Use of Prostaglandin F2α for Controlling the Mare’s Estrous Cycle

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

Prostaglandins belong to a group of modified long-chain fatty acids containing 20 carbons called eicosanoids. The cyclooxygenase pathway uses prostaglandin synthases to convert arachidonic acid into prostaglandins. Arachidonic acid is available through the hydrolysis of phospholipids present in the cell membrane. The breakdown of membrane phospholipids is catalyzed by the enzyme phospholipase A. Two isoforms of prostaglandin synthase exist: constitutive (cyclooxygenase-1) and inducible (cyclooxygenase-2). Systemic administration of prostaglandins (mainly prostaglandin F2α [PGF] in animals) is associated with side effects affecting the central nervous system (incoordination, stupor, and ataxia) and the vascular system (contraction of smooth muscle of organs such as the stomach, intestines, and urinary bladder).

The use of injectable preparations of PGF has revolutionized the breeding management of horses and cattle since its identification as the main luteolytic hormone. Pharmacokinetics of PGF after intravenous administration of 5 mg per mare (large mixed breeds of large ponies) has been recently described as follows: apparent plasma clearance, 3.3 ± 0.5 L/h/kg; distribution half-life of 1.57 ± 0.26 minutes; elimination half-life of 25.9 ± 5.0 minutes; and maximum plasma PGF concentration of 249.1 ± 36.8 ng/mL. The original studies pointed out that mares appeared to be more sensitive to exogenous PGF than cows. Indeed, an in vitro study has shown the affinity of equine luteal cell membrane preparations for PGF to be approximately 10 times greater than that for bovine luteal cell membrane preparations. The relatively high affinity of mare corpus luteum (CL) to binding of PGF along with the relatively slow metabolic clearance documented in mares account for the greater sensitivity of mare CL to the luteolytic effect of PGF when compared with other domestic species.

The luteal phase of the equine estrous cycle can be reliably shortened by the administration of PGF, allowing mares to return to estrus at a relatively predictable time (on average 2–5 days after PGF administration). In horses, a single treatment with PGF (intramuscular or subcutaneous) will induce complete luteolysis if administered at least 5 to 6 days after ovulation. This fact led to the prevailing assumption that the CL is not responsive to PGF luteolytic effects before it is at least 5 days old, despite the fact that some initial studies reported that some mares were responsive to luteolytic effect...
of PGF administration when treated on day 3 after ovulation. In the United States, the natural analogue dinoprost tromethamine—a is the only Food and Drug Administration–approved PGF for use in horses, although equine practitioners commonly use the synthetic analogue cloprostenol in breeding management, mostly owing to the longer half-life than its natural analogue. A review of the effects of PGF on luteal function and characteristics of the induced estrus and ovulation is presented in the subsequent sections. The use of PGF as an abortifacient and ecbolic for breeding management will not be presented.

2. Effects of PGF Administration on the Mare’s Reproductive Cycle

Natural luteolysis begins approximately 14 days after ovulation in mares. In the decade of the 1970s, several studies investigated the effects of PGF treatment on blood progesterone concentration profile and effects of the length of diestrus and interovulatory intervals. Most of these studies were based on examinations of serial blood samples taken before and after treatment with PGF or on the recording of the length of interovulatory intervals in treated and control mares. Studies on subsequent PGF-induced estrus, follicular dynamics, and ovulation were based mainly on findings of serial reproductive examinations by palpation per rectum. More recently, a significant wealth of information on the characteristics of luteal development and regression, follicle growth, and ovulation after PGF-induced luteolysis became available with the advent of transrectal ultrasonography. The information gained with ultrasonography studies on mare reproduction contributed to the understanding of PGF actions on the mare’s reproductive cycle and tract.

Soon after PGF was shown to be the uterine luteolysin in cattle, sheep, and rats, Douglas and Ginther published convincing evidence that exogenous (subcutaneous or intramuscular) or intrauterine administration of PGF had also luteolytic effects in mares. Since then, PGF and its synthetic analogues have been widely used for intensive broodmare management. In the study by Douglas and Ginther, mares received PGF on day 6 after ovulation. Previous studies demonstrated that intrauterine saline solution infusions 6 days after ovulation shortened the mare’s estrous cycle. The shortened cycle was denoted by luteal activity interruption that terminated diestrus. In that study, all mares treated with 1.25, 2.5, 5.0, or 10.0 mg of PGF (intramuscular) had shorter diestrus and shorter interovulatory intervals than did control mares (not treated with PGF). After that report, several other studies confirmed that PGF treatments not only shorten diestrus but also shorten interovulatory intervals. Despite the fact some mares may undergo complete luteolysis when treated on day 3 after ovulation, maximal response to one single-bolus intramuscular injection is expected when at least 5 days have elapsed from ovulation. Anecdotally, some equine practitioners report that whenever the day of ovulation is unknown, daily treatments of PGF are prescribed until treated mares show signs of behavioral estrus.

