Hormones and Breeding

Carlos R.F. Pinto, MedVet, PhD, Diplomate ACT

Author’s address: Theriogenology and Reproductive Medicine, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210; e-mail: crfpinto@gmail.com. © 2013 AAEP.

1. Introduction
The administration of hormones to mares during breeding management is an essential tool for equine practitioners. Proper and timely administration of specific hormones to broodmares may be targeted to prevent reproductive disorders, to serve as an aid to treating reproductive disorders or hormonal imbalances, and to optimize reproductive efficiency, for example, through induction of estrus or ovulation. These hormones, when administered exogenously, act to control the duration and onset of the different stages of the estrous cycle, specifically by affecting duration of luteal function, hastening ovulation especially for timed artificial insemination and stimulating myometrial activity in mares susceptible to or showing delayed uterine clearance. In this discussion, we address the effects and potential indications for the different hormones available to the equine practitioner working with broodmare reproduction.

2. Prostaglandin F₂α
The administration of natural or synthetic prostaglandin F₂α (PGF) analogues causes interruption of luteal function by luteolysis. The diestrus stage of the estrous cycle is then shortened leading to onset of estrus. Characteristics of estrus and ovulation are not different from that occurring in natural cycles. The inherent fertility of mares is not affected by PGF treatment to induce estrus. In other words, once luteolysis takes place, whether induced by PGF treatment or occurring naturally, the events that follow (estrus behavior, ovulation and fertility) are essentially similar or minimally affected (eg, decreased signs of behavioral estrus). Duration of diestrus and interovulatory intervals are shortened after PGF administration. The equine corpus luteum (CL) is responsive to PGF luteolytic effects any day after ovulation; however, only CL >5 days are responsive to one bolus injection of PGF. Luteolysis or antiluteogenesis can be reliably achieved in CL <5 days only if multiple PGF treatments are administered. For that reason, it became a widespread practice to administer PGF as a single bolus injection (subcutaneous or intramuscular) only after 5 to 6 days after ovulation. This common practice perhaps contributed to the prevailing assumption that PGF treatments have only significant effects in mares with CL >5 days.

Mares with a CL >5 days undergo luteolysis when treated with one single injection (subcutaneous or intramuscular) of 5 to 10 mg of dinoprostone tromethamine or 250 to 500 μg of cloprostenol (synthetic PGF analogue). In the United States, dinoprostone tromethamine is the only PGF drug approved by the Food and Drug Administration (FDA) for use in horses, although the use of the racemic formulation...
of d,l-cloprostenol is very common among practitioners working with horse breeding, especially because of its longer half-life than dinoprostone salt preparations—hours versus minutes, respectively. Conversely, in other countries (Europe, South America, etc), formulations of the synthetic analogue cloprostenol are approved and available for use in horses. In those countries, the racemic d,l-cloprostenol and the more potent formulation containing only the d-enantiomer cloprostenol are available; only 25 to 37.5 µg per mare of d-cloprostenol is needed to induce luteolysis in mares at least 5 days after ovulation.4

3. Induction of Ovulation
The ability to induce ovulation is one of the most important strategies used in breeding management. The goal of inducing ovulation in breeding management of mares is to induce ovulation within 48 hours, preferably between 24 to 48 hours after treatment. In mares monitored closely by palpation per rectum and ultrasonography, ovulation occurs within 36 to 48 hours. This fact is particularly important for mares being managed for artificial insemination with frozen-thawed semen. For optimal chances to predict outcome and increase efficacy of the treatment, regardless of the ovulation-inducing agent being used, induction of ovulation should be attempted only in mares found unquestionably to be in estrus and with a growing follicle at least ≥30 mm in diameter. The most common hormones used to induce ovulation include human chorionic gonadotropin (hCG) and the gonadotropin-releasing hormone agonists (GnRH), which are discussed below.

