Effect of repeated administration of oxytocin during diestrus on duration of function of corpora lutea in mares

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<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>CL</th>
<th>Corpora lutea</th>
<th>PG</th>
<th>Prostaglandin</th>
</tr>
</thead>
</table>

**Objective**—To determine whether IM administration of exogenous oxytocin twice daily on days 7 to 14 after ovulation blocks luteolysis and causes prolonged function of corpora lutea (CL) in mares.

**Design**—Prospective study.

**Animals**—12 mares.

**Procedures**—Beginning on the day of ovulation (day 0), jugular blood samples were collected every other day until day 40 for determination of progesterone concentration. On day 7, mares (n = 6/group) were treated with saline (0.9% NaCl) solution (control group) or oxytocin. Beginning on day 7, control mares received 3 mL of sterile saline solution every 12 hours, IM, and oxytocin-treated mares received 60 units of oxytocin every 12 hours, IM, through day 14. Mares were considered to have prolonged CL function if progesterone concentration remained >1.0 ng/mL continuously through day 30.

**Results**—The proportion of mares with prolonged CL function was significantly higher in the oxytocin-treated group (6/6), compared with the control group (0/6). All control mares underwent luteolysis by day 16, at which time their progesterone concentrations were <1.0 ng/mL. In contrast, all 6 oxytocin-treated mares maintained progesterone concentrations >1.0 ng/mL continuously through day 30.

**Conclusions and Clinical Relevance**—IM administration of 60 units of oxytocin twice daily on days 7 to 14 after ovulation was an efficacious method of inhibiting luteolysis and extending CL function in mares. Disrupting luteolysis by administering exogenous oxytocin during diestrus appears to be a plausible and practical method of long-term suppression of estrus in mares. (J Am Vet Med Assoc 2007;231:1864–1867)

Administration of exogenous progesterone or synthetic progestins, such as altretonest, to mares is commonly used to block estrus behavior when that behavior is deemed undesirable or interferes with intended activities.1 Intramuscular injection of 0.2 mg of progesterone/kg (0.09 mg/lb) in oil or oral administration of 0.044 mg of altretonest/kg (0.02 mg/lb) on a daily basis will block estrus behavior; however, the need for daily administration is a drawback of these formulations. In an effort to eliminate the need for daily administration, there have been anecdotal reports of the use in horses of growth-promoting implants containing progesterone and estradiol that are labeled for use in cattle; however, when cattle implants were tested in horses under controlled conditions, they failed to induce blood progesterone concentrations >0.5 ng/mL and they did not suppress estrus behavior.2,6 More recently, a compounded long-acting injectable formula-
addition to the 11 mares that retained the glass ball and never developed extended CL function (ie, continued to cycle normally), 3 of the 7 glass ball–treated mares with extended CL function had 1 or 2 estrous cycles of normal duration after placement of the glass ball before CL function was prolonged. Therefore, on a per-cycle basis, the frequency of prolonged CL function was 11% (7/62 cycles) in the glass ball–treated mares, compared with 8% (47/50 cycles) in the nontreated control mares, which was not significantly different between groups. Because of its variable efficacy among mares and the need to physically remove the glass ball when the resumption of cyclical reproductive activity is desired, placement of an intruterine glass ball does not appear to be an optimal method of suppressing estrus behavior in mares.

In contrast to using an intruterine glass ball to extend CL function, administration of exogenous oxytocin during diestrus is a plausible alternative method of blocking luteolysis to prolong CL function. Endogenous oxytocin secretion is involved in regulating PGF2α secretion from the endometrium during spontaneous luteolysis in mares,8,9 and although administration of exogenous oxytocin to mares around the time of luteolysis (ie, days 11 to 15 after ovulation) stimulates an acute onset of PGF2α secretion,10,12 when oxytocin is administered in the midluteal phase prior to the expected time of luteolysis (ie, before day 10 after ovulation), it does not induce PGF2α secretion and often disrupts luteolysis, causing prolonged CL function.11 Experimentally, continuous infusion of oxytocin by use of a subcutaneous osmotic mini pump from days 8 to 20 after ovulation blocked luteolysis in 4 of 5 mares, whereas luteolysis occurred at the expected time in all 4 control mares that received saline (0.9% NaCl) solution infusion.13 Although it successfully induced prolonged CL function, continuous infusion of oxytocin to disrupt luteolysis would not be a practical method of long-term suppression of estrus behavior. The purpose of the study reported here was to determine whether IM administration of exogenous oxytocin twice daily on days 7 to 14 after ovulation would block luteolysis and cause prolonged CL function in mares.

