Luteal function in mares following administration of oxytocin, cloprostenol or saline on Day 0, 1 or 2 post-ovulation

Gary J. Nie*, Kristina E. Johnson1, James G.W. Wenzel, Timothy D. Braden2

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL 36849-5522, USA

Received 29 October 2002; accepted 20 December 2002

Abstract

Mares (n = 30) were treated in the post-ovulatory period with saline, oxytocin, or cloprostenol (Clo). Dose, administration frequency and treatment day (Day 0, 1 or 2 post-ovulation) were evaluated. Interovulatory interval of control cycles was 22.7 (±0.36) days with a range of 20.6 (±1.44) to 23.8 (±1.39) days among all treatment groups. Mares treated with two micro-doses of cloprostenol on Day 2 post-ovulation had the shortest interovulatory interval. This group also had the lowest mean circulating progesterone concentrations on Days 3–7 and 13, and was the slowest group to reach concentrations of 5 ng/ml. Repeated administration of cloprostenol over 24 h in the early post-ovulatory period may more effectively impair luteal function than single doses. This could negatively affect pregnancy outcome but may be effective for lysing the early post-ovulatory luteal structure when mares are not bred.

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Keywords: Mares; Oxytocin; Cloprostenol; Post-ovulation; Luteal function

1. Introduction

Oxytocin and prostaglandin F2α (PGF2α) promote myometrial contraction in mares [1]. The ecbolic effect these hormones produce enhances mechanical uterine clearance [2,3].
Enhancing uterine clearance with ecbolics has been advocated for improving fertility of mares susceptible to post-breeding endometritis [4,5]. Cloprostenol, a PGF$_{2\alpha}$ analogue, has a much longer duration of action than oxytocin [5], and therefore may be a better choice for some mares.

Clinically, ecbolics are administered throughout the periovulatory period. Recent reports of the effect of ecbolics on luteal function, however, have raised concern about post-ovulatory use of such treatments [6–9]. Cloprostenol administered in the early post-ovulatory period is reported to impair luteal function for several days [6–9]. This conflicts with the long standing belief that the corpora lutea in mares is resistant to the luteolytic effect of PGF$_{2\alpha}$ from Days 0 to 4 following ovulation [10]. If impairing luteal function soon after ovulation shortens luteal lifespan, administration of cloprostenol to treat post-breeding endometritis in the post-ovulatory period may be contraindicated. However, inducing luteolysis prior to Day 4 may be beneficial for mare management in cycles when breeding does not occur.

The effect periovulatory ecbolics have on interovulatory interval is unknown. Luteal function has not been characterized following treatment with oxytocin and cloprostenol administered at doses and frequencies consistent with clinical applications. Additionally, the effect of cloprostenol administered in micro-doses on luteal function has not been evaluated. Micro-doses of cloprostenol are reported to be equally luteolytic to standard doses at Day 6 post-ovulation [11]. Though luteolytic, the ecbolic effect of a micro-dose of cloprostenol is not known and was not evaluated in this study.

The objective of this study was to determine the effect of saline, oxytocin or cloprostenol on luteal function and interovulatory interval in mares. Dose, administration frequency and treatment day were evaluated.

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 ($n = 30$) ranging in age from 3 to 20 years. Estrous cycles had begun for the season for all mares prior to the start of the study. Mares were housed in groups of four to eight in large paddocks. 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).

The reproductive tract of each mare was evaluated every other day by palpation and ultrasonography per rectum for presence of $\geq 30$ mm follicles. When follicles $\geq 30$ mm were observed, the mare was evaluated daily through ovulation. The day of ovulation was considered Day 0 and the endpoint for determination of interovulatory interval. Blood was collected each morning on Days 0–7, 9, 11, 13, and 15 post-ovulation from all the mares throughout the study. 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 were considered the endpoint for effect of treatment on luteal function.

Mares were assigned three treatment cycles in addition to a control cycle. The cycle order was randomized for each mare. All other treatment group and treatment day assignments were randomized among the study mares to avoid duplication within mare. Data were collected from each mare in successive cycles.

