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Original Research

Effect of Cloprostenol Administration on Interval to Subsequent Ovulation and Anovulatory Follicle Formation in Quarter Horse Mares

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A B S T R A C T

Prostaglandin F2α (PGF) treatment is routinely used in the reproductive management of mares to induce luteolysis and allow a subsequent return to estrus. The objective of this retrospective study was to assess the effect of follicle size at the time of administration of cloprostenol on interval to subsequent ovulation. A secondary objective was to determine the incidence of hemorrhagic anovulatory follicle (HAF) formation after PGF administration. Reproductive records of 275 mares monitored over a total of 520 estrous cycles were evaluated. All mares received a single intramuscular dose of 250 μg of the synthetic PGF analog cloprostenol sodium between days 5 and 12 after ovulation. The average interval from PGF to ovulation was 8.4 ± 2.5 days. The interval from PGF administration to subsequent ovulation was inversely proportional to the diameter of the largest follicle at the time of treatment. Administration of cloprostenol to mares with a large (≥35 mm in diameter) diestrous follicle resulted in one of three outcomes—ovulation within 48 hours (13.4%) with variable uterine edema, ovulation after 48 hours usually accompanied by the presence of uterine edema (73.1%), or regression without ovulation followed by emergence and eventual ovulation of a new dominant follicle (13.4%). There was no effect of mare age or season on interval from PGF to ovulation. The overall incidence of HAF development after PGF administration in this study was low (2.5%).

1. Introduction

Prostaglandins (PGFs) are commonly used in the reproductive management of mares as either a luteolytic or ecbolic agent [1–5]. Prostaglandins may be effective at inducing complete luteolysis if administered at least 4 days after ovulation [1], but the general convention in equine clinical practice is that PGFs are not routinely administered until the corpus luteum is at least 5 days old [2,6,7]. The intervals from PGF administration to initial return to estrus and subsequent ovulation are 3–4 days and 6–12 days, respectively [1,8,9]. The size of the dominant follicle at the time of PGF administration has been reported to be inversely correlated with the interval to subsequent ovulation [2,7,10]. For example, mares with small follicles at the time of PGF administration take longer to ovulate after PGF administration than mares with moderately sized follicles. It is important to note that mares with a large diestrous follicle (i.e. greater than 35–40 mm in diameter) may ovulate within 24–72 hours after PGF treatment without coming into behavioral estrus or developing endometrial edema [2,11–13]. In addition, Newcombe et al [7] reported that the interval from PGF to ovulation is shorter when larger doses of PGFs are administered in mid-diestrus, an effect that was observed at all follicle sizes. The dose of

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PGFs and follicle diameter at treatment also influenced the percentage of follicles that regressed after PGF administration, with lower doses and larger follicles more likely to be associated with follicular regression and replacement by another follicle.

Reports by Ginther and Al-Mamun [14] and Cuervo-Arango and Newcombe [15] indicate that the number of large dominant follicles and the subsequent ovulation rates are both higher after PGF administration than after non-treated or spontaneous cycles. In contrast, Samper et al [13] did not detect an increase in ovulation rate in the subsequent estrus after PGF administration. Similar discrepancies have also been reported for pregnancy rates after PGF administration, with some studies noting a decrease in pregnancy rates [10,16], whereas others report no change in pregnancy rate in the estrus after PGF administration [17].

Conflicting reports have also suggested either no association between administration of PGFs and development of a hemorrhagic anovulatory follicle (HAF) [7] or that administration of PGFs in diestrus is associated with an increased risk of development of a HAF [14,18,19]. Development of a HAF was not reported in earlier studies on the effects of PGF administration on subsequent follicular development and ovulation and is not a common observation in our clinical equine reproduction practice.

The primary objectives of the current retrospective study were to reassess the effect of follicle size at the time of administration of cloprostenol on interval to subsequent ovulation. A second objective was to determine incidence of HAF formation after PGF administration.

