Luteal Regression And Follicle Development Following Prostaglandin-F2α Treatment 3 Days After Ovulation In Mares

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Complete and sustained structural and functional demise of the primary corpus luteum (CL) in response to prostaglandin-F$_{2\alpha}$ (PGF) treatment less than 5 days after ovulation has not previously been documented. The present study was designed to compare the morphological and physiological response of the primary CL to PGF given at early diestrus with a more conventional treatment given at about mid-cycle. In addition, follicle status pre- and post-treatment were examined and compared between the treatment groups. On the day of pretreatment ovulation (Day 0), riding-type horse mares were randomly assigned to receive a single dose of PGF (Lutalyse; 10 mg/mare, i.m.) on Day 3 (n=17) or Day 10 (n=17). Beginning on either Days 3 or 10, transrectal ultrasonography was used to determine follicle and CL diameters, luteal tissue gray-scale scores (echogenicity), and to detect ovulation. Follicular and luteal measurements and jugular blood samples were collected daily until the post-treatment ovulation. Structural and functional regression of the CL was indicated by: 1) a progressive decrease (Day effect; P<0.0001) in mean diameter of the CL beginning 24 h after PGF treatment in the Day 3 and Day 10 groups; 2) a precipitous decrease (P<0.009) in mean plasma progesterone concentrations within 24 h in both groups followed by a more gradual decline to basal concentrations by the second day in the Day 10 group or after the fourth day in the Day 3 group; and 3) an increase (P<0.02) in mean luteal tissue echogenicity in both groups after the second day following PGF treatment. The mean intervals from PGF treatment to ovulation were not different (P>0.2) between groups (combined, 9.9 days) but the mean (+SEM) interovulatory interval was shorter (P<0.0001) in the Day 3 group (13.2±0.9 days; range, 7 to 20 days) than in the Day 10 group (19.2±0.7 days; range, 14 to 26 days). The greater the diameter of the largest follicle at the time of PGF treatment the shorter the interval to subsequent ovulation in the Day 3 (r = -0.57, P<0.02) and Day 10 (r = -0.74, P<0.001) groups. Growth rates of the preovulatory follicles were similar (P>0.59) between groups (combined, 3.6 mm/day) but the maximum diameter was smaller (P<0.05) in the Day 3 group (40.5±1.2 mm) compared to the Day 10 group (43.4±0.8 mm). Unexpectedly, more (P<0.03) double ovulations occurred in the Day 3 group (6/17, 35%) than in the Day 10 group (1/17, 6%). In conclusion, an immature CL at early diestrus responded to PGF treatment in a manner comparable to a mature CL at mid-cycle. The Day 3 group ovulated an average of 6 days earlier than the Day 10 group as a result of the difference in timing of the treatments. Thus, these results warrant a reassessment of the prevailing concept that the equine CL is resistant, insensitive or refractory to PGF-induced regression before 5 days after ovulation, especially when considering the potential benefits of a shortened interovulatory interval and an increased double ovulation rate.
Hypothesis and Objectives

Hypothesis

– Mares ovulate at similar intervals after a luteolytic dose of PGF$_{2\alpha}$ regardless of the time of administration during diestrus.

Objectives

– To compare the morphologic and physiologic response of the primary CL to a single dose of native PGF$_{2\alpha}$ administered on Day 3 versus Day 10 post-ovulation.

– Secondarily, compare the follicle status pre- and post-treatment between the Day 3 and Day 10 treatment groups.
INTRODUCTION

• Native prostaglandin-\(F_{2\alpha}\) and analogues (PGF) have been used alone or in combination with gonadotropins (FSH and LH) and steroids (estrogen and progestogens) to manipulate and control ovarian function for basic and applied purposes.

• PGF treatment during the periovulatory period after insemination induced functional regressive changes in corpora lutea (CL) during early diestrus which seems contrary to the prevailing concept that an immature CL is resistant to PGF-induced luteolysis before 5 days after ovulation.

• Day 10 was chosen to compare with Day 3 because, according to previous studies, the middle of the estrous cycle represents a time when PGF consistently induced complete luteal gland regression in the mare.
MATERIALS AND METHODS

- Riding-type horse mares ranging in age from 4 to 16 yr and weighing 400 to 550 kg were kept under natural light in outdoor paddocks during July to September and had free-choice access to alfalfa/grass hay, water and trace-mineralized salt.
- Transrectal ultrasonographic scanning was used to monitor follicle and luteal gland development and to detect ovulation (Day 0).
- On Day 0, mares were randomly assigned to a Day 3 group (n=17) or a Day 10 group (n=17).
- On Days 3 or 10, mares received a single dose of PGF (10 mg/mare, i.m.).
- The dose of PGF was standardized for all mares according the estimated mean body weight and the manufacturer recommended dose (Lutalyse; 0.02 mg/kg body weight).
MATERIALS AND METHODS

• Experimental data were collected beginning on either Day 3 or Day 10.

• Mares in both groups were scanned and had blood collected daily until post-treatment ovulation of the largest follicle.

• Diameters of the two largest follicles and diameter and luteal tissue echo texture score of the primary CL were determined.

• Follicle and CL diameters were determined by calculating the average of two lines of measurement from an image frozen at its maximal cross-sectional area.