4. Human Chorionic Gonadotropin
Human chorionic gonadotropin has been widely and extensively used as an ovulation-inducing agent because of its luteinizing hormone (LH)-like activity. The hormone, hCG, is produced after being extracted from urine of pregnant women. Chemically, hCG is a glycoprotein composed of an α- and a β-subunit; the α-subunit is similar to that of human follicle-stimulating hormone (FSH) and LH; however, the β-subunit, despite containing the same amino acids present in the LH β-subunit, does have an additional group of 23 amino acids and a high degree of sialylation that confers hCG a longer half-life (several hours) than that of LH (20 to 30 minutes). The hCG hormone is then lyophilized and available in the United States in vials containing 10,000 international units (IU). Despite reports of mares being treated with hCG since 1939, hCG is not approved by the FDA for use in horses. Nevertheless, a single bolus dose of 1500 to 3300 IU is typically sufficient to induced ovulation in mares during estrus, with distinct estrous uterine edema and with a viable, growing follicle >30 mm. Mares with more than one presumptive preovulatory follicle do not need to receive a double dose or a second injection during that estrus. It is the author's clinical experience that mares that repeatedly ovulate two or three follicles respond to a single hCG treatment similarly to mares ovulating only one follicle during estrus. Because hCG is available in 10,000-IU vials, it is common to have equine practitioners administering bolus doses of 2000 or 2500 IU to optimize the use of each vial. Although the author has successfully used vials that have been reconstituted for several weeks, it is recommended that reconstituted vials be used within 2 weeks and kept refrigerated during that period. Intramuscular or intravenous routes can be used to administer hCG in mares, but it seems, anecdotally, that fewer ovulation failures are seen in mares receiving hCG intravenously than in mares receiving it intramuscularly. For example, intramuscular doses of 5000 to 10,000 IU should be avoided. Mares do have development of antibodies to the human hormone, but the presence of antibodies does not appear to interfere with ovulation. Nevertheless, some authors do recommend not treating mares with hCG more than once or twice during the same breeding season, whereas others (including this author) have used it successfully (ovulation within 48 hours from administration) in mares treated five to 10 times during the same breeding season.

5. Gonadotropin-Releasing Hormones
Administration of simple gonadotropin-releasing hormone (GnRH) analogues (equivalent to the natural decapeptide GnRH) to cycling mares has failed to consistently induce ovulation. Whereas doses of 50 to 100 µg of GnRH are sufficient to induce ovulation in cattle, doses as high as 1 to 4 mg have failed to reliably induce ovulation in mares when administered once or twice daily during estrus. Furthermore, only pulsatile administration (every hour) of GnRH has been found to be effective in inducing ovulation in the mare; this method is, however, not feasible for widespread clinical application. Since the chemical structure and peptide identification was reported in 1971, more than 2000 GnRH analogues were synthesized in the following 15 years after the 1971 report. Scientists working in research and private pharmaceutical institutions shared two main aims: (1) to create an analogue with high affinity for GnRH receptor binding and (2) to avoid or minimize enzymatic degradation by proteolysis. The GnRH molecule is susceptible to cleavage and inactivation by hypophyseal endopeptidases, especially between amino acids in position 5 and 6 and between 6 and 7. Another enzyme, carboxypeptidase, targets the bond between amino acids in position 9 and 10. For these reasons, several GnRH synthetic analogues used in human and veterinary medicine have (1) D-amino acid substitutions in position 6 (glycine) and (2) the amino acid in position 10 (another glycine) and its amide terminal replaced by an ethylamide residue that is linked to the amino acid 9 proline.  }
Deslorelin

In contrast with the poor or limited efficacy of simple GnRH analogues, more potent GnRH synthetic analogues such as deslorelin have a predictable effect in inducing ovulation within 48 hours when administered as intramuscular injections or by means of subcutaneous implants. Deslorelin differs from the natural decapetide GnRH in two amino acid substitutions: in amino acid position 6, where L-glycine has been replaced with the amino acid D-tryptophan, and in position 10, where glycine and its amino terminal have been replaced by N-ethylamide.

Ovulation rates after deslorelin administration are comparable to those resulting from hCG administration.\(^9\) Commercially, deslorelin became available as implants containing 2.1 mg of deslorelin acetate.\(^3\) After being first available in Australia and other countries, a deslorelin implant\(^8\) became FDA-approved and available in the United States in 1998. After being on the market for approximately two breeding seasons, it was anecdotaly reported that occasionally, mares treated with the implant had prolonged interovulatory intervals, and some actually had their reproductive cyclicity downregulated for months. When exploring this issue, McCue et al\(^10\) reported normal interovulatory intervals in mares that had their implant removed once ovulation occurred. This phenomenon was later elucidated by the fact that prolonged delivery of deslorelin would induce ovulation but its continuous release would downregulate FSH and LH secretion from the pituitary, resulting in prolonged interovulatory intervals. Removing the implant after ovulation thus prevented sustained release of deslorelin. Reportedly because of problems in manufacturing logistics, this deslorelin implant\(^8\) has not been commercially available in the United States since the early 2000s. Interestingly, side effects related to sustained deslorelin release have not been reported in Australia, where the drug originated and still remains available there and in other countries.

