Use of hormones in equine breeding management

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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: 1) to prevent reproductive disorders, 2) to aid in treating reproductive disorders or hormonal imbalances, and 3) to optimize reproductive efficiency, for example, induction of estrus or ovulation. The most common applications of exogenous hormone administration include controlling the duration and onset of the different stages of the estrous cycle (specifically by affecting duration of luteal function or hastening ovulation) and stimulating myometrial activity in mares susceptible to or showing delayed uterine clearance. In this discussion, we will address the effects and potential indications for the different hormones available to the equine practitioner working with broodmare reproduction.

Keywords: $PGF_{2\alpha}$, hCG, GnRH, mare, corticosteroids

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

The administration of natural or synthetic $PGF_{2\alpha}$ analogues causes interruption of luteal function by inducing luteolysis. The diestrus stage of the estrous cycle is then shortened, hastening onset of estrus. Characteristics of estrus and ovulation (e.g., estrus behavior, follicle growth rate, ovulation, fertility) are not different from those occurring in natural cycles.[1-3] However, there is some evidence of increased incidence of hemorrhagic anovulatory follicles (HAF) following exogenous administration of the synthetic $PGF_{2\alpha}$ analogue cloprostenol.[4-6] The potential association between $PGF_{2\alpha}$ administration and the incidence of HAF has not been reported in mares treated with the natural analogue dinoprost tromethamine. Duration of diestrus and interovulatory intervals are shortened following $PGF_{2\alpha}$ administration.[7-18] The equine corpus luteum (CL) is responsive to $PGF_{2\alpha}$ luteolytic effects any day following ovulation; however, only CL >5 days are consistently responsive to the luteolytic effects of one bolus injection of $PGF_{2\alpha}$.[15, 19] Luteolysis or antiluteogenesis can be reliably achieved in CL <5 days only if multiple PGF treatments are administered.[14,15,20] For that reason, it is widespread practice to administer $PGF_{2\alpha}$ as a single bolus injection (sc or im) at least five to six days following ovulation. This common practice perhaps contributed to the prevailing assumption that $PGF_{2\alpha}$ treatments only have significant effects in mares with CL >5 days old.

Mares with a CL >5 days old undergo luteolysis when treated with a single injection (sc or im) of 5 to 10 mg of dinoprost tromethamine or 250 to 500 ug of cloprostenol (synthetic $PGF_{2\alpha}$ analogue). In the USA, dinoprost tromethamine* is the only $PGF_{2\alpha}$ 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 fairly common among practitioners working with horse breeding, especially owing to its longer half-life than dinoprost tromethamine salt preparations, hours vs. minutes, respectively.[21] Conversely, in other countries in Europe, and South America for example, 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 ug per mare of d-cloprostenol is needed to induce luteolysis in mares at least five days after ovulation.[18]

Induction of ovulation

The ability to induce ovulation is one of the most important strategies utilized in breeding management. Ovulation-inducing agents typically trigger ovulation within 48 hours. In mares monitored closely via palpation per rectum and ultrasonography, ovulation occurs mainly within 36 to 48 hours after systemic administration of these agents.[22,23] This fact is particularly important for mares being managed

for artificial insemination with shipped cooled semen or 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 ≥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 will be discussed below.

