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Kaleigh Marie Potter

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**COMPARISON OF SERIAL PGF<sub>2α</sub> PROTOCOLS FOR CONTROL OF THE  
ESTROUS CYCLE IN MARES**

A Masters Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Agriculture

By

Kaleigh M. Potter

May 2017

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# COMPARISON OF SERIAL PGF<sub>2α</sub> PROTOCOLS FOR CONTROL OF THE ESTROUS CYCLE IN MARES

College of Agriculture

Missouri State University, May 2017

Master of Science

Kaleigh M. Potter

## ABSTRACT

Prostaglandin F<sub>2α</sub> is commonly administered to manipulate the estrous cycle in mares, at a dosage of dinoprost tromethamine (Lutalyse®) at 10 mg, administered IM. My objectives were to compare three serial low-dose PGF<sub>2α</sub> protocols, and determine the most effective treatment in terms of number of injections, intensity of protocol, time allocated, and labor. Quarter Horse mares (n=11) were used in a crossover design. Lutalyse was administered at 1.1 mL per injection: treatment 1- PGF<sub>2α</sub> once 6d post-ovulation; treatment 2- PGF<sub>2α</sub> twice daily 0 d, 1 d, and 2 d, then once 3 d and 4 d post-ovulation; treatment 3- PGF<sub>2α</sub> twice daily on 2 d, then once 3 d and 4 d post-ovulation. Teasing scores were assigned daily. Rectal palpation and ultrasound were performed to measure follicular growth and detect ovulation. Blood samples were drawn on 3 d and 6 d post-ovulation to analyze plasma progesterone (P4) levels. One-way ANOVA and Tukey Method Pairwise Comparisons were used for data analysis. Between treatments on 3 d and 6 d post-ovulation, plasma P4 values were not statistically different. Mean intervals from ovulation to ovulation for treatments 1, 2, and 3 were 14.8 d ± 3.1, 12.9 d ± 6.3 d and 14.3 d ± 1.4 respectively. Mean intervals from first day of treatment to ovulation were 9.8 d ± 2.0, 12.9 d ± 6.3, and 12.6 d ± 1.7. Results did not show significant statistical difference. Further research is needed on low-dose administration of PGF<sub>2α</sub>.

**KEYWORDS:** mare, prostaglandin, synchronization, estrous, lutalyse

This abstract is approved as to form and content

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Dr. Gary Webb  
Chairperson, Advisory Committee  
Missouri State University

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May 2017

Approved:

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Dr. Gary Webb

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I dedicate this thesis to the Missouri State University College of Agriculture.

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## INTRODUCTION

### **Justification of the Study**

The equine industry in the United States has an economic impact of approximately \$102 billion, a number recorded in 2005 and since increasing. With over 1.4 million full-time jobs supported by the equine industry and over 9 million horses in the United States, the equine industry is a diverse and powerful substructure of the American economy (American Horse Council, 2017).

Manipulation and control of the estrous cycle of mares is critical to the prosperity and vitality of the equine industry. Implemented estrous and ovulation synchronization protocols allow industry professionals to coordinate and allocate time, money, and resources to maximize success during the breeding season, and ultimately pregnancy rates in mares.

When estrus and ovulations are synchronized, artificial insemination practices can be managed, or natural breeding can be planned efficiently for maximization of each stallion. Estrous synchronization allows embryo transfer and cloning processes to come to fruition with as little disturbance as possible (Klug and Jöchle, 2001).

Administration of Prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) is reportedly the most common method of estrus synchronization in the mare (Coffman and Pinto, 2016). Clinical uses of  $PGF_{2\alpha}$  include estrous synchronization, treatment for persistent CL syndrome, short-cycling, and the termination of unwanted pregnancies (Burden et al., 2015). In the 1970's, research identified  $PGF_{2\alpha}$  as an endogenous luteolysin. The administration of exogenous  $PGF_{2\alpha}$  is widely accepted as an effective method of inducing luteolysis in

order to synchronize the estrous cycle (Coffman and Pinto, 2016). The induction of luteolysis is critical to the success of the reproductive management of the mare (Kuhl et al., 2016a).

A more comprehensive understanding of the effects of various dosage protocols of exogenous prostaglandin given to cycling mares will allow advancement and success in regards to reproductive success of the mare.

### **Statement of Purpose and Objectives of the Study**

The purpose of this study was to determine if a new treatment protocol would hasten the return to estrus and ovulation in cycling mares. Three serial low-dose PGF<sub>2α</sub> treatment protocols were compared in terms of total injections and intensity of protocol, in relation to efficacy of treatment. Results from this study may provide insight into low-dose hormonal manipulation of the estrous cycle, to be implemented into new breeding management practices.

The objective of the study was to determine the efficacy of serial low-dose administration of PGF<sub>2α</sub> designed to prevent luteal development and shorten the interval to ovulation in cycling mares.

### **Hypotheses**

This study was designed around two hypotheses, as follows:

1. Low-dose serial administration of PGF<sub>2α</sub> will not be as effective at inducing luteolysis and shortening the intraovulatory period or preventing development of the CL as the standard dosage rate for dinoprost tromethamine (Lutalyse®) in mares.
2. There will be no difference in efficacy between treatment protocols.

## LITERATURE REVIEW

### Overview of the Estrous Cycle

Cyclicity of the mare is characterized by seasonal polyestrous; there are periods during the year in which most mares do not cycle regularly. Mares are long-day breeders, becoming the most reproductively receptive in the spring and into the summer (Senger, 2012). Regulation of gonadotropin releasing hormone (GnRH) secretion is dependent on photoperiod. When the photoperiod is short, the pineal gland secretes melatonin, which downregulates GnRH production in the hypothalamus. When the photoperiod increases, melatonin production decreases, allowing GnRH to stimulate the release of FSH and LH. (Blanchard et al., 2003).

Research has suggested that leptin and kisspeptin may have a direct effect on GnRH neurons in the hypothalamus. Adipocytes secrete leptin, a hormonal peptide, in relation to the fat content in the body. Leptin may be critical to the hypothalamic signaling that the animal is at an acceptable nutritional status for reproductive performance. Additionally, kisspeptin signaling supplies GnRH cell bodies in the hypothalamus. In response to leptin, kisspeptin neuron activity may be stimulated, which in turn stimulates GnRH neurons (Senger, 2012).

Gonadotropin releasing hormone is secreted in the hypothalamus and released into the hypothalamic-pituitary portal system. In the anterior pituitary, GnRH then stimulates the production and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). When estrogen is high and progesterone is low, LH secretion increases. Progesterone (P4) levels increase in the luteal phase as the CL continues to

develop. Once progesterone levels are above 2 ng/mL, behavioral signs of estrus are not seen in most mares. Ovulation is typically characterized by signs of estrus and plasma progesterone concentrations measuring 1 ng/mL or less. However, ovulation can occur without estrus, referred to as silent heat; low P4 levels allow the subsequent surge of LH and thus ovulation. The largest follicle is normally the one to ovulate; complete ovulation occurs in 2 to 7 minutes following the onset (Blanchard et al., 2003).

The average estrous cycle of the mare is 21 days long. Estrus typically lasts 7 days in mares. After the onset of estrus, ovulation occurs in approximately 5 days. After the LH surge, ovulation should be expected within 2 days (Senger, 2012). The estrous cycle is characterized by two distinct phases- the follicular phase and the luteal phase. The follicular phase occurs from the time the CL regresses, to the time of ovulation. Large antral follicles develop during the follicular phase, one of which will become dominant and later ovulate. Estradiol is secreted by the follicles, and is highest during the follicular phase. During the luteal phase, the CL develops, which creates an increase in progesterone levels. Follicular growth and development occurs during proestrus, along with secretion of estradiol. During estrus, estradiol secretion reaches its peak, and the mare becomes sexually receptive to the male. Progesterone secretion begins during metestrus, as CL formation begins, and progesterone secretion is then sustained during the diestrus period of the estrous cycle (Mottershead 2001; Figures 1 and 2).

During the estrous cycle, follicular development occurs in a wave-like pattern. Several large follicles develop during a major follicular wave; one follicle will continue growth as the dominant follicle while smaller follicles will undergo atresia. The dominant follicle secretes hormones which inhibit FSH, which in turn halts growth of the non-

dominant follicles (Shirazi et al., 2002). Proestrus and estrus both occur during the follicular phase of the estrous cycle, while metestrus and diestrus both occur during the luteal phase of the estrous cycle. The follicular phase accounts for approximately 20% of the estrous cycle. In turn, 80% of the estrous cycle is the luteal phase (Senger, 2012).

### **Biochemistry of Progesterone**

Progesterone is a steroid hormone synthesized from cholesterol. Cholesterol can either be synthesized in the smooth endoplasmic reticulum via de novo synthesis, or synthesized in the liver from acetate and released into the bloodstream in lipid droplets. Pregnenolone, a 21-carbon intermediate, is produced via side-chain hydrolysis of cholesterol.  $3\beta$ -hydroxy- $\Delta^5$ -steroid dehydrogenase then converts pregnenolone to progesterone. Progesterone can later be converted to estrogens, androgens, or corticosteroids (McKinnon and Voss, 1993).