Materials and Methods

This study was conducted in the Northern Hemisphere (latitude, 47° 7’ N) from September through November with nonlactating mares of mixed breeding that were 3 to 12 years old and that weighed 300 to 500 kg (660 to 1,100 lb). The prior reproductive history of the mares was unknown. This study was approved and conducted following the guidelines of the University of Idaho Institutional Animal Care and Use Committee. The reproductive tracts of cycling mares were examined 3 times weekly via transrectal palpation and ultrasonography. Mares with an ovarian follicle ≥ 30 mm in diameter and prominent endometrial edema were examined every day until ovulation was detected. Ovulation was defined as disappearance of ovarian follicles > 35 mm in diameter between 2 successive examinations and subsequent identification of the CL via ultrasonography. Beginning on the day of ovulation (day 0), blood samples were collected from a jugular vein every other day until day 40. Blood samples were allowed to clot at room temperature (22°C), after which the serum was recovered and kept frozen at −20°C until progesterone was measured via radioimmunoassay.

On day 7, mares were randomly assigned to 1 of 2 groups (n = 6/group) to receive saline solution (control group) or oxytocin. Beginning on day 7, control mares received 3 mL of sterile saline solution every 12 hours, IM, and oxytocin-treated mares received 60 units of oxytocin every 12 hours, IM. Treatments were continued through day 14. The dose of oxytocin used in this study was selected for its intermediate concentration between previously published doses or treatment regimens that have induced prolonged CL function when administered beginning in mid-diestrus (10 units, IV, q 24 h13 and 27.5 U/h, continuous SC infusion).13 On day 18, the reproductive tract of each mare was examined via transrectal palpation and ultrasonography to determine whether there was physical evidence that the animal had undergone luteolysis and returned to estrus (ie, flaccid uterine tone, prominent endometrial edema, or both). Mares were considered to have prolonged CL function if progesterone concentrations remained > 1.0 ng/mL continuously through day 30.

Progesterone was measured directly in unextracted serum by use of a progesterone radioimmunoassay validated for equine serum.2 Sensitivity of the assay was 0.15 ng/mL; values less than the assay sensitivity value were assigned a value equal to the sensitivity value. The intra- and interassay coefficients of variation were 3.2% and 12.1%, respectively. Statistical analysis was performed with computer software.4 The proportion of mares in each group with prolonged CL function was compared by use of the Fisher exact test. A value of P < 0.05 was considered significant.

Results

The proportion of mares with prolonged CL function was higher (P = 0.001) in the oxytocin-treated group, compared with the control group (6/6 vs 0/6, respectively). All of the control mares underwent luteolysis by day 16, at which time their progesterone concentrations were < 1.0 ng/mL (Figure 1). In contrast, all 6 oxytocin-treated mares maintained progesterone concentrations > 1.0 ng/mL continuously through day 30 (Figure 2). The progesterone concentration in one of the oxytocin-treated mares decreased precipitously from 6.3 ng/mL on day 12 to 2.3 ng/mL on day 14 and then remained approximately 2.0 ng/mL through day 30. Progesterone concentrations decreased to < 1.0 ng/mL in 2 of the oxytocin-treated mares between days 30 and 40, whereas the other 4 mares had progesterone concentrations between 3.0 and 7.0 ng/mL through day 40 when blood sampling was discontinued. No adverse effects or reactions to the oxytocin treatment were detected in any of the mares. When the mares were examined on day 18, the physical characteristics of the reproductive tract were consistent with hormonal status; mares that had undergone luteolysis had flaccid uterine tone, prominent endometrial edema, or both. In contrast, mares with prolonged CL function had pronounced uterine tone and an absence of uterine edema. One control mare remained anovulatory after undergo-
Results of this study indicated that twice-daily administration of 60 units of oxytocin on days 7 to 14 after ovulation in mares was an efficacious method of disrupting luteolysis because it caused prolonged CL function in all 6 treated mares. In contrast, all 6 control mares underwent luteolysis at the expected time during their treatment cycle. The mares with prolonged CL function had blood progesterone concentrations > 1.0 ng/mL continuously through day 30, which is a sufficient concentration of progesterone to block estrus behavior\(^2\); therefore, disrupting luteolysis by administering exogenous oxytocin appears to be a plausible method of long-term suppression of estrus in mares. However, because estrus behavior was not monitored in this study, further work will be needed to assess estrus behavior when CL function is extended with oxytocin treatment.