Human chorionic gonadotropin

Human chorionic gonadotropin[§] has been widely and extensively used as an ovulation-inducing agent since the 1930's and 1940's.[24-27] The luteinizing hormone (LH)-like activity of hCG is explained by its 85% homology in amino acid sequence in the β-subunit and by its ability to bind to the LH receptor.[28] 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 hCG β-subunit, despite its homology with the LH β-subunit, does have an additional carboxyl-terminal group with 31 amino acids and a high content of sialic acid that provides a substrate for glycosylation. Glycosylation is the post-translational modification of the hCG molecule that confers it a longer half-life (several hours) than that of LH (20 to 30 minutes). For many years, the hormone hCG was only produced after being extracted from urine of pregnant women, lyophilized and marketed in the USA in vials containing 10,000 international units (IU) available for clinical use in human and veterinary medicine. Since the early 2000's a recombinant form of hCG produced from genetically modified Chinese hamster ovary cells is also available commercially in the USA for treatment of women undergoing fertility treatments. There are no reports of use of the recombinant hCG in the horse. Despite reports of mares being treated with hCG since 1939,[26] hCG is not approved by the FDA for use in horses. Nevertheless, a single bolus dose of 750 to 3300 IU of urinary hCG is typically sufficient to induce ovulation in mares during estrus, with distinct estrous uterine edema and with a viable, growing follicle >30 mm.[29,30] Mares with more than one presumptive preovulatory follicle do not need a double dose or a second injection during that estrus. In our experience, 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 for equine practitioners to administer bolus doses of 2,000 or 2,500 IU to optimize the use of each vial. Although the authors have successfully used vials that have been reconstituted for several weeks, it is recommended that reconstituted vials be used within two weeks and kept refrigerated during that period. Alternatively, unused reconstituted hCG can be frozen in separate doses for future use as repeated freezing-thawing reconstituted vials is not recommended. 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 5,000 to 10,000 IU should be avoided. Mares do develop antibodies to the human hormone but the presence of antibodies does not seem to interfere with ovulation.[31] Nevertheless some authors do recommend not treating mares with hCG more than once or twice during the same breeding season[33] whereas others (including the current authors) have used it successfully (ovulation within 48 hours from administration) in mares treated four to seven times during the same breeding season.

Gonadotropin-releasing hormones

Administrations of simple (relative potency = 1) GnRH analogues (equivalent to the natural decapeptide GnRH) to cycling mares have failed to consistently induce ovulation. Whereas doses of 50 to 100 ug of GnRH are sufficient to induce ovulation in cattle, doses as high as 1 to 4 mg of GnRH 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;[33] this method is, however, not feasible for widespread clinical application. Since the chemical structure and peptide identification was reported in 1971,[34-36] more than 2,000 GnRH analogues were synthesized in the following 15 years.[37] 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 positions 9 and 10. For these reasons, several GnRH synthetic analogues used in human and veterinary medicine have 1) D-amino acids 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 aminoacid 9 proline.[38]

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[23,39] or subcutaneous implants.[40-42] Deslorelin differs from the natural decapeptide GnRH in two amino acid substitutions: in amino acid position 6 where L-glycine has been replaced with the aminoacid D-tryptophan and in position 10 where glycine and its amino terminal have been replaced by N-ethylamide.

Ovulation rates following deslorelin administration are comparable with those resulting from hCG administration.[41] Commercially, deslorelin became available as implants containing 2.1 mg of deslorelin acetate.¶ After being first available in Australia and other countries, Ovuplant® became FDA-approved and available in the USA in 1998. After being on the market for approximately two breeding seasons, it was anecdotally reported that, occasionally, mares treated with the implant had prolonged interovulatory intervals and some actually had their reproductive cyclicity downregulated for months; this effect was confirmed in a controlled study.[43] When investigating this issue, McCue et al reported normal interovulatory intervals in mares that had their implant removed once ovulation occurred.[44] This phenomenon was later elucidated by the fact that prolonged delivery of deslorelin would induce ovulation but its continuous release would down regulate FSH and LH secretion from the pituitary,[45] resulting in prolonged interestrous intervals. Removing the implant after ovulation thus prevented sustained release of deslorelin. Reportedly owing to problems in manufacturing logistics, Ovuplant® has not been commercially available in the USA since the early 2000's. Interestingly, side effects related to sustained deslorelin release have not been reported in Australia, where the drug originated and still remains available (as well as in other countries).

