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The use of prostaglandin F$_{2\alpha}$ (PGF) for controlling the mare’s estrous cycle
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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: a constitutive (cyclooxygenase-1) and an inducible (cyclooxygenase-2) isoforms. Systemic administration of prostaglandins (mainly 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 prostaglandin F$_{2\alpha}$ (PGF) has revolutionized the breeding management of horses and cattle since its identification as the main luteolytic hormone. Pharmacokinetics of PGF following 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 94.2 ± 15.9 s, elimination half-life of 25.9 ± 5.0 min, and maximum plasma PGF concentration of 249.1 ± 36.8 ng/ml.$^{1}$ The original studies pointed out that mares seemed 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.$^{2}$ 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 to 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 two to five days after PGF administration). In horses, a single treatment with PGF will induce complete luteolysis if administered at least five to six 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 five 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 USA, the natural analogue dinoprost tromethamine$^3$ is the only FDA-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 subsequent induced estrus and ovulation will be presented in the subsequent sections. Use of PGF as an abortifacient and ecbolic actions of PGF for use during breeding management will not be discussed in this manuscript.

Effects of PGF administration on the mare’s reproductive cycle

Natural luteolysis begins approximately 14 days after ovulation in mares. In the decade of 1970s, several studies investigated the effects of PGF treatment on blood progesterone concentration profile and effects of the length of diestrus and interovulatory intervals.$^{3,4,5}$ Most of these studies were based on examinations of serial blood samples taken before and after treatment with PGF or based 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 utilizing palpation per rectum. More recently, a significant wealth of information on the characteristics of luteal development and regression, follicle growth and ovulation following 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.\textsuperscript{6,7}

Soon after PGF was shown to be the uterine luteolysin in cattle, sheep and in rats, Douglas and Ginther published in 1972 convincing evidence that exogenous (subcutaneous or intramuscular) or intrauterine administration of PGF had also luteolytic effects in mares.\textsuperscript{4} Since then, PGF and its synthetic analogues have been widely used in breeding farms that require intensive management of broodmares and stallions and mares.\textsuperscript{7} In the original study by Douglas and Ginther (1972) mares received PGF treatment on day six after detection of ovulation because it had been previously shown that intrauterine infusions of saline solution performed six days after ovulation would shorten the mare’s estrous cycle as denoted by an interruption of luteal activity that terminated diestrus and by a shortened interovulatory interval.\textsuperscript{4} In that study, all mares treated with 1.25, 2.5, 5.0 or 10.0 mg of PGF had shorter diestrus and shorter interovulatory intervals than control mares (not treated with PGF). Following that report, several other studies confirmed that PGF treatments not only shorten diestrus but also interovulatory intervals. Despite the fact some mares may undergo complete luteolysis when treated on Day 3 after ovulation, maximal response to one single bolus injection is expected when at least five 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.

Luteolytic doses of PGF preparations

Dinoprost tromethamine

For PGF tromethamine salt preparations (PGF tham salt), 1.34 mg of the salt equals 1 mg of free acid PGF. Douglas and Ginther (1972) reported that doses of 1.25, 2.5, 5.0 and 10.0 mg of PGF were all found to shorten the luteal phase of the estrous cycle. Mares in all treatment groups were found in estrus three to four days after treatment. In horse mares, a single bolus dose of 1.25 mg of dinoprost tromethamine per horse mare (~ 2.8 ug/kg for an average 450 kg mare) when administered between days six 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).\textsuperscript{8} Even doses as low as 0.5 mg per mare (~ 1.1ug/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.\textsuperscript{9} In that study, this low dose did not induced 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 to 22.2 ug/kg).

Cloprostenol

In contrast to several other countries, cloprostenol formulations are not FDA-approved for use in horses in the USA. Nevertheless, cloprostenol is widely used in the USA by equine practitioners mainly because of its longer half-life and association with lesser side effects than dinoprost tromethamine. Cloprostenol is available as two optically active isomers (enantiomers), 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.\textsuperscript{10} 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 USA use the racemic mixture\textsuperscript{b} (d- and l-enantiomers) at a dose of 250 to 500 ug per mare. In one study, doses as low as 25 ug of d,l cloprostenol per mare successfully induced luteolysis.\textsuperscript{11} In several countries, the more potent preparations using only the active d-cloprostenol enantiomer\textsuperscript{c} are also available and labeled for use in horses. In a recent report, the bolus dose of 37.5 ug of d-cloprostenol\textsuperscript{c} was found to induce complete luteolysis similar to mares receiving 250 ug of a d,l-cloprostenol preparation.\textsuperscript{12} The recommended labeled doses for d-cloprostenol and d,l cloprostenol are 37.5 ug per mare (0.5 mL injection volume) and 250 ug (1 mL injection volume), respectively, administered subcutaneously or intramuscularly.
Luteolytic effects of PGF and stage of the estrous cycle

