Effect of administering oxytocin or cloprostenol in the periovulatory period on pregnancy outcome and luteal function in mares

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Abstract

Mares (n = 37) were treated from 4 h after breeding through 2 days post-ovulation with oxytocin or cloprostenol. Oxytocin (20 units i.m.) was administered every 6 h and cloprostenol (250 mcg i.m.) daily. Luteal function was impaired for several days following treatment, however, lower progesterone levels among cloprostenol treated mares in this study did not result in decreased pregnancies. Pregnancy outcome at 15 days post-ovulation was not different between the oxytocin (13/18) and cloprostenol (13/19) treatment groups, respectively (P = 0.80). The results of this study indicate cloprostenol can be used to treat post-breeding mares through the second day following ovulation without decreasing pregnancy outcome.

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1. Introduction

Impaired uterine clearance of inflammatory debris is a significant contributor to persistent endometritis in mares. Persistent post-breeding endometritis, in turn, is a major
obstacle to pregnancy. Administration of ecbolics enhances mechanical uterine clearance in mares susceptible to endometritis [1,2]. Enhancing mechanical clearance post-breeding has been advocated for improving pregnancy outcome of mares susceptible to endometritis [3,4].

Oxytocin and cloprostenol are ecbolics administered to enhance mechanical uterine clearance in susceptible mares [1,2]. Cloprostenol has a much longer duration of action than oxytocin [5] and therefore may be a better treatment choice for some mares. Clinically, for a number of years, we have administered either oxytocin or cloprostenol following breeding in most cases. Treatment has continued for up to 48 h post-ovulation in some cases with seemingly good results. Recently however, progesterone concentrations were reported to be lower in mares for 5–7 days following administration of cloprostenol in the post-ovulatory period than in mares receiving oxytocin, saline, sterile water or no treatment [6–9]. This suggests luteal “injury” or incomplete lysis caused by the cloprostenol. Two of the studies reported fewer pregnancies in mares administered cloprostenol in the post-ovulatory period when compared to those receiving oxytocin, saline or sterile water [7,9]. Our clinical observations contradict these findings.

If impairing luteal function shortens luteal lifespan and results in early embryonic death, administration of cloprostenol in the post-ovulatory period would be contraindicated. However, if luteal function is only temporarily impaired without a decrease in pregnancies, cloprostenol could be used safely in mares that would benefit from its ecbolic effect during the post-ovulatory period.

The objective of this study was to determine if luteal function or pregnancy outcome differ among mares administered oxytocin or cloprostenol post-breeding throughout the periovulatory period.

2. Materials and methods

This study was conducted at Auburn University in southeast Alabama between late June and early September in 2001. Horses (Equus caballus) used in the study included light breed mares (n = 37) ranging in age from 2 to 20 years, and a 5-year old Quarterhorse stallion of proven fertility. All mares were judged to be suitable for mating as defined in the breeding terminology section of the American Association of Equine Practitioners Ethics and Professional Guidelines. Mares were housed in groups of four to eight in large paddocks, while the stallion was individually housed in a smaller paddock. All horses were fed a commercial concentrate ration (12% protein) and coastal bermuda grass hay for maintenance of body condition. Horses were maintained in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (First Revised Edition, January 1999). All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at Auburn University (IACUC Protocol No. 0401-R-2373).

Mares were randomized between oxytocin and cloprostenol treatment groups. Each ecbolic was administered at a dose and frequency consistent with our standard post-breeding management of mares. The duration of treatment was meant to simulate our treatment approach for a mare highly susceptible to endometritis and requiring treatment
throughout the post-breeding and periovulatory period. Treatments were administered starting 4 h after insemination and continued through 2 days post-ovulation. All treatment administrations were standardized on the day after insemination to 1200 for each subsequent 24 h period. Mares were treated with oxytocin (20 units i.m.) every 6 h or cloprostenol (Estrumate®, Bayer Corporation, Shawnee Mission, KS, USA; 250 mcg i.m.) every 24 h.

Mares were bred using standard artificial insemination breeding management and techniques [10]. Early in estrus mares were examined every other day by palpation and ultrasonography per rectum for follicles ≥30 mm in diameter. When follicles reached 30–35 mm mares were examined daily. All examinations were conducted in the early morning. When a follicle ≥35 mm was detected, the mare was inseminated with a minimum of 500 million progressively motile sperm and given human chorionic gonadotropin (Novarel®, Ferring Pharmaceuticals, Tarrytown, NY, USA; 2500 units i.v.) to induce ovulation. Semen was collected from the stallion using a CSU model artificial vagina (Animal Reproduction Systems, Chino, CA, USA). All mares were provided routine post-breeding management standard for our unit, including uterine lavage if deemed necessary and daily examinations until the detection of ovulation or resolution of any post-breeding endometritis. The day of ovulation was considered Day 0. Mares were examined for pregnancy on Day 15 following ovulation using palpation and ultrasonography per rectum. The pregnancy examination result on Day 15 was considered the endpoint for effect of treatment on pregnancy outcome.

Blood was collected each morning on Days 0–7, 9, 11, 13, and 15 from all mares and every other day from pregnant mares between Days 15 and 35. Plasma was harvested and frozen for later analysis. Plasma progesterone concentrations were assayed in batches using a commercial RIA kit (Coat-a-Count progesterone radioimmunoassay kit, Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma progesterone concentrations through Day 15 for all mares and Day 35 for pregnant mares were considered the endpoint for effect of treatment on luteal function.