Thecal cells produce  $3\beta$ -hydroxy- $\Delta^5$ -steroid dehydrogenase needed for the conversion of pregnenolone to progesterone; progesterone can then be converted to estradiol in the granulosa cells (Rotstein, 2016). The CL is the primary location of progesterone secretion (McKinnon and Voss, 1993). See Figure 3 for an overview in changes of plasma progesterone concentrations throughout the estrous cycle.

### **Biochemistry of Prostaglandins**

Eicosanoids act at low concentrations, and are critical to reproduction. Eicosanoids do not transport through the bloodstream to their target tissue, but tend to act closer to the cells of production (Voet et al., 1999). Prostaglandins are classified as

eicosanoids, comprised of modified long-chain fatty acids with 20 carbon atoms. Prostaglandin synthases are used in the cyclooxygenase pathway to synthesize prostaglandins from arachidonic acid, which becomes available when phospholipids of the cell membrane are hydrolyzed. The phospholipid degradation is quickened in the presence of phospholipase A, which serves a catalytic role (Coffman and Pinto, 2016).  $\text{PGF}_{2\alpha}$  stimulates cyclooxygenase (*COX*)-2 mRNA expression in not only epithelial cells, but also in stromal cells. Low dose administration of  $\text{PGF}_{2\alpha}$  can increase *COX*-2 expression, in turn inducing luteolysis. The increase in *COX*-2 expression leads to auto-amplification of endometrial  $\text{PGF}_{2\alpha}$  production (Okuda et al., 2014).

Prostaglandin  $\text{F}_{2\alpha}$  and prostaglandin  $\text{E}_2$  ( $\text{PGE}_2$ ) are the two main prostaglandins associated with reproduction in the mare. Endogenous prostaglandins are not only metabolized in the target tissue, but also in the lungs, kidneys, and liver. In mares,  $\text{PGF}_2$  is metabolized to 15-keto-  $\text{PGF}_{2\alpha}$ , then 13,14-dihydro-15-keto-  $\text{PGF}_{2\alpha}$ , then 11-ketotetranor- $\text{PGF}$  compounds. The 13,14-dihydro-15-keto metabolite is more stable when separated from red blood cells in either plasma or serum, despite its 5 minute half-life. Therefore, prostaglandin  $\text{F}_{2\alpha}$  metabolite ( $\text{PGFM}$ ) concentrations can be measured from peripheral blood in order to estimate the amount and rate that  $\text{PGF}_{2\alpha}$  is released from tissues at (McKinnon and Voss, 1993).

### **Role of Prostaglandin in the Estrous Cycle**

Prostaglandin  $\text{F}_{2\alpha}$  is known as the main luteolysin, which is secreted from the endometrium. Prostaglandin  $\text{F}_{2\alpha}$  and progesterone metabolite concentrations can be measured by enzyme immunoassay profiles. When cloprostenol treatment effect on

cycling mares was analyzed, no cross reaction between progesterone and PGFM was demonstrated. Two hours post-treatment, progesterone decreased 50% and by 90% 24 hours post-treatment. An increase in PGFM peaked at 45 minutes post-treatment, then decreased before again increasing at 16 hours post-treatment to the same level as the 45 minute post-treatment level (Okuda et al., 2014). Studies have shown that the ovulatory effect of  $\text{PGF}_{2\alpha}$  not only occurs at the follicular level, but also in the central nervous system at the hypothalamus-hypophysis axis. Following  $\text{PGF}_{2\alpha}$  administration, FSH and LH levels in peripheral blood increase followed by an increase in GnRH (Newcombe et al., 2008).

During luteolysis, oxytocin may be the main inductor of pulsatile secretions of  $\text{PGF}_{2\alpha}$  from the uterus. Prostaglandins are synthesized actively in the equine CL and may serve a critical role in its auto-paracrine regulation (Lukasik et al., 2014). Concentrations  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  were recording throughout the luteal phase, as well as mRNA transcription of PG synthesis enzymes found in the equine CL. Plasma progesterone levels, follicular diameter, and morphological appearance of the CL were all used to classify luteal structures.  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  levels were lowest in the mid-development of the CL ( $P < 0.05$ ), and the highest during the early-development CL ( $P < 0.05$ ).

Prostaglandin-endoperoxide synthase 2 (PTGS-2) and synthase prostaglandin E (PGES) synthesis via mRNA transcription were measured highest in the early-development CL ( $P < 0.05$ ). In a second experiment,  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  stimulated mRNA transcription of PTGS-2, PGES, OXT receptor (OXTR), and  $3\beta$ -hydroxysteroid dehydrogenase ( $3\beta$ HSD) enzymes, while simultaneously stimulating progesterone and oxytocin secretion ( $P < 0.05$ ) (Lukasik et al., 2014).



## **Administration of PGF<sub>2α</sub>**

During the 1970's and 1980's, various PGF<sub>2α</sub> analogues were tested and developed for potential use in the livestock industry. Prostaglandin and fenprostalene, alpha-chain allenic analogs, as well as fluprostenol were developed; fluprostenol being a synthetic PGF<sub>2α</sub> analog similar to cloprostenol. Luprostiol, a synthetic PGF<sub>2α</sub> analogue, is not approved for use in the United States but is available in certain European countries (Coffman and Pinto, 2016).

Dinoprost tromethamine, the natural analogue of PGF<sub>2α</sub>, is the only form of PGF<sub>2α</sub> that has received approval by the FDA in the United States (Lutalyse; Pfizer Animal Health). Dinoprost tromethamine is a tromethamine salt; naturally occurring in the PGF<sub>2α</sub> molecule. 1.34 mg of dinoprost is equal to 1 mg of PGF<sub>2α</sub> free acid. The labeled dosage of dinoprost is 10 mg per mare, although studies have shown significantly smaller doses to have high efficacy rates as well. A single injection of 1.25 mg dinoprost can be effective when administered between days 6 and 12 following ovulation. Lutalyse is diluted to 5 mg/mL, therefore administration does not require dilution. Doses as low as 1.1 ug/kg have been reported as having an effect on luteal function, but this dose may not result in complete luteolysis. After administration of 5 mg PGF<sub>2α</sub> IV, plasma clearance was  $3.3 \pm 0.5$  L/hr/kg, half-life was  $1.57 \pm 0.26$  minutes, elimination half-life was  $25.9 \pm 5.0$  minutes, and maximum plasma PGF<sub>2α</sub> concentration was  $249.1 \pm 36.8$  ng/mL (Coffman and Pinto, 2016).

Cloprostenol is a synthetic analogue of PGF<sub>2α</sub> which is commonly used by industry professionals to shorten the interval to ovulation, due to its longer half-life than dinoprost. Additionally, there are fewer side effects associated with cloprostenol. The

pharmokinetic parameters of cloprostenol in horses have not been published. There are two enantiomers of cloprostenol available; d-cloprostenol and l-cloprostenol. Recommended dosage rates for cloprostenol are noticeably smaller than the recommended dosage rates of dinoprost. The d-enantiomer is pharmacologically active and luteolytic, therefore the dosage needed to induce luteolysis is much lower than the luteolytic dose of d,l-cloprostenol. Estrumate, produced by Merck Animal Health, is a commonly used racemic mixture of d,l-cloprostenol sodium administered at a dosage rate of 250 to 500  $\mu\text{g}$  per mare. In other countries, the active d-cloprostenol enantiomer is used in order to prepare stronger solutions of cloprostenol, though not available in the United States (Coffman and Pinto, 2016).

To shorten the luteal phase of the estrous cycle,  $\text{PGF}_{2\alpha}$  can be administered subcutaneously or intramuscularly to induce luteolysis. Mares treated with  $\text{PGF}_{2\alpha}$  typically display estrus anywhere from 2 to 5 days post-treatment, and ovulate 7 to 10 days post-treatment. However, if a large follicle is present at the time of  $\text{PGF}_{2\alpha}$  administration, mares may ovulate as soon as 48-72 hours following treatment with  $\text{PGF}_{2\alpha}$  (Coffman and Pinto, 2016). In mares with a confirmed fully functional CL,  $\text{PGF}_{2\alpha}$  has approximately an 80% luteolytic rate when given as a single dose to mares 5 days post-ovulation. Following  $\text{PGF}_{2\alpha}$  treatment, progesterone levels decrease drastically, and the CL undergoes lysis. Endometrial edema will develop, and the interovulatory interval will also decrease. The interovulatory interval is also decreased when partial luteolysis occurs. However, no endometrial edema will develop, which may increase the incidence of silent heat (Newcome and Cuervo-Arango, 2014).

If an atretic or small preovulatory follicle is present at the time of PGF<sub>2α</sub> treatment, the interval from treatment to ovulation may be as long as 15 days. A single dose of PGF<sub>2α</sub> given during the early (0-4 days post-ovulation) diestrus period did not induce luteolysis. Following the trials, the conclusion was drawn that the equine CL is not fully responsive to the luteolytic actions of PGF<sub>2α</sub> until the CL is, at minimum, 5 days old (Coffman and Pinto, 2016).