• Luteal tissue echo textural scores were determined by assigning a gray-scale score to the luteal portion of the gland.
  – Scores ranged from 0 to 6 (lower scores represented darker, less dense or anechoic tissue and higher scores represented lighter, more dense or echoic tissue).
MATERIALS AND METHODS

- Growth rates of the largest follicles were determined by subtracting the smallest follicle diameter concomitant with the beginning of a consistent increase in diameter from the maximum diameter and dividing by the number of days between observations for each mare.
- Blood was collected from the jugular vein into heparinized tubes, centrifuged, decanted and stored frozen (-20°C) until assayed for progesterone.
- Plasma progesterone concentrations were determined using a commercial kit modified and validated for use in horses in our laboratory (19).
- Over 5 assays, the intra- and inter-assay CV and mean sensitivity were 13.3%, 12% and 0.04 ng/ml, respectively.
MATERIALS AND METHODS

• SAS MIXED for repeated measures were used to determine the main effects of group and day and the group-by-day interaction for the day-to-day follicle, luteal and progesterone data.

• If significant main effects or interactions were detected, unpaired t-tests were used to determine mean differences between groups within days and unpaired t-tests were used to determine mean differences between days with groups.

• One-way ANOVA was used to determine the differences between groups for single-point measurements.

• Pearson’s correlation coefficient was used to determine the relationship of concentrations of progesterone and diameter of the largest follicle at the time of PGF treatment with the length of the interval to post-treatment ovulation.

• Chi-square was used to compare the number of mares with double post-treatment ovulations between groups.
## Results

<table>
<thead>
<tr>
<th>End points</th>
<th>Groups</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Number of mares</td>
<td></td>
<td>Day 3</td>
<td>Day 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Number of days from:</td>
<td></td>
<td></td>
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<tr>
<td>PGF treatment to last detection of CL</td>
<td>5.8 ± 0.7</td>
<td>4.3 ± 0.6</td>
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<tr>
<td>PGF treatment to ovulation</td>
<td>10.2 ± 0.9</td>
<td>9.2 ± 0.7</td>
<td></td>
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<tr>
<td>Ovulation to ovulation</td>
<td>13.2 ± 0.9</td>
<td>19.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td></td>
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<tr>
<td>Largest follicle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (mm) at PGF treatment</td>
<td>19.0 ± 1.0</td>
<td>20.2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Maximum diameter (mm)</td>
<td>40.5 ± 1.2</td>
<td>43.4 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Growth rate (mm/day)</td>
<td>3.7 ± 0.2</td>
<td>3.5 ± 0.3</td>
<td></td>
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<tr>
<td></td>
<td>x</td>
<td>y</td>
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<tr>
<td>Second-largest follicle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (mm) at PGF treatment</td>
<td>14.9 ± 0.9</td>
<td>17.2 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Maximum diameter (mm)</td>
<td>28.6 ± 1.6</td>
<td>26.6 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Number of mares with double ovulations</td>
<td>6 (35%)</td>
<td>1 (6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>x</td>
<td>y</td>
<td></td>
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</tbody>
</table>

*Differences between groups abP<0.0001 and xyP<0.05*
Progesterone

Plasma concentration (ng/ml)

Days from PGF treatment

Day 10 group

Day 3 group

G: P<0.001
D: P<0.0001
GD: P<0.0001
Corpus Luteum Size

- Cross-sectional diameter (mm)
- Days from PGF treatment

- G: P<0.05
- D: P<0.0001
- GD: P<0.05
Luteal Tissue Echo Texture Score

Gray scale (score)

Days from PGF treatment

G: $P<0.002$
D: $P<0.002$
GD: NS
Day 3 Group

Number of mares ovulating vs Days from ovulation

PGF
Day 10 Group

Number of mares ovulating

Days from ovulation

PGF
Day 3 Group

Diameter (mm) of the largest follicle

Days from PGF treatment

$r = -0.57$

$P < 0.02$
Day 10 Group

Diameter (mm) of largest follicle vs. Days from PGF treatment

$r=-0.74$

$P<0.001$
Follicular Profiles

Largest follicle

Day 10 group

Day 3 group

Second-largest follicle

Day 10 group

Day 3 group

G: P<0.04
D: P<0.0001
GD: NS

G: NS
D: P<0.0001
GD: P<0.007

G: P<0.02
D: P<0.0001
GD: NS

G: P<0.08
D: NS
GD: NS

Days from PGF Treatment

Days Preceding Ovulation
Summary

The hypothesis that mares ovulate at similar intervals after a luteolytic dose of PGF regardless of the time of administration during diestrus (Day 3 versus Day 10 post-ovulation) was supported as indicated by:

- Significant precipitous decrease in mean plasma progesterone concentrations within 24 h of treatment.

- Significant progressive decrease in mean diameter of the CL within 24 h treatment.

- Significant increase in mean luteal tissue echo texture 48 h after treatment.
Summary

Early demise of the CL was associated with:

• Significantly shorter interovulatory interval in the Day 3 group compared to the Day 10 group. (*regardless of group, the greater the diameter of the largest follicle at the time of PGF treatment the shorter the interval to subsequent ovulation*).

• Significantly more double ovulations in the Day 3 group compared to the Day 10 group. (*which may be attributed to the smaller maximum diameter of the largest follicle in the Day 3 group compared to the Day 10 group*).
Conclusion

• A single dose of native PGF (*10 mg/mare*) can induce complete physiological and morphological regression of the primary CL regardless of the stage of diestrus in a majority of mares such that the interovulatory interval was shortened and more double ovulations occurred following treatment on Day 3 *versus* Day 10 post-ovulation.

• The prevailing concept that the primary CL in the mare is resistant, insensitive or refractory to PGF-induced regression before 5 days after ovulation needs to be reassessed, especially considering the practical benefits of administering treatment during early diestrus.