3. Luteolytic Doses of PGF Preparations

Dinoprost Tromethamine

For PGF tromethamine salt (PGF tham salt) preparations, 1.34 mg of the salt equals 1 mg of free acid PGF. Douglas and Ginther reported that doses of 1.25, 2.5, 5.0, and 10.0 mg of PGF administered intramuscularly shortened the luteal phase of the estrous cycle. Mares in all treatment groups were in estrus 3 to 4 days after treatment. A single bolus dose of 1.25 mg of dinoprost tromethamine per horse mare (~2.8 μg/kg for an average 450-kg mare) when administered between days 6 and 12 after ovulation has been shown to be luteolytic and induce normal ovulatory estrus periods, which in turn were followed by normal luteal function (diestrus). Even doses as low as 0.5 mg per mare (~1.1 μg/kg) has been shown to affect luteal function; however, complete luteolysis (21/21 mares) was only achieved when mares were treated twice, 24 hours apart.

In that study, this low dose did not induce common side effects (sweating, colicky behavior) generally associated with PGF treatment. Most commercial preparations of dinoprost tromethamine, however, recommend a single intramuscular or subcutaneous bolus administration of 5 to 10 mg per mare (~11.1–22.2 μg/kg).

Cloprostenol

In contrary to several other countries, cloprostenol formulations are not Food and Drug Administration–approved for use in horses in the United States. Nevertheless, cloprostenol is widely used in the United States by equine practitioners because of the longer half-life and association with fewer side effects than dinoprost tromethamine. Cloprostenol is available as two optically active isomers (enantio- mers), combined, d-cloprostenol and l-cloprostenol. The recommended luteolytic doses of these synthetic analogues are much lower than that recommended for the natural analogue dinoprost tromethamine. Luteolytic doses for d-cloprostenol are further lower than that needed for d,l-cloprostenol–induced luteolysis. The dosage difference between these two cloprostenol analogues is explained by the fact that only the d-enantiomer is pharmacologically active (luteolytic). Most popular preparations of cloprostenol in the United States use the racemic mixture (d- and l-enantiomers) at a dose of 250 to 500 μg per mare. In one study, doses as low as 25 μg of d,l-cloprostenol per mare successfully induced luteolysis. In several countries, the more potent preparations with only the active d-cloprostenol enantiomer are also available and labeled for use in horses. In a recent report, the bolus dose of
37.5 μg of d-cloprostenol induced complete luteolysis, similar to that in mares receiving 250 μg of a d,l-cloprostenol preparation. The recommended labeled doses for d-cloprostenol and d,l-cloprostenol are 37.5 μg per mare (0.5-mL injection volume) and 250 μg (1-mL injection volume), respectively, administered subcutaneously or intramuscularly.

4. Luteolytic Effects of PGF and Stage of the Estrous Cycle
The results presented in the early studies in the 1970s provided the basis for the assumption that PGF formulations would not induce luteolysis or affect CL function if administered before day 5 or 6 after ovulation. Interestingly, some of these studies reported that some mares actually responded to PGF-induced luteolysis when treated on day 3 after ovulation; however, the notion that the early CL was not responsive to PGF administration remained ingrained in the scientific and veterinary professional community. In 1974, Thompson and Witherspoon briefly reported another phenomenon that has recently gained attention: the ability of PGF to induce partial luteolysis followed by resurgence in CL function characterized by a transient increase in concentrations of blood progesterone. In that study, two mares receiving a relatively low dose of a synthetic PGF analogue 9 days after ovulation began to have a decrease in concentrations of plasma progesterone at 12 hours after PGF treatment, followed by a resurgence in progesterone concentrations at 48 hours after treatment; progesterone concentrations then remained at 30% to 50% of that before PGF treatment. More recently, 32 years from that initial report, Bergfelt et al (2006) compared the pattern of luteolysis after PGF treatment as a single bolus injection on day 3 after ovulation with that of mares treated on day 10. In the day 3 group, 75% (12/16) of mares had CL resurgence. Among those, six mares with “minor” progesterone resurgence had treatment-to-ovulation intervals similar to that in control mares. In summary, CL resurgence after PGF treatment results in partial luteolysis of the CL. Partial luteolysis is evident by decreasing concentrations of blood progesterone and followed by CL function resurgence. The resurgence is denoted by a moderate increase in progesterone concentrations. Partial luteolysis followed by CL resurgence may occur after administration of sub-luteolytic boluses doses of PGF during mid diestrus, or after administration of single injections at day 3 after ovulation.

