stimulates the release of endogenous LH and FSH from the anterior pituitary, and the sudden release of LH stimulates follicle maturation and ovulation. In an analysis of six studies involving more than 1000 ovulatory cycles, injection of hCG or deslorelin induced ovulation in approximately 90% of mares within 48 hours.\(^{a}\) While injection of hCG induced ovulation in 83% of mares within 48 hours, 94% of deslorelin-treated mares ovulated within the same time frame.\(^{13}\) However, in a more recent study, the percentages of hCG- and deslorelin-treated mares ovulating within 48 hours were 88%

\(^{a}\)Burns PJ. Personal communication. Lexington, KY: BET Pharm; 2007.
Neither progestogens nor prostaglandins alone are very effective for precise synchronization of estrus between mares. And 90%, respectively. In comparison, only a small percentage (7%) of saline-treated mares ovulated within 48 hours. Thus, the average untreated mare ovulates 1 to 2 days later than a mare treated with hCG or deslorelin. In a field trial, deslorelin-treated mares had a 13% higher pregnancy-per-cycle rate than hCG-treated mares (62% versus 49%, respectively). When a deslorelin implant was used, pregnancy rates were 64% compared with 55% in mares injected with hCG, but the difference was not statistically significant. Embryo recovery rates increased by 14% when ovulation was induced with injection of deslorelin (69%) compared with treatment with hCG (55%).

The following question often arises when these types of treatment are being considered: Can an agent used to induce ovulation be used repeatedly during a single breeding season? Because hCG is a large glycoprotein, it induces antibody formation after repeated injections. This may account for the reduction noted in mares receiving repeated injections of hCG in some studies; other studies have not reported this effect. Nevertheless, it is recommended that hCG not be used more than twice during a breeding season. In contrast, deslorelin is a small molecule that does not induce antibody formation when administered repeatedly. This is particularly important when donor mares are used for embryo collections and are inseminated in several consecutive heat cycles.

Postbreeding Endometritis
Some degree of inflammatory response occurs in virtually every mare after breeding and is characterized by an influx of polymorphonuclear leukocytes into the lumen of the uterus. This inflammatory response begins within 0.5 to 1 hour after insemination, peaks at 6 to 12 hours, and persists for 24 hours. In most mares, the response should be greatly diminished by 48 hours. In mares susceptible to postbreeding endometritis, a persistent inflammatory response results in accumulation of inflammatory cell-rich fluid within the uterus. Accumulation of this fluid and inflammatory leukocytes may adversely affect fertility by interfering with motility and viability of sperm or by causing failure of embryonic survival if the response persists beyond day 5 after ovulation.

Sperm migration into the oviducts is completed within 4 hours after breeding. Therefore, postbreeding endometritis can be treated by lavaging the uterus with warm saline solution (2 to 3 L) to remove the intraluminal fluid as early as 4 hours after breeding, followed by administration of ecbolic agents to induce uterine contractions and promote expulsion of the uterine contents. Subcutaneous administration of oxytocin (5 IU) increases uterine contractility for 20 to 50 minutes; this treatment can be repeated every 6 hours. Administration of the prostaglandin analogue cloprostenol (250 μg IM) in the evening may be preferred because it produces sustained (2 to 4 hours) uterine contractions. However, cloprostenol has been shown to impair the function of the early corpus luteum and reduce the serum concentration of progesterone for several days. When administered within 48 hours after ovulation, cloprostenol did not affect mare fertility in one study, but the pregnancy rate was decreased in another study involving the drug. If an infection is diagnosed based on positive cytology and culture results, antimicrobials can be administered into the lumen of the uterus after uterine contractility has diminished (i.e., 1 hour after oxytocin administration or at least 4 hours after cloprostenol administration). The first choice for intruterine therapy, especially until culture and sensitivity results are available, is the combination of penicillin G potassium (20 million U dissolved in 500 mL of lactated Ringer’s solution) and gentamicin.