2. Materials and Methods

2.1. Mares

Reproductive records of American Quarter Horse mares housed and managed at the Equine Reproduction Laboratory, Colorado State University, between 2006 and 2013 were evaluated retrospectively. Mares and individual cycles were included in the study only if (1) the diameter of the largest follicle on each ovary was measured at the time of PGF administration; (2) serial reproductive evaluations were subsequently performed to monitor follicular development and determine whether the mare ovulated, the number of ovulations, and the day of ovulation; and (3) no hormones, such as human chorionic gonadotropin (hCG) or deslorelin acetate, were administered in the subsequent estrous period to induce an early ovulation.

2.2. Cloprostenol Treatment

A single intramuscular dose of 250 μg (1.0 mL) of the synthetic prostaglandin F2α analog cloprostenol sodium (Estrumate; Merck Animal Health, Summit, NJ) was administered between days 5 and 12 after ovulation. All mares were treated with cloprostenol; there was no untreated control group in this retrospective evaluation of privately owned mares in our clinical practice.

2.3. Reproductive Evaluations

An ultrasound examination (7.5 mHz; EXAGO; Echo Control Medical, Angoulême, France) was performed immediately before PGF administration, and the diameters of the two largest follicles on each ovary were recorded. Mares with follicle(s) less than 30 mm in diameter were initially evaluated 4 days after PGF administration. Mares with follicles 30–34 mm in diameter were examined 2 days after PGF treatment, and mares with a diestrous follicle of ≥35 mm in diameter were examined the day after PGF treatment. Ultrasound examinations were performed daily on all mares once the dominant follicle was at least 30 mm in diameter and continued until ovulation, follicle regression, or formation of a HAF was detected. Follicle regression was defined as a decrease in diameter of the dominant follicle without ovulation (i.e., atresia) and replacement by development of a new dominant follicle. A HAF was defined as a dominant follicle that initially developed echogenic particles in the follicular lumen, followed by echogenic strands and eventually complete infiltration with echogenic tissue in the absence of a discernible ovulation.

2.4. Statistical Analysis

Diameter of the largest follicle at the time of PGF administration was divided into subcategories for statistical analysis (<10, 10–14, 15–19, 20–24, 25–29, 30–34, and ≥35 mm). Continuous data were compared using a one-way analysis of variance with post hoc analysis by Student t test using the statistical software (Graphpad). Significance was set at $P < .05$. Categorical data were analyzed by chi-square analysis. All data presented are expressed as mean ± standard error of the mean.

3. Results

Reproductive records of 275 mares monitored over a total of 520 estrous cycles were evaluated. Mares ranged in age from 4 to 14 years.

Overall, the average interval to ovulation after cloprostenol administration to diestrous mares was 8.4 ± 2.5 days. The interval from PGF administration to subsequent spontaneous ovulation was inversely proportional to the diameter of the largest follicle at the time of treatment, if the follicle was <35 mm in diameter and went on to ovulate (Table 1).

Table 1
Interval to ovulation after administration of cloprostenol sodium to diestrous mares with a dominant follicle less than 35 mm in diameter.

Follicle Size (mm)	Number of Cycles	Interval to Ovulation (d)
<10	6	11.8 ± 1.1[a]
10–14	74	10.2 ± 0.2[b]
15–19	83	9.1 ± 0.2[c]
20–24	118	9.1 ± 0.2[c]
25–29	122	8.0 ± 0.2[d]
30–34	37	7.8 ± 0.5[d]

[a,b,c,d]Data within column with different superscript letters are significantly different ($P < .05$).

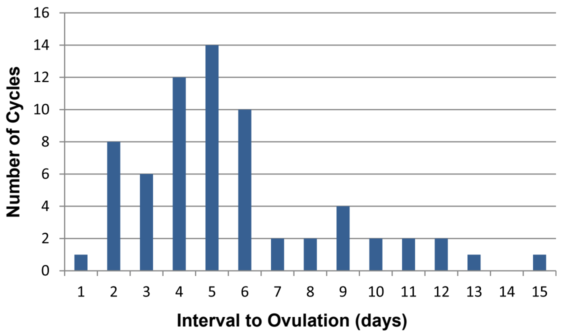

Fig. 1. Interval from cloprostenol to subsequent ovulation in mares with a large diestrous follicle (≥35 mm in diameter).