5. Effects of Exogenous PGF on Steroid and Gonadotropin Secretion
Administration of PGF in mares with a functional CL >5 days is followed by functional luteolysis (significant decrease in progesterone) 24 hours after treatment that is, however, preceded by an immediate, transient rise in progesterone shortly after PGF treatment. Noden et al reported that functional luteolysis was preceded by a transient increase in progesterone, estradiol, and leuteinizing hormone at 10, 30, and 60 minutes after PGF treatment of diestrual mares. In a more recent study by Ginther et al, administration of a single luteolytic intravenous bolus of PGF resulted in an immediate increase in circulating progesterone concentrations within 10 minutes after the bolus injection, accompanied by an increase in concentrations of follicle-stimulating hormone, leuteinizing hormone, and cortisol. Conversely, mares infused with PGF for 2 hours, mimicking a natural pulse of endogenous PGF action, did not show increases in the same hormones; however, both treatments, bolus injection and infusion, resulted in similar luteolytic effects. These effects on steroids and gonadotropin secretion associated with supraphysiologic doses of PGF may partially explain the results of one study that found that mares treated in estrus with a synthetic PGF, fenprostalene, had shorter estrus-to-ovulation intervals than did control mares.

6. PGF Treatment and Antiluteogenesis
Recently, it has been reported that luteolysis or prevention of luteal formation may be accomplished with PGF administration beginning as early as the day ovulation is detected. This effect is dependent on the dose and frequency of PGF treatments. On the basis that the early developing CL <5 days is actually responsive to luteolytic effects of PGF, a series of experiments conducted in our laboratory produced data that support the hypothesis that the early developing CL is indeed responsive to exogenous PGF as early as within the first 24 hours from ovulation. Because of this early luteolytic responsiveness to PGF administration before the CL is fully functional, we named this phenomenon as (PGF-induced) “antiluteogenesis.” Mares treated once or twice daily for 3 days with 2.5 or 10 mg of PGF dinoprost failed to show a significant rise in concentrations of plasma progesterone during the treatment period. Approximately 60% of mares treated twice daily for 3 days with 10 mg of PGF had complete luteolysis; all mares receiving once-daily 2.5 mg of PGF for 3 days showed CL resurgence. Therefore, the antiluteogenesis effect of PGF is dependent on the dose and frequency of PGF treatments.

7. Clinical Applications of PGF in Broodmare Management
Use of PGF to Induce Luteolysis and Return to Estrus
Termination of the luteal phase (“short-cycling”) with exogenous PGF may be attempted for planned breeding of a single mare or as an approach to synchronize estrus and ovulation in a group of mares. If reproductive examinations with palpation per rectum and transrectal ultrasonography are available, the predictability of onset of estrus and ovula-
tion increases. Prediction of the next ovulation in the PGF-induced estrus is not predictable as it is the return to estrus. For example, it has been shown that the diameter of follicles present in the ovaries at the time of PGF treatment may influence when the mare would ovulate.\textsuperscript{22} When a relatively large follicle (≥35 mm) is present at the time of PGF administration, the onset of estrus and ovulation will depend on the follicular status (growing phase versus undergoing atresia). Accordingly, mares with follicles approaching the diameter of preovulatory follicles may come in estrus and ovulate within 2 to 5 days after PGF treatment, whereas the mean interval from treatment to ovulation in mares during mid diestrus and with follicles <25 mm may vary from 7 to 12 days from treatment.\textsuperscript{22} For example, in some extreme instances, mares will ovulate in 2 to 3 days; mares ovulating within 48 hours from PGF treatment often show no signs of behavioral estrus. Conversely, large follicles present at the time of PGF treatment may be already undergoing atresia and will slowly regress, and the mare may not ovulate until 10 to 14 days after the treatment. In most cases, however, mares will come into estrus and the large follicles at the time of PGF treatment will continue to grow and ovulate within 4 to 6 days after PGF treatment.

Obviously, the prediction of PGF-induced estral events requires that treated mares have an active corpus luteum at the time of administration. If reproductive examination is not available, horse owners may be instructed to administer a single dose of PGF 5 days after the mare ceases behavioral signs of estrus, or, alternatively, if teasing is not feasible, daily administration of a single PGF treatment may be prescribed until the mare shows signs of estrus or a reproductive examination by a veterinarian becomes available. Another alternative if veterinary assistance, or teasing information were not available, would be to recommend administration of a single dose of PGF at any given day and to repeat it in 5 days if the mare is not observed to be in estrus.