For several years, deslorelin was available either through importation from countries that manufactured the deslorelin implant\(^8\) or through compounding of injectable formulations of deslorelin (1.5 mg/mL; dose 1 mL per mare administered intramuscularly). Recently, an injectable controlled release form of deslorelin acetate\(^6\) (1.8 mg/mL) was approved by the FDA in 2010 for induction of ovulation in mares and became commercially available in the United States in 2011. It contains 1.8 mg of deslorelin acetate equivalent to 1.7 mg of deslorelin in a sucrose acetate isobutyrate and propylene carbonate matrix that promotes controlled release of the drug. This formulation has been shown to induce ovulation at comparable rates to those from hCG or deslorelin implant administrations. In the only published peer-reviewed report on the efficacy of this FDA-approved deslorelin injectable formulation, 151 of 168 (~90%) mares treated in estrus with 1.8 mg of deslorelin acetate (1 mL IM) ovulated on average 41 hours after treatment, whereas 111 of 134 mares (~83%) treated with hCG (2500 IU IV) ovulated on average 44 hours after treatment.\(^11\)

Histrelin

Once an injectable form of deslorelin became FDA-approved and commercially available, some compounding pharmacies began to accept requests to compound another synthetic GnRH analogue, histrelin (or historelin). Histrelin is an even more potent GnRH analogue than deslorelin (Table 1), but with similar clinical efficacy in inducing ovulation in horses as deslorelin or hCG. Histrelin differs from the natural decapetide GnRH in two amino acid substitutions. In amino acid position 6, where L-glycine has been replaced with the amino acid D-histidine, and in position 10, where glycine and its amino terminal have been replaced by N-ethylamide.

Recently, a sustained-release (biorelease) formulation of histrelin was found to effectively induce ovulation within 2 days after administration.\(^12\) The efficacy of histrelin treatment (0.5 or 1.0 mg bolus injections) in inducing ovulation within 48 hours after administration has been found to be no different from that promoted by hCG or deslorelin. In a more recent study,\(^13\) a sustained-release (biorelease) formulation of histrelin was also found to effectively induce ovulation within 2 days after administration. There were no differences among maiden, barren, and foaling mares treated throughout the ovulatory seasons; similarly, there were no significant differences for the two doses used in that study, 0.25 and 0.5 mg, administered as single intramuscular bolus injections to mares in estrus. The overall ovulation rate at 48 hours was 93% (43/46) and 85% (44/52) for doses of 0.5 mg and 0.25 mg of histrelin, respectively. In summary, there does not appear to be differences in the efficacy, relative to ability to induce ovulation, among deslorelin, histrelin, and hCG.

Buserelin

Buserelin is another GnRH analogue that also contains modification in the amino acid position 6 by having the natural occurring L-glycine replaced by a tertiary butylated D-serine and the amino acid 10 glycine and its amino terminal replaced by N-ethylamide, which is similar to the amino terminal modification present in deslorelin and histrelin.

Table 1. Comparative Rank Order Potency of Synthetic GnRH Analogue in Relation to Natural GnRH

<table>
<thead>
<tr>
<th>GnRH</th>
<th>Buserelin</th>
<th>Deslorelin</th>
<th>Histrelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative potency</td>
<td>1</td>
<td>20</td>
<td>144</td>
</tr>
</tbody>
</table>

IN-DEPTH: REPRODUCTIVE ENDOCRINOLOGY
The moderate potency of buserelin (Table 1) perhaps may explain why a single administration of a labeled dose of buserelin (40 to 100 μg per mare) is not sufficient to induce ovulation in mares. For many years, buserelin (not available in the United States, except through compounding pharmacies) has had limited use and efficacy in inducing ovulation only when used in daily or twice-daily injections for 2 to 4 days. This protocol probably has prevented veterinarians and horse owners from favoring its use in broad mare management. In contrast with this knowledge, Levy and Duchamp\textsuperscript{14} (2007) recently showed that much higher doses than those originally used resulted in ovulation rates comparable to that of hCG. In that study, 6 mg of buserelin was injected subcutaneously to cyclic mares, resulting in 89% to 95% ovulation rates that did not differ from hCG administration (86%). Considering the relative potencies of deslorelin, histrelin, and buserelin (Table 1), we calculate that buserelin is at least 7 and 10 times less potent than deslorelin and histrelin, respectively. This may partially explain why in this study a buserelin dose ~60 times greater (6000 μg versus 100 μg) than that recommended in veterinary commercial preparations of injectable buserelin\textsuperscript{*} was able to effectively promote ovulation in treated mares at a similar rate reported for hCG, deslorelin, and histrelin.