Critical Point
Neither progestogens nor prostaglandins alone are very effective for precise synchronization of estrus between mares.
sulfate (2 g added to the solution immediately before infusion).

A novel approach to treating mares with postbreeding endometritis is based on the immunostimulatory effects of *Propionibacterium acnes* and a cell-wall extract of *Mycobacterium phlei*. These immunostimulants induce a non-specific, cell-mediated response by activating macrophages and releasing cytokines, which increase general immune system activity. It has been suggested that treatment with *P. acnes* should be initiated the day before breeding (day 0; when ovulation is induced; 1 mL/250 lb IV), followed by additional treatments at 2 days (when ovulation is detected) and 6 days after the start of the protocol.20 Similarly, treatment with *M. phlei* cell-wall extract is best initiated 1 day before breeding; this immunostimulant can be administered intravenously (1.5 mL per mare) or infused into the uterus (1.5 mL diluted in 25 to 50 mL of saline). *P. acnes* and *M. phlei* cell-wall extract can increase pregnancy rates in mares susceptible to postbreeding endometritis and can be combined with traditional treatment options as described above.20,24

**Maintaining Pregnancy**

Despite the lack of data supporting its efficacy in individual cases, progesterone is widely used in practice to maintain pregnancies in mares. This therapy is used in mares with a history of repeated pregnancy failure when no specific cause for pregnancy loss can be identified. It is also often used after insemination with frozen semen or after embryo transfer. The synthetic progesterone altrenogest is most commonly administered daily by mouth. Alternatively, a long-acting injectable formulation of progesterone can yield a blood level exceeding 2 ng/mL for 10 days. In one study,25 administration of a long-acting prostaglandin analogue (cloprostenol) every 7 days to eliminate endogenous luteal function was effective in maintaining pregnancy between days 18 and 45 in mares. Recently, a long-acting injectable formulation of altrenogest became available; this medication is administered every 10 days.

On day 12 of pregnancy, circulating progesterone concentrations are lower in nonpregnant than in pregnant mares; a progesterone serum concentration of 2.5 ng/mL was used to distinguish normal from insufficient luteal function.26 However, in another study,27 pregnancies were maintained in ovariectomized mares if progesterone serum concentrations exceeded 4.0 ng/mL, suggesting that this might be the “critical” concentration needed in early pregnancy. Thus, administration of exogenous progesterone is justified in mares with evidence of insufficient luteal function (i.e., a progesterone serum concentration <4 ng/mL) before the endometrial cups are established (days 38 to 40 of pregnancy). After the endometrial cups have formed and have stimulated supplemental corpora lutea, endogenous production of progesterone should be sufficient to maintain pregnancy.28 However, it is important to remember that luteal production of progesterone ceases in approximately 10% of altrenogest-treated mares. In these mares, progesterone supplementation must continue until the placenta takes over progestogen production around day 80 of gestation. Therefore, before a clinician decides to curtail progestin supplementation after day 40 and before day 80 of pregnancy, the endogenous progesterone concentration should be measured.29

Progesterone therapy is also justified in mares that have a colic episode. During the first 40 days of gestation, endotoxemia associated with colic may induce luteolysis through the release of PGs.28 If the mare has a colic episode later in gestation, short-term progesterone administration may help maintain a quiescent uterus, as prolonged exposure to PGs during endotoxemia may induce myometrial contractions and abortion.29

There are some adverse effects of progestin supplementation. For example, progesterone impairs phagocytosis of bacteria by uterine neutrophils. Because the mare’s cervix closes under the influence of progesterone, clearance of debris from the uterus is impaired, and persistent endometritis may occur in mares with a residual uterine infection that are treated with progestins in the early luteal phase of the cycle.29

GnRH not only is important for the induction of ovulation but also regulates progesterone synthesis in luteal cells by inducing LH secretion from the anterior pituitary.20 It has been suggested that treatment with the GnRH agonist buserelin may decrease early embryonic losses in mares. In a large field study involving more than 2000 mares, pregnancy rates increased by 10% with administration of 20 or 40 μg buserelin between days 8 and 12 after ovulation and repro-