Cloprostenol can decrease the time needed for follicular maturation, lysis of the CL, and ovulation in mares. Efficacy of treatment was dependent on the dose of cloprostenol administered. For each treatment group, the interval to ovulation decreased as the size of the pre-ovulatory follicle at time of treatment increased. A synergistic relationship was present between follicular diameter and dose efficacy (Newcombe et al., 2008). The dose of cloprostenol administered to each mare effects the interval to ovulation; hence the use of cloprostenol may be a method of more precisely controlling the estrous cycle, as well as the time of ovulation, in the mare (Newcombe et al., 2008).

When compared to cloprostenol, a larger number of mares ovulated within 48 hours of hCG administration when given luprostitol (P<0.01). Additionally, mares given luprostitol displayed a shorter interval from hCG treatment to ovulation (P<0.01). However, no difference was shown in the interval from estrus to hCG treatment, or the interval from estrus to ovulation. Preovulatory follicles in both treatment groups measured smaller at the time ovulation was induced when compared to the control group (Kuhl et al., 2016a).

The rate of double ovulations was not significantly impacted in mares treated with either luprostitol or cloprostenol. Furthermore, fertility and pregnancy rates were not

affected by administration of PGF<sub>2α</sub> analogs luprostirol or cloprostenol. In 2016, the interval from PGF<sub>2α</sub> administration to ovulation showed less variation using luprostirol rather than cloprostenol, when given to mares in the luteal phase of the estrous cycle (Kuhl et al., 2016a).

When PGF<sub>2α</sub> was given in the early post-ovulatory period, luteal function was inhibited, due to low levels of plasma progesterone. Inhibition of luteal function should induce the return to estrus and ovulation, followed by a normal diestrus period of the estrous cycle. Subsequently, mares treated with PGF<sub>2α</sub> during the study were expected to conceive at normal fertility rates when artificially inseminated following treatment. PGF<sub>2α</sub> is an efficient way of inhibiting luteal function, or promoting anti-luteogenesis. In this study, no effect of PGF<sub>2α</sub> treatment on fertility rate was found in the estrus period following treatment (Pinto et al., 2014).

When administered to mares with a 28 mm or larger follicle, ovulation may occur in less than 4 days; concerns have surfaced that PGF<sub>2α</sub> may alter the fertility of those manipulated cycles. When evaluating the effect of the interval from PGF<sub>2α</sub> treatment to ovulation on embryo recovery rates, as well as pregnancy rates in mares, the interval from treatment to ovulation (ITO) was found to have a significant (P=0.01) effect on the embryo recovery rate (ERR) in the mares, with the highest ERR recorded for the mares with an ITO of 6 to 10 days. Additionally, the ERR in mares with an ITO of less than 6 days was reduced, when compared to the ERR of mares with a longer ITO. In this study, no association was found between ITO and pregnancy rate (Pietrani et al., 2016).

## **PGF<sub>2α</sub> Synchronization Protocols**

As reviewed by Coffman and Pinto (2016), Douglas and Ginther conducted an early study, with mares administered either 1.25, 2.5, 5.0, or 10.0 mg of PGF<sub>2α</sub> intramuscularly (IM) 6 days after ovulation. All mares displayed shorter diestrus and interovulatory intervals, when compared to control mares that were not treated with PGF<sub>2α</sub>. In subsequent trials, some mares underwent complete luteolysis 3 days after ovulation, though this is not a reliable measure of the efficacy of synchronization utilizing PGF<sub>2α</sub> injections. Recommendations to administer PGF<sub>2α</sub> 5 days after ovulation were implemented following the study. When the date of ovulation is unknown, PGF<sub>2α</sub> treatments should be repeated for 2-3 days to minimize the risk of CL resurgence (Coffman and Pinto, 2016).

In 1974, research revealed the ability of PGF<sub>2α</sub> to induce partial luteolysis, followed by a subsequent resurgence of a functional CL. Two mares were given a low dose of PGF<sub>2α</sub> 9 days after ovulation, with plasma progesterone decreasing approximately 12 hours post-treatment. However, a rise in progesterone levels was noted at 48 hours post-treatment (Coffman and Pinto, 2016). In a 2014 study, mares given between 4 and 13 treatments of PGF<sub>2α</sub> at a dosage rate of 37.5 µg of d-cloprostenol per injection displayed a significant decrease in the percentage of mares which underwent full luteolysis ( $P < 0.01$ ). Data analysis suggested that the number of treatments received, as well as the length of treatment could both factor into the decreased luteolytic response in the mares. PGF<sub>2α</sub> receptors on the CL may be physiologically inclined to abnormal luteolysis when under the influence of more intensive PGF<sub>2α</sub> treatment plans. Following

treatment, the number of mares with a longer diestrus period also increased (Newcombe and Cuervo-Arango, 2014).

A common assumption in the equine breeding industry is that the early CL is not responsive to luteolytic actions of PGF<sub>2α</sub> administration. Repeated PGF<sub>2α</sub> treatment during the early diestrus period may produce a similar return to estrus to those mares administered a single PGF<sub>2α</sub> injection during the mid-diestrus period of the estrous cycle. Additionally, fertility of the early diestrus induced mares was hypothesized by Coffman et al. (2014) to not differ from those mares treated conventionally with one injection. In group 1, mares were given a single 10 mg (2.0 mL) dose of dinoprost tromethamine on day 10 post-ovulation. In group 2, mares were administered 10 mg PGF<sub>2α</sub> twice daily on days 0, 1, and 2, then given 10 mg PGF<sub>2α</sub> once daily on days 3 and 4 post-ovulation. Once a 35 mm or larger follicle was detected, mares in estrus were artificially inseminated with a minimum insemination dose of 2 billion motile sperm. Pregnancy was then determined after a growing embryonic vesicle was detected on 2 consecutive examinations, 14 days post-ovulation (Coffman et al., 2014).

In group 1 mares, Coffman et al. (2014) concluded that luteolysis occurred within 48 hours of treatment. When PGF<sub>2α</sub> is administered at a higher frequency during early diestrus, the antiluteogenic effects are similar to those seen when a single injection is given in the mid-diestrus period of the cycle. In group 2, the treatments given on days 3 and 4 prevented luteal resurgence and prolonged luteal formation, in turn increasing the response rate of the treatment group. Luteal cells are responsive to PGF<sub>2α</sub>, almost immediately following ovulation. The diestrus period of the cycle was shortened for mares administered PGF<sub>2α</sub> in both the early and mid-diestrus phases of the estrous cycle.

PGF<sub>2α</sub> administration during early diestrus provided a shorter interval to ovulation when compared to the mares given PGF<sub>2α</sub> during the mid-luteal phase (Coffman et al., 2014).

### **Synchronization of Estrus in Other Livestock Species**

The ovarian anatomy of the mare displays distinct differences from the bovine ovarian anatomy. The cortex and medulla of the ovary are reverse of what is seen in cattle; the cortex is located inside the ovary. While ovulation occurs from various locations of the bovine CL, ovulation only occurs at the ovulation fossa in the mare. Additionally when rectal palpation is performed in the mare, follicles may be palpated but the CL is not easily palpated manually because of its location in the ovarian medulla (Senger 2012).

The equine CL is 18-fold more sensitive to PGF<sub>2α</sub> than the bovine CL. The equine luteal cell membrane has a 10-time greater affinity for PGF<sub>2α</sub> when compared to the bovine cell membrane. The higher sensitivity of the equine CL to PGF<sub>2α</sub> may be attributed to the strong binding affinity of PGF<sub>2α</sub> to the CL, as well as the slower metabolic passage rate in mares (Coffman and Pinto, 2016). In cattle, PGF<sub>2α</sub> metabolite peaks are followed by an increase in progesterone concentrations every 8 hours. Progesterone will increase until reaching the pre- PGFM pulse concentration (Ginther and Santos, 2015).

In cattle treated with a PGF<sub>2α</sub> analog, nitric oxide metabolites may be detected in an ovarian vein sooner than detected in a jugular vein. Because of the earlier detection in the ovarian vein, changes in the systemic concentration of nitric oxide metabolites can be utilized in order to identify nitric oxide production changes in the ovary. Research in

heifers has suggested that LH is critical for the systemic increase in nitric oxide metabolites, which is associated with a simulated PGFM pulse (Ginther et al., 2015).

Similar to what has been done with cattle and sheep, nitric oxide metabolite plasma concentrations were collected from equine jugular veins in order to measure nitric oxide metabolite output. The measured changes in jugular plasma nitric oxide metabolite concentration were, in this study, associated with a PGFM pulse, although only at a certain stage of luteolysis. Although this study did not find the CL to be the primary source of nitric oxide production, nitric oxide production during luteolysis may be analyzed by researching the nitric oxide metabolite concentration within the general circulation of the equine system (Ginther et al., 2015).