Cloprostenol was administered to 67 mares with a large (≥35 mm in diameter) diestrous follicle (Fig. 1). Three outcomes were observed after PGF treatment to these mares—ovulation within 48 hours (13.4%), ovulation after 48 hours accompanied by the presence of uterine edema (73.1%), or regression without ovulation followed by emergence and eventual ovulation of a new dominant follicle (13.4%; Table 2). This follicular response after PGF administration was reflected in the average interval to subsequent ovulation. Six of the nine mares (67%) that ovulated within 48 hours after PGF treatment developed uterine edema before or on the day of ovulation.

Formation of a HAF was observed in 13 of 520 cycles (2.5%) after PGF administration (Table 3). There was no correlation between follicle size at the time of PGF administration and incidence of HAF formation. There was no correlation between the day of PGF administration and incidence of HAF formation. No mares exhibited more than one HAF during any one breeding season.

4. Discussion

Administration of exogenous PGFs is used in broodmare practice to either cause destruction of the corpus luteum (luteolytic effect) or stimulate uterine contractions (ecbolic effect) [2,5]. Clinical uses of PGFs that are based on the luteolytic effect are short-cycling, estrous synchronization, treatment of a persistent corpus luteum, and termination of an unwanted pregnancy. Complete luteolysis is generally achieved if PGFs are administered when the ovarian corpus luteum is mature, or from approximately 5 days postovulation onward. Administration of a single dose of PGFs either the day ovulation is detected or 1–2 days after ovulation will adversely affect development of the corpus luteum but will not result in complete luteolysis [20–24]. However, twice daily administration of PGFs in the early postovulation period may result in failure of a corpus luteum to develop (i.e., an anti-luteogenic effect) [25].

Mares in this study were specifically selected that did not receive an ovulation induction agent, such as hCG or deslorelin, as the timing and administration of such an agent would alter the natural interval from PGF treatment to subsequent ovulation. In addition, fertility after PGF administration was not determined as mares were not bred during the subsequent estrous period.

The relationship between follicle size at the time of PGF administration and subsequent interval to ovulation has been described previously [8,11–13]. Results from the present study are in agreement with results previously published in that mares with small follicles at the time of PGF treatment take longer to develop a dominant follicle and ovulate than mares with a larger diestrous follicle. In addition, the present study reconfirms earlier reports that some mares with large (i.e. >35 mm) diestrous follicles at the time of PGF administration will ovulate within 48 hours after PGF administration [6,11,12]. In the present study, 67% of mares that ovulated within 48 hours after PGF developed uterine edema before or at the time of ovulation.

Samper et al [13] noted that the rate and degree of uterine edema development after PGF administration can be used to differentiate between a viable diestrous follicle and an atretic diestrous follicle. Results of the present study are in agreement with the study by Samper et al in that a majority of mares with a large viable diestrous follicle developed uterine edema within 24 hours after PGF treatment.

Overall, breeding management can be optimized by evaluation of follicle size at the time of PGF treatment and subsequent monitoring of follicular dynamics after treatment. The eventual fate of a large diestrous follicle cannot be routinely predicted at the time of PGF administration, but a reexamination of the mare 48 hours after treatment is usually diagnostic.

Administration of PGFs has been reported to increase the ovulation rate in mares [18,14]. The average ovulation

Table 2
Interval to ovulation (days) after cloprostenol administration to mares with a diestrous follicle ≥35 mm in diameter.

Category	Number of Cycles	Interval to Ovulation (d)
Ovulation within 48 hr after PGF administration	9	1.9 ± 0.1^a
Ovulation more than 48 hr after PGF administration	49	5.3 ± 0.3^b
Regression and replacement of dominant follicle	9	10.5 ± 0.9^c

Abbreviation: PGF, prostaglandin F2α.
[a,b,c]Data within column with different superscript letters are significantly different ($P < .05$).

Table 3
Incidence of hemorrhagic anovulatory follicle formation relative to follicle diameter at the time of cloprostenol administration to mares in diestrus.