In mares with spontaneously prolonged CL function, the typical duration of extended CL function is approximately 60 days (range, 35 to 95 days).\(^14\) In the present study, progesterone decreased to < 1.0 ng/mL between days 30 and 40 in 2 of the mares with prolonged CL function, whereas the other 4 mares had progesterone concentrations > 3.0 ng/mL through day 40, when blood sampling was discontinued. The cessation of CL function before day 40 in these 2 mares may reflect a seasonal effect on CL function because this study was completed at the end of the physiologic breeding season when gonadotropin secretion wanes and because CL function (ie, progesterone secretion) is dependent on adequate support from endogenous gonadotropin secretion.\(^15\) It is likely that a similar seasonal effect was responsible for one of the control mares remaining anovulatory after undergoing luteolysis during the treatment cycle. Further work will be needed to more fully characterize the duration of CL function when exogenous oxytocin is used to inhibit luteolysis earlier in the physiologic breeding season. The mean duration of prolonged CL function induced by oxytocin treatment will have a direct bearing on the clinical usefulness of this treatment protocol for suppressing estrus behavior in mares.

In nonpregnant mares, the ability of the endometrium to secrete PGF\(_{2\alpha}\) in response to oxytocin (endogenous or exogenous) increases markedly between days 10 and 15 after ovulation concomitantly with an increase in the concentration of oxytocin receptors\(^12,18\) and PGF\(_{2\alpha}\) synthetic enzymes\(^19\) in the endometrial cells. Through day 10 after ovulation, the concentrations of endometrial oxytocin receptors\(^12,18\) and PGF\(_{2\alpha}\) synthetic enzymes\(^19\) are low, which effectively blocks the ability of oxytocin to induce PGF\(_{2\alpha}\) secretion. It is postulated that when continuous (or repeated, as in this study) administration of exogenous oxytocin is initiated prior to day 10 after ovulation, it prevents luteolysis by inhibiting the increase in endometrial oxytocin receptor concentration that would otherwise permit oxytocin-induced PGF\(_{2\alpha}\) secretion at the time of luteolysis\(^13\); however, further work will be needed to confirm that hypothesis. Regardless of the exact mechanism, continuous\(^13\) or repeated administration of oxytocin appears to be an effective method of disrupting spontaneous luteolysis in mares. It is interesting that one of the oxytocin-treated mares in the present study appeared to undergo partial luteolysis because the progesterone concentration decreased precipitously from 6.3 ng/mL on day 12 to 2.3 ng/mL on day 14, which seemed to indicate that the luteolytic mechanism was not completely abrogated.

Figure 1—Serum progesterone concentrations from the day of ovulation (day 0) through day 40 after ovulation in 6 mares treated with 3 mL of sterile saline (0.9% NaCl) solution, IM, twice daily on days 7 to 14 after ovulation.

![Image](image1.png)

Figure 2—Serum progesterone concentrations from the day of ovulation (day 0) through day 40 after ovulation in 6 mares treated with 60 units of oxytocin, IM, twice daily on days 7 to 14 after ovulation.

![Image](image2.png)
by the oxytocin treatment in that mare; despite that, the mare’s progesterone concentration was sufficient to block estrus behavior through day 30.

Intramuscular administration of 60 units of oxytocin twice daily on days 7 to 14 after ovulation was an efficacious method of inhibiting luteolysis and extending CL function in mares. All mares with prolonged CL function had progesterone concentrations > 1.0 ng/mL continuously for > 30 days after ovulation, which is a sufficient concentration of progesterone to inhibit estrus behavior; therefore, disrupting luteolysis by administering exogenous oxytocin appears to be a plausible and practical method of long-term suppression of estrus in mares. An advantage of this method of prolonging CL function is that it should be readily reversible by administering a luteolytic dose of PGF2α (compared with retrieving an intrauterine glass ball), which will initiate resumption of estrous cyclicity (during the physiologic breeding season). To facilitate professional communication of the details of this treatment regimen, we have designated it the “HeatBlock” protocol for extending luteal function in mares.

References

b. DSL-3400, Diagnostic Systems Laboratories Inc, Webster, Tex.
c. SAS, version 9.1, SAS Institute Inc, Cary, NC.