### **Anovulatory Follicles**

Hormonal treatment with  $\text{PGF}_{2\alpha}$  may increase the risk of either double ovulations or anovulatory follicles. In order to determine the frequency of double ovulations and anovulatory follicles occurring in cycling mares treated with  $\text{PGF}_{2\alpha}$ , mares were given 5 mg  $\text{PGF}_{2\alpha}$  at 10 days post-ovulation. Transrectal ultrasounds were performed every 2 days until a 25 mm follicle was detected, then once every day until determination of ovulation. Double ovulations occurred in 15% more cycles within the treatment group when compared to the control group, which was not treated with  $\text{PGF}_{2\alpha}$ . The frequency of either a double ovulation or a single ovulation in concurrence with an anovulatory follicle was 6 times greater in the treatment group when compared to the control. (Ginther and Al-Mamun, 2009).



The occurrence of hemorrhagic anovulatory follicles following cloprostenol administration has been evaluated for its impact on the interval to ovulation in mares (Burden et al., 2015). Mares in the study were given a single IM injection of 250 µg cloprostenol, at varying times between days 5 and 12 post-ovulation. The average interval from treatment to ovulation recorded for the study was  $8.4 \pm 2.5$  days ( $P < 0.05$ ). An inverse relationship between the interval from treatment to ovulation and the diameter of the largest follicle at time of treatment was observed. Three different treatment outcomes were observed: ovulation within 48 hours post-treatment (13.4%) with varying levels of uterine edema, ovulation after 48 hours post-treatment (73.1%) with larger uterine edema, or follicular regression without ovulation (13.4%), with a new dominant follicle eventually developing. Both age of the mare as well as season had no effect on the interval from treatment to ovulation. Development of hemorrhagic anovulatory follicles was low overall, at 2.5% (Burden et al., 2015).

Uterine pathology and decreased  $\text{PGF}_{2\alpha}$  production can both contribute to persistent CL syndrome. Spontaneous or idiopathic persistent CL syndrome may also occur with normal uterine pathology. Persistent CL Syndrome progesterone concentrations are similar to the levels observed before the CL resurgence that occurs during pregnancy, with the concentration remaining constant approximately halfway between the levels recorded at day 8 and the end of luteolysis (Ginther and Santos, 2015).

Eighty-five percent of anovulatory follicles in mares are hemorrhagic; the likelihood of anovulatory follicles may increase with age of mare as well as hormonal manipulation of the estrous cycle (Cuervo-Arango et al., 2009). When hemorrhagic anovulatory follicles are present, bleeding into the lumen of the dominant follicle occurs,

which is typically followed by eventual luteinization. Because of the anticoagulant factors found in equine follicular fluid, the blood does not clot as quickly. Once clotting begins, granulosa and theca cells enter the lumen, multiply, and then luteinize; in turn producing the luteinized anovulatory follicle which is responsible for progesterone production (Burden et al., 2015).

A reported 15% of anovulatory follicles develop atresia, or become persistent anovulatory follicles. No significant hemorrhage occurs in the follicular lumen; the follicle does not luteinize and in turn does not produce progesterone (Burden et al., 2015). Hemorrhagic anovulatory follicle formation may differ between studies as influenced by: breed of mares used, number of mares involved in the study, location of the experiment, timing and dosage of PGF<sub>2α</sub> treatments, as well as the extensity of reproductive management involved in the study (Burden et al., 2015).

Following PGF<sub>2α</sub> treatment and subsequent luteolysis, a decrease in progesterone leads to an increase in LH at an earlier phase of follicular development, which may increase the probability of hemorrhagic anovulatory follicle development. Follicular growth and ovulation were not disrupted by increased and premature exposure to LH while fluid factors were altered, which could lead to lapses in maturation of the follicle or oocyte (Burden et al., 2015).

Over a 17-year period, 319 combined estrous cycles from two mares were compared and analyzed. In 204 of the 319 cycles, luteolysis was induced with cloprostenol at varying doses (25 to 1,000 µg). Cloprostenol-manipulated cycles were more likely (P<0.000) to develop hemorrhagic anovulatory follicles than non-manipulated cycles, with 25.1% total occurrence of hemorrhagic anovulatory follicles. In

both mares, the number of multiple dominant follicles, potentially leading to double ovulations, was higher ( $P < 0.00$  and  $P = 0.004$ ), respectively for the cloprostenol-treated cycles when compared to the naturally occurring cycles (Cuervo-Arango and Newcombe, 2009). Small doses ( $8.75 \mu\text{g}$ ) of cloprostenol have the same luteolytic effect as higher doses, with no difference in clinical response with the highest incidence recordings of hemorrhagic anovulatory follicles occurred when cloprostenol was given to mares at either 625 or 250  $\mu\text{g}$  per dose (Newcombe et al., 2008).

### **Physiological Effects of $\text{PGF}_{2\alpha}$ Administration**

Common side effects associated with  $\text{PGF}_{2\alpha}$  treatment include sweating, abdominal cramping, and other behaviors associated with colic (Coffman and Pinto, 2016). Listed behaviors, including abdominal cramping, diarrhea, and sweating may occur for as long as 20 minutes in approximately 10% of mares treated with  $\text{PGF}_{2\alpha}$  (McKinnon and Voss, 1993). Low dose (5 mg/mare) administration of the  $\text{PGF}_{2\alpha}$  analog Lutalyse may not induce the side effects associated with higher dose (10 mg/mare) administration in mares (Coffman and Pinto, 2016).

Eight mares received luprostitol (3.75 mg IM), D-cloprostenol (22.5  $\mu\text{g}$  IM), or saline on day 8 post-ovulation in an alternating order. In both luprostitol and cloprostenol treatment groups, plasma progesterone concentration decreased to the baseline level within two days following treatment ( $P < 0.05$ ). Salivary samples were collected to analyze cortisol levels, heart rate and heart rate variability were measured, skin temperature was recorded, behavior was monitored, and fecal output was recorded and scored for consistency.  $\text{PGF}_{2\alpha}$  treatment significantly increased the concentration of

salivary cortisol when measured 60 minutes after treatment ( $P < 0.001$ ). Over time, heart rate decreased independently of treatment and no changes were recorded in heart rate variability post-treatment. Administration of  $\text{PGF}_{2\alpha}$  analogs, either cloprostenol or luprostiol, is an efficient way of inducing luteolysis with minimal significant side effects, aside from the increase in salivary cortisol levels (Kuhl et al., 2016b).

When treated with cloprostenol (0.075 mg), 25 mares displayed significant ( $P < 0.01$ ) increase in intestinal motility. The increase in motility led to diarrhea on a short term basis, with symptoms subsiding within two hours following onset. Heart rate and respiratory rate were not significantly altered by administration of cloprostenol ( $P > 0.05$ ). Sweating and restlessness were also reported in 40-52% of mares involved in the study (Alcántara et al., 2005).

Because the reproductive cycle of the mare and the timing of physiological events has such a large impact on the economic success of the equine industry, pregnancy rates are of utmost concern. After concerns arose that the use and administration of exogenous prostaglandins may lower conception may lead to lower pregnancy rates in mares, Metcalf and Thompson (2010) further researched the subject. The 2010 study concluded that the synchronization and induction of estrus using a prostaglandin analogue, cloprostenol, did not have a negative impact on pregnancy rate in treated mares (Metcalf and Thompson, 2010).

### **Additional Uses of Exogenous Prostaglandins**

For mares with delayed uterine involution and endometritis,  $\text{PGF}_{2\alpha}$  may be given during the peri-ovulatory period due to its ecobolic effects, though this may lead to luteal

resurgence (Coffman and Pinto, 2016). In mares treated with PGF<sub>2α</sub> 24-72 hours post-ovulation, no decrease in pregnancy rates occurred. During early embryonic growth and development, mares are capable of maintaining pregnancy with decreased progesterone concentrations (Newcombe and Cuervo-Arango, 2014).

In mares, an increase in progesterone following a PGFM pulse has not been recorded. However, it is widely accepted that the equine primary CL undergoes natural resurgence at day 35 of pregnancy. In pregnant mares, resurgence of the CL is linked to equine chorionic gonadotropin (eCG) from the endometrial cups and its luteotropic characteristics. As the size and vascularity of the CL increase, progesterone production also increases. During CL growth, partial resorption of the corpus hemorrhagicum will also occur (Ginther and Santos, 2015).

The BAI HUI acupuncture point, in the sacral lumbar space, is commonly used in the treatment of ovarian conditions in veterinary acupuncture. A 1990s study was conducted to determine the efficacy of either low or micro doses of PGF<sub>2α</sub>, when administered at the BAI HUI acupuncture point. In this study, a micro dose (0.05 mg) was shown to be equally as effective (P<0.05) as the conventional dose (5.0 mg) when given to mares in the mid-luteal phase at inducing luteolysis. The BAI HUI acupuncture point provides an efficient pathway from administration to ovarian effect (Alvarenga et al., 1998).

### **Other Protocols Utilized in Breeding Management**

Before PGF<sub>2α</sub> gained recognition as a natural luteolysin, uterine lavage using saline solution was often implemented for open mares showing signs of a prolonged

luteal phase in order to gain a return to estrus. This protocol then became a treatment for mares with a persistent CL. Additionally, intrauterine saline treatment 6 days after ovulation was shown to shorten the luteal phase of estrous in cycling mares (Coffman and Pinto, 2016).