Follicle Size	Total Number of Cycles	HAF Formation (%)
<10	6	0 (0)
10–14	77	3 (3.9)
15–19	84	1 (1.2)
20–24	120	2 (1.6)
25–29	122	0 (0)
30–34	39	2 (5.3)
>35	69	2 (2.9)
Not recorded	3	3
Overall	520	13 (2.5)

Abbreviation: HAF, hemorrhagic anovulatory follicle.

rate in the present study (1.1 ± 0.3 ovulations per cycle) was similar to that reported previously at our facility for untreated mares (1.1 ± 0.2 to 1.2 ± 0.5 ovulations per cycle) [26,27] and was therefore not apparently influenced by PGF administration.

Mares may develop one of two subtypes of anovulatory follicles [28]. A majority of anovulatory follicles (85%) are associated with hemorrhage and eventual luteinization. These so-called "HAFs" occur when significant bleeding occurs into the lumen of the dominant follicle. Initially, the blood does not clot because of anticoagulant factors present in equine follicular fluid [29]. Eventually, the blood does clot and the fibrin scaffolding within the blood clot allows granulosa and theca cells to invade, multiply, and luteinize, forming a "luteinized anovulatory follicle" that produces progesterone. A lower percentage (15%) of anovulatory follicles remain as a nonviable or atretic "persistent anovulatory follicle" without significant hemorrhage into the follicular lumen and do not luteinize and therefore do not produce progesterone [28].

The overall incidence of HAF formation after PGF administration in the present retrospective study was 2.5%. This percentage is notably different than data published by Cuervo-Arango and Newcombe [18] in which an increased incidence of hemorrhagic anovulatory formation was documented after cloprostenol administration to two mares (35.8% incidence rate) compared with untreated cycles (6.1% incidence rate). The differences in HAF formation between the studies may be because of the number of mares in each study, breed of mare, geographic location, timing of PGF treatment, dose of PGF administered, or degree of reproductive management.

A hypothesis for HAF formation after PGF administration is that a decline in progesterone levels after luteolysis allows for prolonged increased levels of luteinizing hormone (LH) at an earlier stage of follicular growth, which predisposes the follicle to later development into an HAF [14,30]. However, a recent study reported that premature exposure of mares to increased concentrations of LH did not disrupt follicular growth patterns or ovulation, but did alter follicular fluid factors that could disrupt oocyte or follicle maturation [31].

In our breeding program, a reproductive evaluation, consisting of manual palpation and an ultrasound examination, is performed on all mares before administration of PGFs. Reasons for the pre-PGF examination include (1) correct identification of mare to avoid inadvertent administration of PGF to the wrong mare; (2) confirmation that the mare is not pregnant; (3) determination if a corpus luteum is present; (4) evaluation for the presence of uterine or cervical tone and uterine edema; and (5) measurement of the diameter of the largest follicle on each ovary. The objectives of the latter three qualifiers are to determine whether an active corpus luteum is present and enable a prediction of when the mare will return to estrus and an approximate interval to subsequent ovulation.

Administration of PGFs is used in clinical practice to lyse the corpus luteum and allow for an early return to estrus and early ovulation as compared with letting the mare go through a normal luteal phase. Information on the clinical response of mares to PGFs based on follicle size at the time of treatment provided in this study can be used to predict when a mare is likely to return to estrus and when she is likely to ovulate after PGF administration. In some instances, administration of PGFs can be scheduled so that return to estrus and potential ovulation will match stallion availability and semen shipment options, as well as avoid time periods when the stallion is not available, such as weekends, shows, or other events. An ability to predict interval from PGF to ovulation is also useful in avoiding additional shipment costs associated with counter–counter semen shipments on weekends or holidays.

In summary, the average interval from PGF administration to subsequent ovulation was 8.4 ± 2.5 days. There was an inverse correlation between follicle diameter at the time of PGF treatment and subsequent interval to ovulation. Mares with large (>35 mm) diestrous follicles present at the time of PGF administration may ovulate within 48 hours or may ovulate at a variable interval after 48 hours depending on if the large follicle continues to develop or if that follicle regresses and is replaced by another dominant follicle. The incidence of HAF formation after PGF treatment was low.

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