For several years, deslorelin was available either via importation from countries that manufactured Ovuplant® or via 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¶ (Sucromate™;1.8 mg/mL) was approved by the FDA in 2010 for induction of ovulation in mares and became commercially available in the USA in 2011. Sucromate™ is a product of and manufactured by Thorn BioScience, Louisville, KY, a division of CreoSalus and it is marketed by Bioniche Animal Health USA, Inc., Athens, GA. 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 of 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 out of 168 (~ 90%) mares treated in estrus with Sucromate™ (1 mL, i.m.) ovulated on average 41 hours after treatment whereas, 111 out of 134 mares (~83%) treated with hCG (2,500 IU, i.v.) ovulated on average 44 hours after treatment.[39]

Histrelin. Another synthetic GnRH analogue, histrelin (or historelin) is available in the USA from compounding pharmacies. Histrelin is an even more potent GnRH analogue than deslorelin (Table), but with similar clinical efficacy in inducing ovulation in horses as deslorelin or hCG.[46,47] Histrelin differs from the natural decapeptide 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.

Table. The comparative rank order potency (affinity for GnRH receptor) of synthetic GnRH analogues in relation to natural GnRH

	GnRH	Buserelin	Deslorelin	Histrelin
Relative Potency	1	20	144	210

Recently, a sustained release (biorelease) formulation histrelin was found to effectively induce ovulation within two days following administration.[47] Doses of 0.25-1.0 mg of a sustained release (biorelease) formulation histrelin effectively induce ovulation within two days following administration.[47-49] In a more recent study,[48] 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 91% (42/46) 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. The moderate potency of buserelin (see Table) perhaps may explain why a single administration of a labeled dose of buserelin (40 to 100 ug per mare) is not sufficient to induce ovulation in mares. For many years, buserelin (not available in the USA, except via compounding pharmacies) has had limited use and efficacy in inducing ovulation only when used in daily or twice daily injections for two to four days.[49,50] This protocol likely has prevented veterinarians and horse owners from favoring its use in brood mare management. In contrast with this knowledge, Levy and Duchamp recently showed that much higher doses than those originally used resulted in ovulation rates comparable to those of hCG.[51] In that study, 6 mg of buserelin were 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), 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 ug vs. 100 ug) than that recommended in commercial preparations of buserelin (Receptal) was able to effectively promote ovulation in treated mares at a similar rate reported for hCG, deslorelin and histrelin.

Ecbolic 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 four to eight hours following artificial or natural insemination to avoid interferences with sperm transport that could potentially affect fertility.[52,53] 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 prostaglandin-$F_{2\alpha}$.

Oxytocin

Oxytocin is a nonapeptide synthetized in the magnocelullar neurons in the hypothalamus that extend their axons into the neurohypohysis where it is stored until released into the blood stream for biological action in the mare. Oxytocin has several known physiologic effects, such as milk ejection, promotion of maternal behavior, and induction of myometrial contractions. Because delayed uterine clearance is commonly seen in mares experiencing 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,[54] it seems 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 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, i.e., < 4 hours.[55] Recently, our laboratory reported on the pharmacokinetics of carbetocin, a long-acting oxytocin analogue.[56] Carbetocin has a half-life of ≈ 17 minutes. Carbetocin is available and approved for use in horses in several countries but not in the USA. 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 $PGF_{2\alpha}$. A direct comparison between the ability of oxytocin and carbetocin to promote uterine clearance has not been reported for in vivo treatment in mares. Currently, it is not known whether the reported prolonged half-life of carbetocin would translate into a greater and/or more productive biologic response than oxytocin in mares that may require prolonged myometrial stimulation. Steckler et al have recently reported on the results of a comparison of oxytocin vs. carbetocin in eliciting contractions in ex vivo uterine tissues collected from mares at different reproductive stages (e.g., anestrus, estrus and diestrus).[57] The results indicated that the effect of oxytocin on equine myometrial tissue was greater during anestrus and diestrus than during estrus, whereas carbetocin seemed 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 $F_{2\alpha}$