The efficacy of using estradiol to synchronize estrus compared to the use of estradiol and progesterone was evaluated. Mares in group 1 received 50 mg estradiol IM, and mares in group 2 received 50 mg estradiol in addition to 1.5 g sustained release progesterone. All mares included in both treatment groups were given 10 mg PGF<sub>2α</sub> IM 10 days post-treatment. Examinations were performed on all mares via transrectal ultrasonography on days 1 and 10 post-treatment, then every 1 or 2 days thereafter in order to determine follicle size. After detection of a follicle larger than 30 mm and uterine edema, mares were given 0.5 mg histrelin, an analogue of GnRH. After administration of histrelin, mares were examined ultrasonographically every day until ovulation was detected. Group 1 mares did not display synchronization of estrus following treatment. However, mares in group 2 had a much more uniform and synchronized interval to ovulation of  $20.4 \pm 1.5$  days, with a range of 17-22 days. Researchers concluded that administration of sustained release estradiol and progesterone, followed by administration of PGF<sub>2α</sub> 10 days post-treatment, is effective in synchronization of estrus and ovulation, without daily injections being necessary (Sudderth et al., 2013).

Equine follicle stimulating hormone (eFSH) is often incorporated into superovulation protocols. A 2010 study (Meyers-Brown et al., 2010) was conducted to determine whether or not administering varying doses of recombinant equine follicle stimulating hormone (reFSH) to mares would increase the number of ovulatory follicles

per cycle, and in turn increase the number of embryos recovered per mare. Because reFSH can be cloned and synthesized in reliable and consistent quantities, its use over eFSH is much preferred. As part of the experimental protocol, treatment groups were given varying doses of eFSH, reFSH, and a combination thereof. The largest number of ovulations was recorded for the group receiving 12.5 mg eFSH, 0.5 mg reFSH, and 0.65 mg reFSH. The treatment group receiving eFSH and 0.65 mg reFSH had the highest recorded number of embryos recovered per flush. Also noted in the conclusions was the similar embryo per ovulation rates between all treatment groups, including the control group. After data analysis, researchers concluded that administration of reFSH is just as effective as eFSH, when given to increase the number of follicles greater than 35 mm, ovulation rates, and the number of embryos recovered per flush (Meyers-Brown et al., 2010).

In order to simulate a natural PGFM pulse that occurs during luteolysis, oxytocin (OT) may be administered on day 13 post-ovulation to induce the PGFM pulse. Five different OT doses (1-10 IU/mare) were used for bolus treatment in the mares. Mares that received 1.25, 2.5, and 5 IU/100 kg displayed the largest decrease in progesterone within 8 hours of treatment ( $P < 0.05$ ). Although cyclooxygenase inhibitor treatment with flunixin meglumine decreased progesterone concentration ( $P < 0.008$ ), treatment 2 hours prior to OT administration did not inhibit OT-induced PGFM pulses and the subsequent decrease in progesterone. A 2 hour OT infusion was sufficient in the study to induce the PGFM pulse, however the pulse was not stimulated by a single bolus OT treatment. The OT-stimulated PGFM pulse caused a decrease in progesterone, which was not inhibited

by flunixin meglumine treatment. In turn, OT has a role in luteolysis in mares, as well as other species (Santos et al., 2015).

Human chorionic gonadotropin (hCG) dosage rate varies between 1500 to 4000 IU, which can be given either IV or IM. Typical administration of hCG occurs after a follicle measuring 30 mm or larger is detected. Lower synchronization rates may occur when hCG is administered on days 1-3 of estrus. Additionally, administration of hCG may reduce the length of estrous in cycling mares (McKinnon and Voss, 1993).

In the presence of a 35 mm or larger follicle, hCG has been shown to induce ovulation within 48 hours following treatment. Because hCG is a glycoprotein, antibodies may develop over the course of repeated treatments, leading to a decrease in the efficacy of hCG-induced ovulation (Berezowski et al., 2004). Gonadotropin releasing hormone has been shown to double plasma LH concentrations following administration. GnRH can be given in order to induce the secretion of FSH and LH, and in turn inducing follicular growth and development, CL maturation, and ovulation. GnRH may also serve as a nonantigenic substitute for the administration of hCG to induce ovulation.

Human chorionic gonadotropin is commonly used in Europe for the induction of ovulation in mares. Mares are often used several times throughout each breeding season; antibodies against hCG may develop, in turn reducing the efficacy of the treatment (Newcombe and Cuervo-Arango, 2016). Desorelin does not increase production of hCG antibodies, but the interovulatory interval for each cycle may increase. Deslorelin is also less cost efficient per mare when compared to hCG. Suprefact©, (Sanofi; 1 mg/mL), is a buserelin compound used in human medicine and experimentally used in mares. A large (5.5 mL), single dose of buserelin was shown to be just as effective at inducing ovulation



within 48 hours of treatment as hCG. When comparing mares treated with buserelin and hCG, a larger number of mares ( $P < 0.05$ ) ovulated when given buserelin than hCG. Because buserelin is effective when given as a single dose and is relatively inexpensive, its use provides an alternative to multi-dose hCG ovulation induction (Newcombe and Cuervo-Arango, 2016). Ovulation may be induced efficiently via hCG administration when given after estrus is induced with luprostiol (Kuhl et al., 2016a).

In 1999, deslorelin (Ovuplant, Fort Dodge Animal Health, Overland Park, KS), a GnRH agonist, was approved for use in mares when administered as a subcutaneous controlled-release implant. When compared to hCG, repeated treatment with GnRH has not been shown to decrease efficacy of ovulation induction. In mares with a 30 mm or larger follicle, 88% of mares were shown to ovulate within the 48 hours following treatment when induced with Ovuplant; however Ovuplant is not available for use in mares in the United States (Berezowski et al., 2004).

Although Ovuplant is not available for use, an injectable deslorelin analog, Sucromate™ (Thorn BioScience, Lexington, KY) has been approved by the FDA for use in the United States. A 2012 study compared the efficacy of deslorelin to induce ovulation in mares when compared to hCG. After detection of a follicle measuring 35 mm or larger, 1.0 mL of Sucromate was administered intramuscularly. Approximately 89% of mares ovulated within 48 hours following treatment, a rate similar to that seen following administration of hCG. The study concluded that Sucromate, injectable deslorelin, is an effective method of inducing ovulation in mares (Ferris et al., 2012).

Although less common, fractionated coconut oil can be implemented in intrauterine infusion protocols for mares. Plant oil infusion has been shown to be a safe,

reversible, and inexpensive method of lengthening the luteal phase of the estrous cycle when administered to mares on day 10 of the cycle. Diel de Amorim et al. (2016) hypothesized that administration of intrauterine coconut oil infusion would lead to higher serum progesterone levels at the time of luteolysis when compared to untreated mares. For the study, half of the mares (n=5) were administered 1.0 mL fractionated coconut oil infused into the uterus using an artificial semination pipette on day 10 of the estrous cycle; the remaining mares (n=5) were given 0.5 mL fractionated coconut oil infused into the uterus using an embryo transfer gun on day 10 of the estrous cycle. Intrauterine administration of 1.0 mL fractionated coconut oil did not lengthen the luteal phase in the treated mares, and lowered diestrus progesterone levels ( $P < 0.05$ ). In a 2013 preliminary study comparing sesame, coconut, and fractionated coconut oils, the same results were found; progesterone levels decreased at the time of luteolysis, with a faster return to estrus (Diel de Amorim et al., 2016).

Flunixin meglumine (FM), a  $PGF_{2\alpha}$  inhibitor, can be administered in order to analyze the occurrence of prolonged luteal activity in treated mares. As part of Santos et al.'s study (2013), FM was administered every 8 hours at 1.0 mg/kg body weight on days 14-16.7.  $PGF_{2\alpha}$  metabolite concentration over an 8 hour time period was lower in the mares treated with FM on day 14 post-ovulation, compared to the untreated mares. The interval from ovulation to a decrease in progesterone and the end of luteolysis ( $P < 1$  ng/mL) was prolonged by approximately 1 day in the mares treated with FM. This study confirmed that  $PGF_{2\alpha}$  synthesis inhibition effects luteolysis, and if inhibition occurs at the time luteolysis is expected, prolonged luteal activity may be present (Santos et al., 2013).