Veterinarians should use the FDA approved products for this indication. Certain compounded products can vary significantly in potency and stability. Consequently results may vary substantially. There can also be legal and ethical issues with using a compounded product when there are FDA approved products available in the appropriate dosage form and with the appropriate indication.

6. Ecobic Hormones for Treatment of Delayed Uterine Clearance

In general, the use of ecbolic hormones to correct deficiencies or weakness in myometrial contractions commonly seen in mares with delayed uterine clearance and susceptible to endometritis are safely initiated at 4 to 8 hours after artificial or natural insemination to avoid interferences with sperm transport that could affect fertility. These treatments are then continued at the discretion of the prescribing veterinarian; ecbolics are often administered twice daily or more often if necessary and depending on the recorded response for an individual mare. Commonly used ecbolics in broodmares include oxytocin and PGF.

Oxytocin

Oxytocin is a nonapeptide synthetized in the magnocellular neurons in the hypothalamus that extend their axons into the neurohypophysis, where it is stored until released into the blood stream for biological action. Oxytocin has several known physiologic effects, such as milk ejection, promoting maternal behavior, and inducing myometrial contractions. Because delayed uterine clearance is commonly seen in mares with persistent mating-induced endometritis, oxytocin is one important therapeutic option for treating abnormal intrauterine accumulation of fluid. Despite oxytocin’s relatively short-life of ~7 minutes, it appears that its biological effect far exceeds this time. For that reason, treatment protocols varying from two to four daily treatments have been shown to be relatively efficacious in promoting uterine clearance. Boluses of doses of 10 to 20 IU administered subcutaneously, intramuscularly, or intravenously are frequently used to treat mares with mating-induced intrauterine fluid accumulation. Oxytocin treatments are administered during estrus before or after ovulation, with treatments scheduled to not coincide with breeding to avoid interfering with sperm transport, especially if given shortly after artificial insemination, for example, <4 hours. Recently, our laboratory reported on the pharmacokinetics of carbocetin, a long-acting oxytocin analogue.\textsuperscript{15} Carbocetin has a half-life of ~17 minutes. Carbocetin is available and approved for use in horses in several countries but not in the United States. Despite its longer half-life than oxytocin, multiple daily treatments may still be required for optimal effects in promoting uterine clearance as is required with oxytocin or PGF. A direct comparison between oxytocin’s and carbocetin’s ability to promote uterine clearance has not been reported for in vivo treatment in mares. Currently, it not known whether the reported prolonged half-life of carbocetin would translate into a greater and/or more productive biologic response than oxytocin in mares that may require prolonged myometrial stimulation. Steckler et al\textsuperscript{16} have recently reported on the results of a comparison of oxytocin versus carbocetin in eliciting contractions in ex vivo uterine tissues collected from mares at different reproductive stages (eg, anestrus, estrus, and diestrus). The results indicated that the effect of oxytocin on equine myometrial tissue was greater during anestrus and diestrus than during estrus, whereas carbocetin appeared to elicit myometrial contractions independent of the reproductive status; however, there were no differences between their ability to induce myometrial contractions in the ex vivo strips of myometrial tissues.

Prostaglandin F\textsubscript{2α}.

In addition to being known for its luteolytic actions, PGF is also an effective promoter of myometrial contractions. Because of its longer half-life than oxytocin, especially the synthetic PGF analogue cloprostenol (~2–3 hours), PGF has been favored by equine practitioners, especially for use in mares that anecdotally appear to be refractory or do not respond appropriately to oxytocin treatment. Cloprostenol does elicit less vigorous myometrial contractions than oxytocin but for prolonged periods. For example, one study reported that oxytocin induced strong myometrial contractions that lasted ~30 minutes,
whereas cloprostenol induced low-amplitude myometrial contractions that lasted 4 to 5 hours. This sustained effect is particularly desirable to enhance lymph flow and consistent intraluminal evacuation of accumulated fluid (inflammatory transudate). Despite its indisputable efficacy as an ecbolic hormone, the use of PGF during estrus, especially in the early post-ovulatory period, was found to transiently affect luteal function. Some studies confirmed this effect on luteal function that was followed by a resurgence in CL function. It is unknown whether this early effect in suppressing luteal function would affect the fertility of treated mares. For this reason, if PGF is to be used as an ecbolic to treat delayed clearance during estrus and early postovulatory period, it is important that equine practitioners remain cognizant of its effects on luteal function. Most mares so treated should have resurgence in luteal function and not have deleterious effects on their luteal function and subsequent fertilities. Moreover, our laboratory has recently reported that the effect of PGF administration on early luteal function can induce luteolysis or antiluteogenesis, depending on the duration of treatment after ovulation and on dosage used. For these reasons, treatments with PGF during estrus should not continue beyond 24 hours after ovulation. Whenever indicated, low doses of PGF (eg, 62.5–125 μg cloprostenol or 1.25 mg dinoprost), which are capable of inducing uterine contractions, are recommended for treating mares with delayed uterine clearance.