In addition to being known for its luteolytic actions, $PGF_{2\alpha}$ is also an effective promoter of myometrial contractions. Owing to its longer half-life than oxytocin, especially the synthetic $PGF_{2\alpha}$ analogue cloprostenol (≈ 2-3 hours), $PGF_{2\alpha}$ has been favored by equine practitioners, especially for use in mares that anecdotally seem 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 found that oxytocin induced strong myometrial contractions that lasted approximately one hour; whereas, cloprostenol induced low-amplitude myometrial contractions that lasted four to five hours.[59] 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 $PGF_{2\alpha}$ during estrus, especially in the early post-ovulatory period, was found to transiently affect luteal function followed by resurgence in CL function.[59,60] However, these studies had conflicting results on the effect of such treatment on the resulting fertility of treated mares. For this reason, if $PGF_{2\alpha}$ is to be used as an ecbolic to treat delayed clearance during pre-ovulatory estrus and early postovulatory period, it is important that equine practitioners remain cognizant of its effects on luteal function. Most mares so treated should experience resurgence in luteal function[61] and may not suffer deleterious effects on their long-term luteal function and subsequent fertility. Moreover, our laboratory has recently reported that the effect of $PGF_{2\alpha}$ administration on early luteal function can induce luteolysis or antiluteogenesis, depending on the duration, timing, and dosage utilized.[15,20] For these reasons, treatments with $PGF_{2\alpha}$ during estrus should not continue after ovulation. Blood sampling for monitoring of progesterone secretion and supplementation with exogenous progestagens may be considered during that period.

Modulation of mating-induced endometritis with steroids

Endometritis following artificial insemination or mating is one important cause of infertility especially in mares susceptible to persistent mating-induced endometritis. In addition to therapies aiming at combating endometritis by utilization of intrauterine or systemic antibiotic treatment and stimulation of myometrial function via administration of ecbolic hormones (mainly oxytocin and $PGF_{2\alpha}$), 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.[62] A significantly higher pregnancy rate was seen in barren mares treated five times with 0.1 mg/kg of prednisolone acetate (≈ 50 mg per mare) administered every 12 hours with four treatments occurring before artificial insemination and one at the time of insemination. More recently, Bucca et al reported the use dexamethasone given as a single bolus administration of 50 mg per mare at time of breeding.[63] 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 and increased fertility in mares displaying multiple risk factors for developing post-breeding inflammation. Ferris and McCue studied the effects of multiple (twice daily for five days) glucocorticoid treatments (prednisolone or dexamethasone) in mares during early estrus.[64] They reported that mares treated with dexamethasone showed reduced uterine edema and ovulation rate (40% vs. 83%, respectively) than mares treated with prednisolone. In addition, only two out five dexamethasone-treated mares had unaltered LH hormonal profiles, whereas five out 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.[65] 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 glucorticoid agents on 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.[65] Mares treated with a single bolus intravenous injection of 50 mg of dexamethasone within one hour of breeding and concurrent induction of ovulation with 1,500 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.

Conclusions

Several pharmacologic agents are now available for use in the breeding management of broodmares. Shortening of the diestrus stage and induction of estrus with $PGF_{2\alpha}$ can be routinely achieved at virtually any stage of the cycle. 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 impact the outcome of breedings by increasing the odds of timed ovulation and pregnancy.

Footnotes

* Dinoprost tromethamine; Lutalyse®; Pfizer Animal Health, Kalamazoo, MI
† d,l cloprostenol sodium; Estrumate®; Merck Animal Health, Union, NJ
‡ d, cloprostenol; Genestran®; FORTE Healthcare Limited; Naul, Dublin, Republic of Ireland
§ human chorionic gonadotropin; Chorulon®; Merck Animal Health, Millsboro, DE
¶ deslorelin acetate; Sucromate™ Equine; manufactured by Thorn BioScience, Louisville, KY; marketed by Bioniche Animal Health USA, Inc., Athens, GA

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