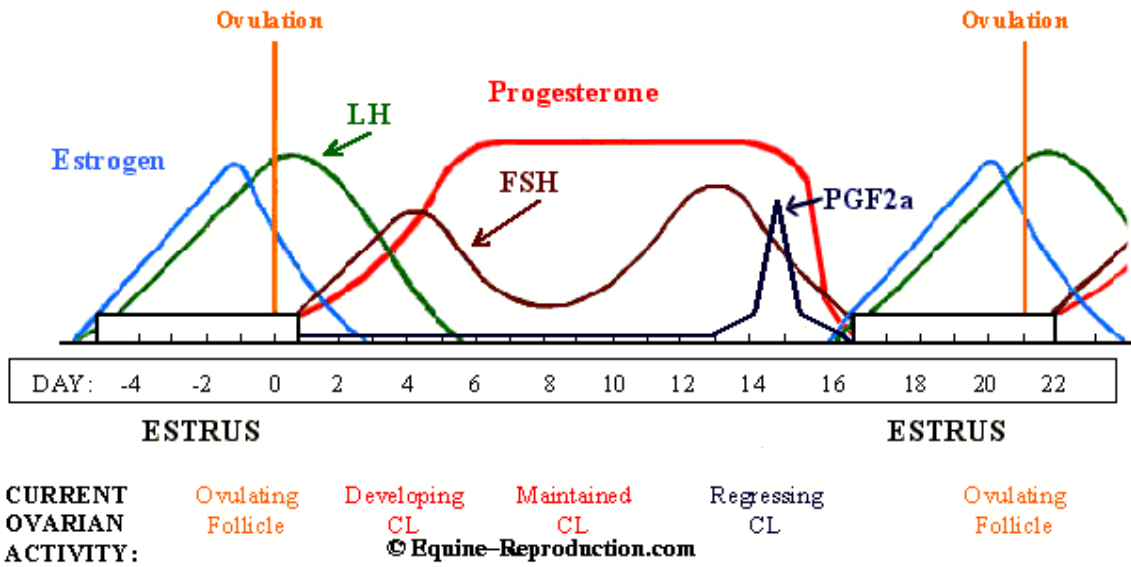


Figure 1. Stages of the estrous cycle (Mottershead, 2001).

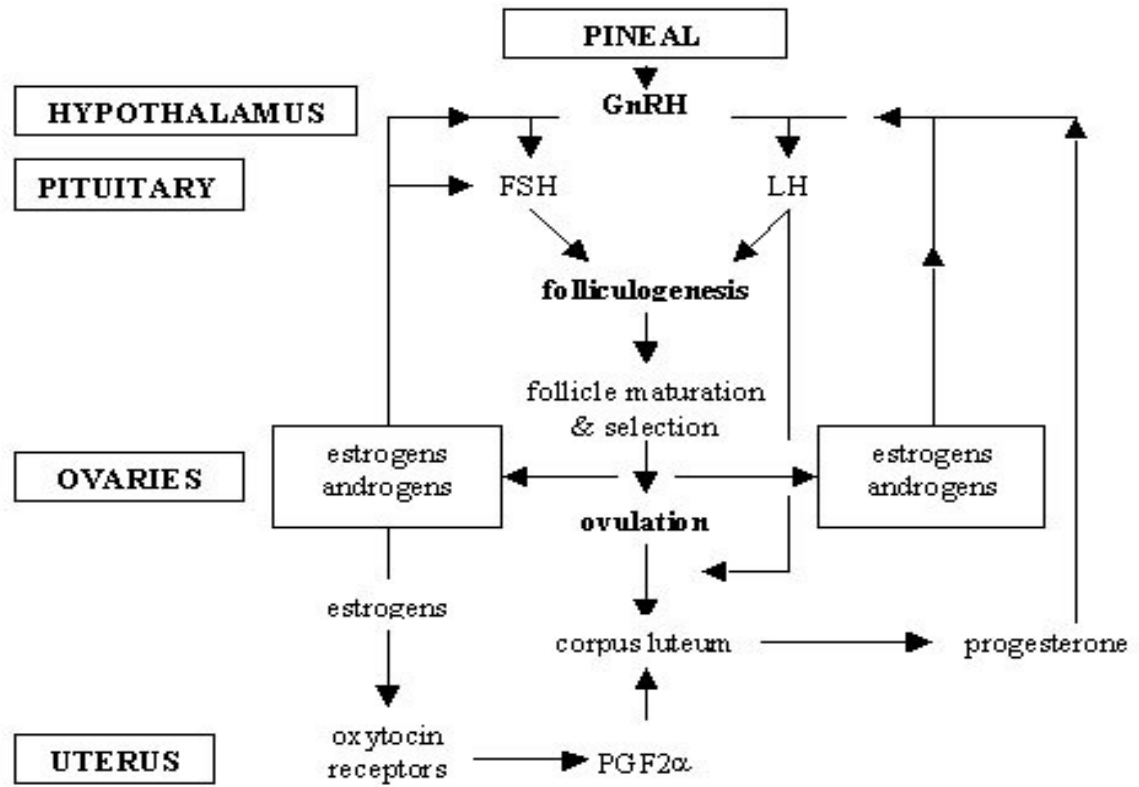


Figure 2. Endocrinology of the estrous cycle (Mottershead, 2001).

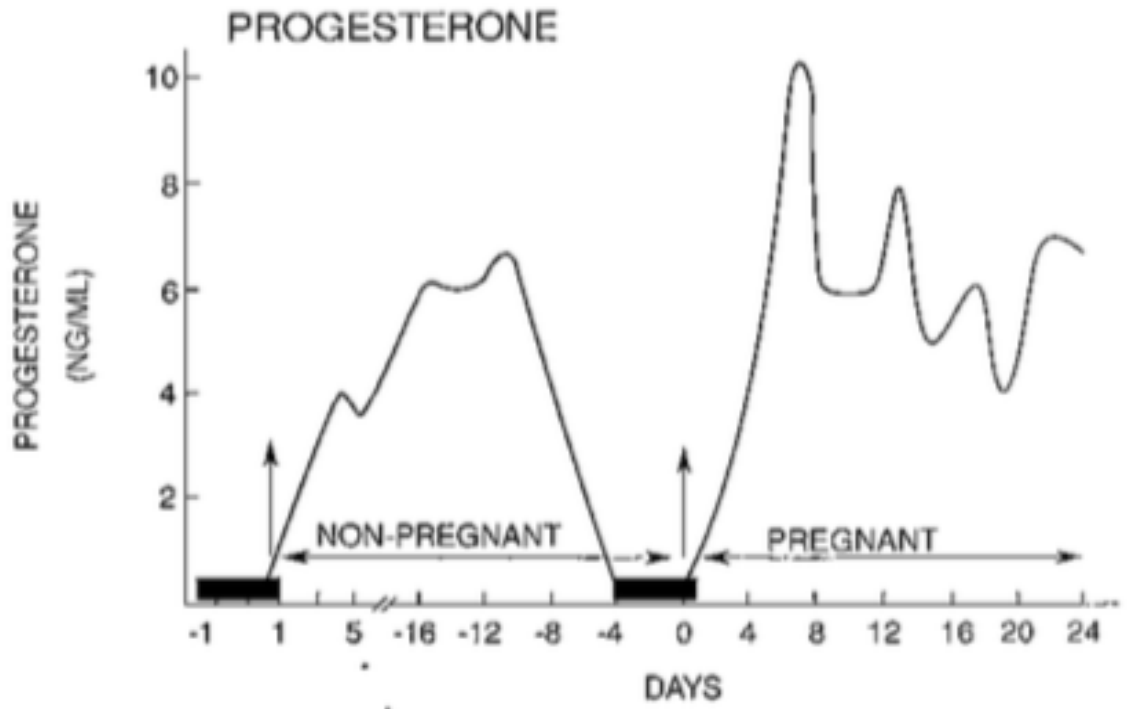


Figure 3. Plasma progesterone levels (ng/mL) in pregnant vs non-pregnant mares (McKinnon and Voss, 1993)

## MATERIALS AND METHODS

### IACUC Compliance

This project was funded by the USDA NLGCA Capacity Building Grant and approved by IACUC Protocol 15-025.0-B. Research was conducted at the Missouri State University Darr Agriculture Center in Springfield, Missouri. Research began in May 2017 and concluded in July 2017. Eleven Quarter Horse mares (468- 572 kg, 7-23 years old) were used for the study.

### Experimental Design and Procedures

Mares were teased using a teaser stallion, and teasing scores (T0-T4) were assessed daily. A description of criteria for each teasing score is as follows:

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0	Mare shows no signs of receptivity; possibly some aggression toward the stallion- striking, kicking, squealing, etc.
1	No aggression toward the stallion.
2	Some interest; mare may approach the stallion and exhibit some winking of the vulva and tail raising.
3	More interest, tail raising, squatting, urination.
4	Intense interest; turns hindquarters toward the stallion with continuous winking and frequent urination.

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(Coleman and Powell, 2004)

Once a T3 teasing score was detected, rectal palpation and ultrasound were both performed. A digital ultrasound display and transrectal probe were used in order to

perform ultrasound, and follicular diameter was measured (mm) during each examination. Individual record books were utilized for each mare in order to record teasing scores as well as follicular growth and location (right or left ovary) of each follicle. When an ovarian follicle measured 30 mm or larger, mares were examined daily, including rectal palpation, ultrasound, and measurement of follicles, until ovulation was detected.

In a crossover design, each mare was subjected to all treatments. Mares were assigned to one of three treatment groups. Treatment protocols were as follows: treatment 1- PGF<sub>2α</sub> administered once on day 6 post-ovulation; treatment 2- PGF<sub>2α</sub> administered twice daily on days 0, 1, and 2, then once on days 3 and 4 post-ovulation; treatment 3- PGF<sub>2α</sub> administered twice daily on day 2, then once on days 3 and 4 post-ovulation. Dinoprost tromethamine (Lutalyse®) was administered at a dosage rate of 1 mg/45.5 kg body weight, and concentrated in a 5 mg/mL solution. Lutalyse was administered IM to each mare for each dose of the assigned treatment. To ensure that each mare received a full 1.0 mL of Lutalyse for each dose of each treatment, 1.1 mL was administered per injection. Treatment 1 was used as a control protocol; a single injection of PGF<sub>2α</sub> is typically administered to induce luteolysis. Treatments 2 and 3 were included in the study to repeat the protocols of a previous studies utilizing higher dosage rates of Lutalyse (Coffman et al., 2014; Pinto et al., 2014).