**Use of PGF in Postpartum Mares**

Several factors associated with complications during foaling could compromise the fertility of the mare’s foal heat. For most mares with dystocia or retention of the fetal membranes, it may be prudent to not breed on the first estrus after parturition (foal heat). In this scenario, instead of having horse owners waiting for mares to come into their second postpartum estrus (“30-day heat”), one strategy would be to treat mares with PGF approximately 5 to 7 days after ovulation in the foal heat.

**Use of PGF in Mares With Prolonged Luteal Phases**

Occasionally, mares may have prolonged diestrus periods because of the presence of a persistent CL. Persistent CLs may occur in mares that failed to express their endogenous luteolytic mechanism (rare), or, more commonly, in mares that have early embryonic loss after maternal recognition takes place. In general, prolonged diestrus is often associated with another unique phenomenon of the mare’s estrous cycle, the diestrus ovulation. Prolonged diestrus is diagnosed as a diestrus period lasting more than 16 days after ovulation. A single luteolytic dose of PGF (5–10 mg dinoprost) should induce mares to return to estrus.

**Use of PGF in Estrus Synchronization**

One of the most basic methods to attempt estrus synchronization is to treat mares with PGF and repeat the treatment approximately 2 weeks from the first injection. If teasing is available, mares can then be teased every other day beginning 2 days after PGF treatment. The efficacy of the use of PGF in estrus synchronization programs is greatly enhanced with the concomitant use of progestagens and estrogens.

**8. Non-Reproductive Effects Associated With PGF Administration**

In general, prostaglandins have significant effects on vascular and non-vascular smooth muscle, the central nervous system, and carbohydrate and lipid metabolism.\textsuperscript{23} The administration of exogenous PGF is relatively safe, and doses 20 to 40 times greater than the therapeutic dose (typically 5–10 mg of dinoprost) do not elicit toxic effects.\textsuperscript{24} Even doses up to 800 mg were not fatal to mares despite being associated with intense side effects such as recumbency; in that study, severe side effects subsided by 4 to 5 hours after PGF overdose treatment. This increased sensitivity is also reflected by the appearance of side effects after administration of a conventional luteolytic dose in mares in 20% to 40% of mares treated with PGF. Sweating, restless behavior, diarrhea, or even colic-like signs are commonly observed in mares but not in cattle. One of the most common side effects is a pronounced sweating seen within minutes after PGF administration. The results of most research studies indicate that equine sweating occurs by stimulation of adrenoreceptors on the sweat gland cells.\textsuperscript{25} Adrenaline-induced sweating is primarily mediated by β2 adrenoreceptors. Horses given PGF intramuscularly sweat but do not shiver, although shivering occurs in horses treated with adrenaline. This may explain why rectal temperature significantly decreased in horses after PGF administration.\textsuperscript{24} Because concentrations of plasma adrenaline and noradrenaline become elevated after administration of PGF, it has been accepted that PGF-related sweating is associated with release of adrenaline from the adrenal medulla. Some mares may also have abdominal discomfort resembling colic-like symptoms. Abdominal discomfort is a result of hypergastromotility. Occasionally, some mares also show locomotor incoordination and ataxia.
These side effects typically subside within 20 to 30 minutes after PGF treatment. The appearance and duration of these aforementioned side effects appear to vary among mares. It is important to note that these side effects are dose-dependent and typically subside within the first hour after PGF treatment. Irvine et al.\(^9\) reported that the administration of two low doses of PGF 24 hours apart did not elicit any appreciable side effects, including elevation in heart rate.

9. Conclusions

Manipulation of the mare's estrous cycle with PGF is an important strategy in the breeding of mares. The CL is sensitive to PGF treatment throughout the whole estrous cycle. A single bolus injection of PGF can reliably induce luteolysis when administered in mares with a CL >5 days. Serial injections of PGF for several days beginning (q 12 h or q 24 h) as early as within 24 hours from ovulation will prevent CL formation (antiluteogenesis), as evidenced by the absence of a rise in progesterone. Not only these side effects subside within the first hour after PGF treatment. These side effects typically subside within 20 to 30 minutes after PGF treatment. These side effects are dose-dependent and typically subside within the first hour after PGF treatment. Irvine et al.\(^9\) reported that the administration of two low doses of PGF 24 hours apart did not elicit any appreciable side effects, including elevation in heart rate.

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