The effect of treatment on pregnancy outcome was analyzed using PROC CATMOD (Statistical Analysis System, SAS Institute, Cary, NC, USA). Repeated measures analysis of progesterone concentrations comparing treatments, oxytocin versus cloprostenol, or outcome, pregnant versus nonpregnant, was performed using PROC GLM (Statistical Analysis System, SAS Institute, Cary, NC, USA).

3. Results

In total, 26/37 mares were diagnosed pregnant on Day 15. Pregnancy outcome was not different between the oxytocin (13/18) and cloprostenol (13/19) treatment groups, respectively (P = 0.80). Embryonic loss was detected in two mares treated with oxytocin. Losses occurred between Days 19 and 21 in the first mare and Days 23 and 25 in the second mare.

The interassay and intra-assay coefficient of variation for the progesterone assay was 6.6 and 3.1%, respectively. The sensitivity of the assay was 0.02 ng/ml. Mean (±S.E.M.) plasma progesterone concentrations are reported in Figs. 1–4 for study mares based on treatment assignment and outcome. Progesterone concentrations rose steadily and were
similar following ovulation in the pregnant and nonpregnant mares. Progesterone concentrations in nonpregnant mares fell below that of the pregnant mares on Day 15 ($P < 0.007$; Fig. 1).

Progesterone concentrations in oxytocin treated mares also rose steadily following ovulation, while concentrations in cloprostenol treated mares rose more slowly and were lower between Days 2 and 9 ($P < 0.01$; Fig. 2). Among mares that became pregnant, oxytocin treated mares had higher circulating progesterone concentrations than did

Fig. 1. Mean plasma progesterone concentrations ($\pm$S.E.M.) in pregnant (■, $n = 26$) and nonpregnant (▲, $n = 11$) mares from Day 0 through Day 15 ($P < 0.007$).

Fig. 2. Mean plasma progesterone concentrations ($\pm$S.E.M.) in oxytocin (Oxy, ▲, $n = 18$) and cloprostenol (Clo, ■, $n = 19$) treated mares from Day 0 through Day 15 ($P < 0.01$).
Fig. 3. Mean plasma progesterone concentrations (±S.E.M.) in oxytocin (Oxy, ▲, \(n = 13\)) and cloprostenol (Clo, ■, \(n = 13\)) treated pregnant mares from Day 0 through Day 35 (*\(P < 0.05\)).
cloprostenol treated mares between Days 2 and 7 \((P < 0.05)\). Concentrations in the cloprostenol treated mares rose to the level of oxytocin treated mares by Day 9 and did not differ throughout the rest of the monitoring period (Fig. 3). Progesterone concentrations were also different among mares grouped by treatment and outcome on Days 2–9 and 15 \((P < 0.05; \text{Fig. 4})\).

### 4. Discussion

The intent of this study was to simulate a worst-case scenario in which a mare highly susceptible to endometritis required ecbolic treatment daily from shortly after insemination through the second day following ovulation. This allowed us to determine whether luteal function or pregnancy outcomes differ in mares administered either oxytocin or cloprostenol following breeding and through the periovulatory period. In a simultaneous study conducted in another group of mares we found results similar to those previously reported, with no differences in progesterone concentrations between oxytocin and saline treated mares \([6,7,11]\).

When cloprostenol is administered through the second day following ovulation luteal function appears to be impaired by Day 2, but seems to rebound by Days 7–9. This finding was consistent with previous reports \([6,7,11]\). The impaired luteal function, however, does not appear to negatively impact pregnancy outcome, because similar proportions of mares were pregnant regardless of the ecbolic administered. This contradicts previous reports in which pregnancy outcome was lower in cloprostenol treated mares than in mares treated with oxytocin, saline or sterile water \([7,9]\). There were some key differences, however,
between our study and the studies previously reported. One study reported using a larger dose of cloprostenol (500 mcg) than was used in our study (250 mcg; [9]). Another study reported administering oxytocin (20 units) only once or twice a day [7]. Treatments were not started until the day of ovulation in the previously reported studies [7,9]. In our study mares were treated from 4 h post-breeding through Day 2 post-ovulation. This more closely simulated clinical reality, as it is unlikely that in a clinical setting treatment would only begin after ovulation had occurred. These differences may have contributed to the improvement in pregnancy outcome observed in our study.

The drop in progesterone concentration observed in nonpregnant mares on Day 15 was expected as the mares returned to estrus (Fig. 1). It is curious though that luteal function in the cloprostenol treated nonpregnant mares did not rebound, following the last treatment, to the extent that was observed in the pregnant mares (Fig. 4). This may suggest a key feedback was absent following luteal injury but present in the pregnant mares and necessary for more complete luteal development.

The early embryonic deaths observed in two of the oxytocin treated mares did not appear to be a result of the periovulatory treatments. Progesterone concentration profiles of the two mares were similar to the mean for all oxytocin treated mares.

Results of our study indicate cloprostenol can be used to treat mares through the second day following ovulation without decreasing pregnancy outcome. Luteal function, however, is impaired for several days. Nevertheless, lower circulating progesterone concentrations for several days among cloprostenol treated mares in our study did not result in decreased pregnancy outcome.

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**References**


Nie GJ, Johnson KE, Wenzel JGW, Braden TD. Luteal function in mares following administration of oxytocin, cloprostenol or saline on Days 0, 1 or 2 post-ovulation. Theriogenology 2003;60:1119–25.