In order to track fluctuations in P4 levels for each mare, blood samples were collected using jugular venipuncture on days 3 and 6 post-ovulation. Blood was collected using 6.0 mL syringes with 18.0 gauge needles. Samples were transferred to 10 mL BD Vacutainer® vials, then set in test-tube rack in order to clot, for approximately ten

minutes. Once coagulation occurred, vials were centrifuged for five minutes in order to separate serum from the clot. Serum was removed from the vials using 3 mL disposable pipettes, and placed in 1.5 mL microcentrifuge vials. Each 1.5 mL vial with serum was labeled with the date and the identifying number of the horse, then frozen. Progesterone ELISA kits ADI-900-011 and ADI-901-011 (Enzo Life Sciences) were used to analyze samples by Northcutt Laboratories in Middletown, Missouri. The assay was run at a dilution rate of 1 to 125, 1 to 250, or 1 to 400 in order to reach a 50% binding rate for each sample. Results were recorded in picograms per milliliter then converted to nanograms per milliliter for analysis in this study.

### **Statistics**

Minitab 17 (Minitab Inc., State College, PA) software was used for data analysis, with significant difference set at  $p = 0.05$ . One-way ANOVA and the Tukey Method pairwise comparisons were used in order to analyze the intervals from ovulation to ovulation and treatment to ovulation between treatment groups.



## RESULTS AND DISCUSSION

### Data Analysis

The age and approximate weight of mares used in the study were recorded in T1. The order of treatments received for each mare in the study were random, and recorded in T2. Mean intervals from ovulation to ovulation for treatments 1, 2, and 3 were  $14.8 \text{ d} \pm 3.1$ ,  $12.9 \text{ d} \pm 6.3 \text{ d}$  and  $14.3 \text{ d} \pm 1.4$  respectively, and recorded in T3. Mean intervals from first day of treatment to ovulation for treatments 1, 2, and 3 were  $9.8 \text{ d} \pm 2.0$ ,  $12.9 \text{ d} \pm 6.3$ , and  $12.6 \text{ d} \pm 1.7$ , and recorded in T4. ANOVA analysis of these variables revealed no significant differences ( $p > 0.05$ ).

Mean intervals from ovulation to the detection of a 30+mm follicle for treatments 1, 2, and 3 were  $13.2 \text{ d} \pm 2.2$ ,  $9.2 \text{ d} \pm 3.0$ , and  $12.0 \text{ d} \pm 2.2$  respectively, and recorded in T5. Mean intervals from the first day of treatment to the detection of a 30+ mm follicle for treatments 1, 2, and 3 were  $8.1 \text{ d} \pm 1.1$ ,  $9.5 \text{ d} \pm 2.7$ , and  $10.3 \text{ d} \pm 1.7$  respectively, and recorded in T6. Mean intervals from ovulation to the display of estrus (teasing score  $\geq$  T3) for treatments 1, 2, and 3 were  $10.6 \text{ d} \pm 4.4$ ,  $6.9 \text{ d} \pm 2.6$ , and  $10.3 \text{ d} \pm 6.8$  respectively, and recorded in T7. Mean intervals from the first day of treatment to the display of estrus (teasing score  $\geq$  T3) for treatments 1, 2, and 3 were  $5.9 \text{ d} \pm 3.6$ ,  $8.1 \text{ d} \pm 5.2$ , and  $6.0 \text{ d} \pm 3.9$  respectively, and recorded in T8.

Mean intervals from ovulation to ovulation for treatments 1, 2, and 3 with outliers removed ( $n=1$ ) from analysis were  $14.8 \text{ d} \pm 3.1$ ,  $11.1 \text{ d} \pm 3.1$ , and  $14.3 \text{ d} \pm 1.4$  respectively. Under Tukey Method pairwise comparison, treatment 2 was significantly different from treatments 1 and 3 ( $p = 0.013$ ). Mean intervals from first day of treatment

to ovulation for treatments 1, 2, and 3 with outliers removed from analysis were  $9.8 \text{ d} \pm 2.0$ ,  $11.1 \text{ d} \pm 3.1$ , and  $12.6 \text{ d} \pm 1.7$  respectively. Treatments 2 and 3 were significantly different from treatment 1, though not significantly different from each other ( $p= 0.052$ ).

Mean plasma progesterone levels on day 3 post-ovulation for treatments 1, 2, and 3 were  $6.0 \text{ d} \pm 2.5$ ,  $4.5 \text{ d} \pm 1.9$ , and  $6.4 \text{ d} \pm 1.6$  respectively, and recorded in T9. Mean plasma progesterone levels on day 6 post-ovulation for treatments 1, 2, and 3 were  $10.9 \text{ d} \pm 10.1$ ,  $3.9 \text{ d} \pm 2.0$ , and  $3.9 \text{ d} \pm 1.9$  respectively, and recorded in T10. Plasma progesterone levels were not statistically difference between treatments from day 3 to day 6 post-ovulation. However, progesterone levels numerically increased within treatment 1 from day 3 to day 6 post-ovulation. Progesterone levels decreased between days 3 and 6 post- ovulation for treatments 2 and 3.

### **Data Limitations**

Statistical outliers may have contributed to a lack of significance in results. When One-way ANOVA and Tukey Method analysis were performed removing statistical outliers, some results of the study became statistically significant.

Plasma progesterone immuno-assay test levels were not all attained at the desired 50% binding rate. Values recorded at less than 50% binding may be less accurate, and may have contributed to statistical skew and lack of significance.

### **Discussion**

Although results were not significantly different, these findings agree with previous studies (Coffman et al., 2014; Pinto et al., 2014) utilizing a dose of 10 mg

dinoprost tromethamine. On treatment 2, one mare displayed estrus and maintained a follicle greater than 30 mm for 13 days, and had an intra-ovulatory interval of 29 days. On treatment 3, one mare failed to return to estrus or develop a dominant follicle measuring larger than 30 mm. Statistical outliers as described may have contributed to a lack of significance in statistical analysis; however results of treatment 2 became significantly different from treatments 1 and 3 with outliers removed.

Previous studies have utilized administration of hCG following PGF<sub>2α</sub> to ensure timely ovulations. Mares develop antibodies against hCG with repeated treatment, in turn decreasing efficacy rates. Because mares were used in a crossover design with three subsequent estrous cycles, hCG was not administered to mares following each PGF<sub>2α</sub> protocol. If hCG or Delsorelin had been administered to mares along with PGF<sub>2α</sub> for each treatment protocol, evaluation of the efficacy of low-dose serial administration of PGF<sub>2α</sub> might have been compromised.

Mares in this study were characterized by a wide range in age, as seen in Table 1. A more uniform age range for mares used in subsequent studies may provide more consistent results, potentially with statistical significance. In this study, maiden mares were used alongside broodmares. Increasing uniformity of mares used by decreasing the range in ages may have improved results of the study. Additionally, a larger sample size would have most likely increased the consistency of results seen in the study.

Plasma progesterone levels were analyzed in order to track changes throughout the estrous cycle for each mare for each given treatment. Also, plasma progesterone levels were evaluated as a measure of luteal tissue development for each treatment. If serial low-dose administration of PGF<sub>2α</sub> had prevented luteal tissue development during

the early diestrus period, progesterone levels should have verified the absence of a functional CL. However, plasma progesterone results were not significantly different among treatment groups. Most serum samples were run at varying dilutions in attempts to reach a minimum 50% binding rate for each sample. However, not all samples were re-run to obtain a higher binding rate, as opposed to other samples which were re-run two and three times. With binding rates as low as 11.1% and as high as 91.2%, a wide range of plasma progesterone levels were recorded. Because of such a wide range in recorded results, statistical analysis of progesterone levels showed no significance. If serum samples had reached a more uniform range of percent antibody binding rate, results may have become significant and supported the hypothesized prevention of luteal development.