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**Critical Point**

As soon as sperm migration into the oviducts is complete (i.e., 4 hours after breeding), post-breeding endometritis can be treated by lavaging the uterus with warm saline solution (2 to 3 L) to remove the intraluminal fluid, followed by administration of ecbolic agents to induce uterine contractions and promote expulsion of the uterine contents.
Critical Point

Administration of exogenous progesterone is justified in mares with evidence of insufficient luteal function (i.e., a progesterone serum concentration <4 ng/mL) before the endometrial cups are established (days 38 to 40 of pregnancy).

References

Conclusion

Manipulation of the estrous cycle and precise timing of breeding are commonly used to improve reproductive efficiencies in cattle. Although the mare’s reproductive cycle is more complex and, therefore, harder to predict, equine practitioners can use several techniques to optimize reproductive performance during the relatively short equine breeding season. For induction of early cyclicity in mares, exposure to artificial light can be combined with progesterone or domperidone therapy; a combination of progesterone and estradiol can be used to control the mare’s estrous cycle. Deslorelin and hCG can be used to induce ovulation within 48 hours when a dominant follicle ≥35 mm in diameter and uterine edema are present; immunostimulants are a novel approach to treating postbreeding endometritis in mares susceptible to this problem. After successful establishment of pregnancy, short-term use of exogenous progesterin is justified during stressful events. However, long-term progestogen administration should be based only on the analysis of endogenous progesterone. In most cases, supplementation with progestogens is not necessary after day 40 and is certainly questionable after day 80 of gestation, unless it is indicated for a late-term mare with placentitis or undergoing significant stress, such as colic surgery.
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and progesterone concentrations in mares treated with a GnRH ag-

1. The transition period in mares can be
 shortened by treatment with
 a. artificial light.
 b. progestogens.
 c. deslorelin.
 d. LH.

2. The effect of domperidone on the induc-
tion of cyclicity in mares is mediated
 through
 a. increased secretion of prolactin.
 b. a decrease in ovarian LH receptors.
 c. higher sensitivity of the ovaries to FSH.
 d. equine chorionic gonadotropin.

3. In mares, the corpus luteum is mature
 and responsive to PGF\(_2\alpha\) at _____ days
 after ovulation.
 a. 7 to 17
 b. 2 to 18
 c. 5 to 12
 d. all of the above

4. After estrus synchronization induced by
 progestogens or PGF\(_2\alpha\), ovulation can
 occur after _______ in mares.
 a. 2 day
 b. 7 days
 c. 13 days
 d. all of the above

5. Estrus in cycling mares is synchronized
 best if treatment begins with
 a. progestogen for 15 days.
 b. equine FSH for 5 days.
 c. progesterone and estradiol for 10 days.
 d. domperidone.

6. Ovulation can be induced in mares
 within 48 hours by injection of
 a. equine chorionic gonadotropin.
 b. the GnRH analogue deslorelin.
 c. dinoprost.
 d. a combination of FSH and LH.

7. In mares susceptible to postbreeding
 endometritis, the uterus can be lavaged
 with lactated Ringer’s solution _____
 after insemination.
 a. immediately
 b. 2 hours
 c. 4 hours
 d. 8 hours

8. Antimicrobials can be administered
 into the uterus _____ after the use of
 oxytocin to evacuate uterine intralumi-
nal fluid in a mare with postbreeding
 endometritis.
 a. 1 hour
 b. 4 hours
 c. 6 hours
 d. 12 hours

9. To maintain pregnancy in the beginning of
gestation before endometrial cup forma-
tion, the endogenous progesterone con-
centration should be at least _____ ng/mL.
 a. 10
 b. 5
 c. 4
 d. 1

10. Adverse effects of progestin supplemen-
tation include
 a. impairment of phagocytosis of bacteria
    by uterine neutrophils.
 b. impairment of uterine clearance.
 c. exacerbation of a residual uterine
    infection.
 d. all of the above