7. Modulation of Mating-Induced Endometritis With Steroids

Endometritis after artificial insemination or mating is one important cause of infertility especially in mares susceptible to persistent mating-induced endometritis. In addition to therapies aimed at combating endometritis by use of intraterine or systemic antibiotic treatment and stimulation of myometrial function through administration of ecbolic hormones (mainly oxytocin and PGF), another strategy is to control or modulate the exacerbated inflammation response seen in these mares. Dell’Aqua et al first reported on the efficacy of prednisolone treatment of mares during estrus that benefited from the glucocorticoid modulatory effects on barren mares afflicted with endometritis. A significantly higher pregnancy rate was seen in barren mares treated 5 times with 0.1 mg/kg of prednisolone acetate (~50 mg per mare) administered every 12 hours with four treatments occurring before artificial insemination (AI) and one at AI. In 2008, Morris reported promising results used the same protocol described by the Dell’Aqua, except she used a dose of 200 mg of oral prednisolone per mare. More recently, Bucca et al reported on the use dexamethasone given as a single bolus administration of 50 mg per mare at time of breeding. In that study, the authors concluded that dexamethasone administered at the time of artificial insemination was safe and effective for modulating persistent mating-induced endometritis in susceptible mares; the glucocorticoid treatment decreased endometrial edema, accumulation of intraluminal uterine fluid and its turbidity, without altering the amount of polymorphonuclear cells seen in preparations obtained from endometrial cytology. Ferris and McCue studied the effects of multiple (twice daily for 5 days) glucocorticoid treatments (prednisolone or dexamethasone in mares during early estrus. They reported that mares treated with dexamethasone showed reduced uterine edema and ovulation rate (40% versus 83%, respectively) than mares treated with prednisolone. In addition, only two of five dexamethasone-treated mares had unaltered LH hormonal profiles, whereas five of six mares treated with prednisolone had LH surges within normal limits. The differences noted between dexamethasone and prednisolone may derive from their differences in relative potencies in relation to cortisol (dexamethasone = 30; prednisolone = 4). Nevertheless, it is unlikely that a single treatment with dexamethasone as prescribed by Bucca and Carli would result in ovulation failure as only mares with multiple, daily treatments with dexamethasone have been shown to have altered endogenous LH release that resulted in significant ovulation failures. It is important, however, to ensure that mares being treated with glucocorticoids during estrus are also treated with ovulation-inducing agents to prevent any potential negative effects of glucocorticoid agents in the hypothalamic-hypophyseal axis that may result in anovulation. Bucca et al provided supporting evidence to the efficacy of associating induction of ovulation with glucocorticoid treatment in mares susceptible to persistent post-mating endometritis. Mares treated with a single bolus intravenous injection of 50 mg of dexamethasone within 1 hour of breeding and concurrent induction of ovulation with 1500 IU of hCG exhibited normal ovulation rates (~97% of mares ovulated within 48 hours). The minimal dose of prednisolone and dexamethasone to combat inflammation in mares during estrus has yet to be determined.

8. Conclusions

Several pharmacologic agents are now available for use in the breeding management of broodmares. Strategic utilization of hormones to induce estrus and ovulation and to modulate/prevent inflammatory processes in mares susceptible to mating-induced endometritis and delayed uterine clearance can significantly affect the outcome of breeding by increasing the odds of timed ovulation and pregnancy.

References and Footnotes


aLutalyse, Pfizer Animal Health, Kalamazoo, MI 49001.
bEstrumate, Merck Animal Health, Summit, NJ 07901.
cGenestrain, FORTE Healthcare Limited, Naul, Dublin, Republic of Ireland.
dChorulon, Merck Animal Health, Millsboro, DE 19966.
eSucromate is a product of and manufactured by Thorn Bioscience, Louisville, KY, a division of CreoSalus; it is marketed by Bioniche Animal Health USA, Inc, Athens, GA 30601.

AAEP PROCEEDINGS