Table 1. Ages (years) and weights (kg) of Quarter Horse mares treated with serial injections of PGF<sub>2α</sub>

Mare	Age	Weight
1	22	558
2	6	457
3	22	564
4	6	395
5	18	518
6	10	498
7	15	505
8	12	573
9	15	493
10	24	470
11	12	550

Table 2. Order of treatment protocol received for mares treated with serial injections of PGF<sub>2α</sub>

Mare	Treatment 1 <sup>*</sup>	Treatment 2 <sup>**</sup>	Treatment 3 <sup>***</sup>
1	A	B	C
2	C	A	B
3	C	A	B
4	A	C	B
5	C	A	B
6	B	A	C
7	B	C	A
8	C	A	B
9	C	B	A
10	B	A	C
11	A	B	C

A- First treatment

B- Second treatment

C- Third treatment

\* - One injection of PGF<sub>2α</sub> 6 d post-ovulation

\*\* - Injections of PGF<sub>2α</sub> twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

\*\*\* - Injections of PGF<sub>2α</sub> twice daily on d 2, then once daily on d 3 and 4 post-ovulation

Table 3. Mean number of days recorded from ovulation to ovulation for mares treated with serial PGF<sub>2α</sub> injections, by treatment

Treatment	Mean	SD	Range	Covariance
1 <sup>a</sup>	14.8	3.1	9-18	8.76
2 <sup>b</sup>	12.9	6.3	4-29	36.29
3 <sup>c</sup>	14.3	1.4	12-17	1.78

<sup>a</sup>- One injection of PGF<sub>2α</sub> 6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2α</sub> twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2α</sub> twice daily on d 2, then once daily on d 3 and 4 post-ovulation

Table 4. Mean number of days recorded from first day of treatment to ovulation for mares treated with serial injections of PGF<sub>2α</sub>, by treatment

Treatment	Mean	SD	Range	Covariance
1 <sup>a</sup>	9.8	2.0	5-12	3.76
2 <sup>b</sup>	12.9	6.3	4-29	36.29
3 <sup>c</sup>	12.6	1.7	10-16	2.47

<sup>a</sup>- One injection of PGF<sub>2α</sub> 6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2α</sub> twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2α</sub> twice daily on d 2, then once daily on d 3 and 4 post-ovulation

Table 5. Mean number of days recorded from ovulation to detection of a 30+ mm follicle for mares treated with serial injections of PGF<sub>2α</sub>, by treatment

Treatment	Mean	SD	Range	Covariance
1 <sup>a</sup>	13.2	2.2	9-16	4.17
2 <sup>b</sup>	9.2	3.0	3-13	8.16
3 <sup>c</sup>	12.0	2.0	9-15	3.56

<sup>a</sup>- One injection of PGF<sub>2α</sub> 6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2α</sub> twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2α</sub> twice daily on d 2, then once daily on d 3 and 4 post-ovulation

Table 6. Mean number of days recorded from first day of treatment to detection of a 30+ mm follicle for mares treated with serial injections of PGF<sub>2α</sub>, by treatment

Treatment	Mean	SD	Range	Covariance
1 <sup>a</sup>	8.1	1.1	7-10	1.11
2 <sup>b</sup>	9.5	2.7	3-13	6.65
3 <sup>c</sup>	10.3	1.7	7-13	2.44

<sup>a</sup>- One injection of PGF<sub>2α</sub> 6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2α</sub> twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2α</sub> twice daily on d 2, then once daily on d 3 and 4 post-ovulation



Table 7. Mean number of days recorded from ovulation to detection of the onset of estrus (teasing score  $\geq$  T3) for mares treated with serial injections of PGF<sub>2 $\alpha$</sub> , by treatment

Treatment	Mean	SD	Range	Covariance
1 <sup>a</sup>	10.6	4.4	1-16	17.64
2 <sup>b</sup>	6.9	2.6	3-11	5.86
3 <sup>c</sup>	10.3	6.8	4-24	39.35

<sup>a</sup>- One injection of PGF<sub>2 $\alpha$</sub>  6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2 $\alpha$</sub>  twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2 $\alpha$</sub>  twice daily on d 2, then once daily on d 3 and 4 post-ovulation

Table 8. Mean number of days recorded from first day of treatment to detection of the onset of estrus (teasing score  $\geq$  T3) for mares treated with serial injections of PGF<sub>2 $\alpha$</sub> , by treatment

Treatment	Mean	SD	Range	Covariance
1 <sup>a</sup>	5.9	3.6	0-12	11.49
2 <sup>b</sup>	8.1	5.2	3-20	23.88
3 <sup>c</sup>	6.0	3.9	1-12	12.67

<sup>a</sup>- One injection of PGF<sub>2 $\alpha$</sub>  6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2 $\alpha$</sub>  twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2 $\alpha$</sub>  twice daily on d 2, then once daily on d 3 and 4 post-ovulation

Table 9. Mean plasma progesterone concentrations on day 3 post-ovulation for mares treated with serial injections of PGF<sub>2α</sub>, by treatment (ng/mL)

Treatment	Mean	SD	Range	Covariance
1 <sup>a</sup>	6.0	2.5	3.3-9.1	4.57
2 <sup>b</sup>	4.5	1.9	2.6-7.0	3.07
3 <sup>c</sup>	6.4	1.6	3.7-8.9	2.39

<sup>a</sup>- One injection of PGF<sub>2α</sub> 6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2α</sub> twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2α</sub> twice daily on d 2, then once daily on d 3 and 4 post-ovulation

Table 10. Mean plasma progesterone concentrations on day 6 post-ovulation for mares treated with serial injections of PGF<sub>2α</sub>, by treatment (ng/mL)

Treatment	Mean	SD	Range	Covariance
1 <sup>a</sup>	10.9	10.1	2.7-34.2	4.62
2 <sup>b</sup>	3.9	2.0	1.6-7.0	3.33
3 <sup>c</sup>	3.9	1.9	1.8-6.2	7.29

<sup>a</sup>- One injection of PGF<sub>2α</sub> 6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2α</sub> twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2α</sub> twice daily on d 2, then once daily on d 3 and 4 post-ovulation

## CONCLUSIONS

In this study, no significant difference was shown between single and multiple-dose PGF<sub>2α</sub> treatment protocols used to prevent luteal development and shorten the interval to ovulation in cycling mares. However, low-dose administration of PGF<sub>2α</sub> may provide an alternative to the 10.0 mg dose normally given. Low-dose administration of PGF<sub>2α</sub> during the early diestrus period of the estrous cycle has the potential to improve the efficiency of breeding programs in terms of total injections administered and intensity of the protocol.

Previous studies implementing a single-dose treatment as a control have administered PGF<sub>2α</sub> as a single injection between days 5 and 10 post-ovulation, during the mid-diestrus period. However, the CL has been shown to be responsive to PGF<sub>2α</sub> when the CL reaches maturity at 5 days post-ovulation; therefore the single dose treatment used in this study administered one injection of PGF<sub>2α</sub> on day 6 post-ovulation.

Results of the study were similar to previous studies implementing the protocol used for Treatment 3. Treatment 3 could be utilized by owners or breeding facility professionals if an ovulation goes undetected. However, if the exact date of ovulation is unknown, treatment with serial PGF<sub>2α</sub> may not be as effective at shortening the interval to ovulation when the treatment is started a few days later. In terms of efficacy, PGF<sub>2α</sub> treatment is most successful when ovulation can be tracked and recorded, and a treatment protocol implemented accordingly.

Although overall results were not significant, the statistical analysis with outliers removed supports the hypothesis that serial administration of PGF<sub>2α</sub> may prevent luteal

development and may provide a means of faster manipulation of the mare in order to successfully complete time-sensitive breedings and embryo transfer programs.

Breeding facilities often charge owners for individual daily expenses of management- boarding, feed and supplements, palpation, ultrasound, injections, insemination, pregnancy checks, and more. With high costs associated with long-term boarding at breeding facilities, shortening the interval to ovulation by even 2 days could provide financial savings for equine owners. Example costs associated with daily mare care and management at a breeding facility are as follows:

Description	Cost
Dry Mare Board	\$12.00-20.00/day
Dry Mare Board, Under Lights	\$17.00-20.00/day
Private Care Board	\$20.00-29.00/day
Special Care Board	\$20.00-29.00/day
Palpation	\$15.00-16.00 each
Palpation and Breeding	\$10.00-25.00-each
Ultrasound	\$40.00-65.00/breeding season
Progesterone Assay	\$30.00-35.00 each
Regumate	\$3.50-6.00/day

(Granada Farms, 2017; Lazy E Ranch, 2017; Oswood Stallion Station, 2017)

Lutalyse, when administered at 1.1 mL per dose, is approximately \$0.64 per injection. With minimal daily mare care starting approximately \$17 per day, utilization of a low-dose serial PGF<sub>2α</sub> treatment protocol may provide significant savings. At a low-cost, serial administration of PGF<sub>2α</sub> may provide a relatively inexpensive method of

shortening the interval to ovulation. The estimated costs per treatment protocol is described as follows:

Description	Cost
1.0 mL (5.0 mg) Lutalyse	\$0.57-0.67/dose
2.0 mL (10.0 mg) Lutalyse	\$1.15-1.36/dose
Treatment 1 Protocol <sup>a</sup>	\$0.57-0.67/mare
Treatment 2 Protocol <sup>b</sup>	\$4.56-5.36/mare
Treatment 3 Protocol <sup>c</sup>	\$2.28-2.68/mare

(Valley Vet Supply, 2017)

<sup>a</sup>- One injection of PGF<sub>2α</sub> 6 d post-ovulation

<sup>b</sup>- Injections of PGF<sub>2α</sub> twice daily on d 0,1, and 2, then once daily on d 3 and 4 post-ovulation

<sup>c</sup>- Injections of PGF<sub>2α</sub> twice daily on d 2, then once daily on d 3 and 4 post-ovulation

Further research is needed to determine the effects of serial low-dose administration of a PGF<sub>2α</sub> analog on the interval to ovulation in mares.

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