The results presented in the early studies in the 1970’s provided the basis for the assumption that PGF formulations would not induce luteolysis or affect CL function if administered before Day 5 or 6 post-ovulation. Interestingly, some authors reported that some mares actually responded to PGF-induced luteolysis when treated on Day 3 post-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 only recently has gained attention: the ability of PGF to induce partial luteolysis followed by resurgence in CL function that is 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 nine days after ovulation began to experience 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 following 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 experienced CL resurgence. Among those, six mares experiencing “minor” progesterone resurgence had similar treatment-to-ovulation intervals to control mares. In summary, the phenomenon of CL resurgence following PGF treatment reflects a condition by which the CL to undergo partial luteolysis, as denoted by decreasing concentrations of blood progesterone followed by resurgence of the CL function, denoted by a modest but significant transient increase in progesterone concentrations. Partial luteolysis followed by CL resurgence may occur following administration of sub-luteolytic boluses doses of PGF during mid diestrus, or following administration of single injections at Day 3 after ovulation.

Effects of exogenous PGF on steroid and gonadotropin secretion

Administration of PGF in mares with a functional CL >5 days after ovulation 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 (1978) reported that functional luteolysis was preceded by a transient increase in progesterone, estradiol and luteinizing hormone (LH) at 10, 30 and 60 min after PGF treatment of diestrual mares. In a more recent study by Ginther et al (2009), administration of a single luteolytic intravenous bolus of PGF resulted in an immediate increase in circulating progesterone concentrations within 10 minutes following the bolus injection accompanied by an increase in concentrations of follicle stimulating hormone (FSH), LH, and cortisol. Conversely, mares infused with PGF for two 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 control mares.

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. Based on the fact 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 three days with 2.5 or 10 mg of...
dinoprost failed to show a significant rise in concentrations of plasma progesterone during the treatment period. Approximately 60% of mares treated twice daily for three days with 10 mg of PGF experienced complete luteolysis where all mares receiving once daily 2.5 mg of PGF for three days showed CL resurgence. Therefore, the antiluteogenesis effect of PGF is dependent on the dose and frequency of PGF treatments.

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 ovulation 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. When a relatively large follicle (35 mm or greater) is present at the time of PGF administration, the onset of estrus and ovulation will depend on the follicular status (growing phase vs. undergoing atresia). Accordingly, mares with follicles approaching the diameter of preovulatory follicles may come in estrus and ovulate within two to five days following PGF treatment, whereas the mean interval from treatment to ovulation in mares during mid diestrus and with follicles <25 mm may vary from seven to 12 days from treatment. For example, in some extreme instances, mares will ovulate in two to three days; mares ovulating within 48 hours from PGF treatment often show no signs of behavioral estrus. Conversely, large follicle present the time of PGF treatment may be already undergoing atresia will slowly regress and the mare may not ovulate until ten to 14 days after the treatment. In most cases, however, mares will come into estrus and the large follicle at the time of PGF treatment will continue to grow and ovulate within four to six days after PGF treatment.

Obviously the prediction of PGF-induced estrual 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 five 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 five days if the mare is not observed 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 experiencing dystocia or retention of the fetal membranes, it may be prudent to not breed on the first estrus following parturition (foal heat). In this scenario, instead having horse owners waiting for mares to come into their second postpartum estrus (“thirty day heat”), one strategy would be to treat mares with PGF approximately five to seven days after ovulation in the foal heat.

Use of PGF mares with prolonged luteal phases

Occasionally, mares may experience prolonged diestrus periods owing to the presence of a persistent CL. Persistent CLs may occur in mares that failed to express their endogenous luteolytic mechanism (rare), or more commonly occur in mares that experience early embryonic loss after maternal recognition of pregnancy 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 dose of PGF 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 two weeks from the first injection. If teasing is available, mares can then be teased every other day beginning two 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.

Non-reproductive effects associated with PGF administration

In general, prostaglandins have significant effects on vascular and non-vascular smooth muscle, central nervous systems and carbohydrate and lipid metabolism. The administration of exogenous PGF is relatively safe and doses 20-40 times greater than the therapeutic dose (typically 5 to 10 mg of dinoprost) do not elicit toxic effects. 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 four to five hours after PGF overdose treatment. This increased sensitivity is also reflected by the appearance of side effects following 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 pronounced sweating seen within minutes following PGF administration. The results of most research studies indicate that equine sweating occurs by stimulation of adrenoreceptors on the sweat gland cells. 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 decreases in horses after PGF administration. 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 experience 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 seem to vary among mares. It is important to note that these side effects are dose dependent and typically subside within the first hour following PGF treatment. Irvine et al (2002) 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.

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 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 diestrus is shortened in mares treated with PGF but interovulatory intervals are also reduced in relation to normal, untreated cycles. Estrus and ovulation occurring after PGF treatments are normal and the inherent fertility of mares treated is not affected.

Footnotes:

a. Dinoprost tromethamine; Lutalyse®; Pfizer Animal Health, Kalamazoo, MI
b. d,l cloprostenol sodium; Estrumate®; Merck Animal Health, Union, NJ
c. d, cloprostenol; Genestran®; FORTE Healthcare Limited; Naul, Dublin, Rep of Ireland

References