Treatments included saline (1 ml i.m.), oxytocin (Oxy4x and Oxy8x) (20 units i.m.), standard cloprostenol (Estrumate®, Bayer Corporation, Shawnee Mission, KS, USA) (250 µg i.m.), and micro-dose cloprostenol (Clo1x and Clo2x) (25 µg i.m.) groups. Treatments were administered on Day 0, 1, or 2 (D0, D1 or D2) following ovulation in order to evaluate the interaction of treatment and administration day. Saline, Clo and Clo1x group mares were treated once on the assigned treatment day. The Oxy4x, Oxy8x and Clo2x group mares were treated every 6, 3 and 12 h on the assigned treatment day, respectively. During a separate control cycle, no treatment was administered but each mare was monitored by the same protocol as for all other treatments to establish interovulatory and progesterone endpoints for the group of mares used in the study. All treatments were standardized to 1200 on the assigned treatment day. Based on the treatment and day of administration the 19 treatment group designations were Saline-D0, Saline-D1, Saline-D2, Oxy4x-D0, Oxy4x-D1, Oxy4x-D2, Oxy8x-D0, Oxy8x-D1, Oxy8x-D2, Clo-D0, Clo-D1, Clo-D2, Clo1x-D0, Clo1x-D1, Clo1x-D2, Clo2x-D0, Clo2x-D1, Clo2x-D2, and control, respectively.

Interovulatory intervals were compared among treatments and days of treatment using PROC GLM (Statistical Analysis System, SAS Institute, Cary, NC, USA). Repeated measures analysis of progesterone concentration, comparing treatments and days of treatment, was also performed using PROC GLM (Statistical Analysis System).

3. Results

A total of 120 estrous cycles, 90 treatment and 30 control, were evaluated during the study. Each treatment was represented by five estrous cycles on each of 3 treatment days. The mean interovulatory interval of control cycles was 22.7 (±0.36) days with a range among treatment groups of 20.6 (±1.44) to 23.8 (±1.39) days for mares treated with Clo2x-D2 and Saline-D0, respectively. However, the interval was not significantly different among any of the treatment groups ($P = 0.80$).

A total of 1440 plasma samples were assayed for progesterone concentration. The inter-and intra-assay coefficient of variation was 6.6 and 3.1%, respectively. The sensitivity of the assay was 0.02 ng/ml. Differences in circulating progesterone concentrations were observed among treatment groups on Days 2 ($P < 0.054$), 3 ($P < 0.002$), and 4 ($P < 0.01$) post-ovulation (Figs. 1–3). However, the treatment and treatment × day interaction was not significant. Mean circulating progesterone concentrations observed during control cycles were significantly greater than Clo-D1, Clo-D2, Clo2x-D2 and Clo-D2, Clo2x-D2 cycles only on Days 3 and 4, respectively.

Mean progesterone concentrations rose above 5 ng/ml by Day 5 post-ovulation in all treatment groups except Clo2x-D2. By Day 6 progesterone reached 5 ng/ml in the
Clo2x-D2 group though mean concentrations were the lowest observed in any treatment group on Days 3–7 and 13 post-ovulation. It was notable that progesterone concentrations in Clo2x-D2 and Clo-D0 mares consistently hovered near the low end of the range throughout diestrus (Figs. 1 and 3). When comparing only treatments administered on
Day 2, Clo2x-D2 mares had the lowest progesterone concentration on Days 3–13 post-ovulation (Fig. 3). Among treatments administered on Day 0, progesterone concentrations were lower in Clo-D0 mares on Days 2–13 post-ovulation than all other mares (Fig. 1).

### 4. Discussion

In this study we compared the effect of saline, oxytocin and cloprostenol on luteal function and interovulatory interval. Treatments included ecbolics administered at doses and frequencies consistent with our clinical practice as well as micro-doses of cloprostenol. Treatment effect was also compared among administration days. Control cycles from the herd provided baseline data for comparison with the treatment groups while the saline group controlled for the i.m. treatment.

The effect of ecbolics on interovulatory interval has not been evaluated in previous studies [6–9]. In this study, the range of mean interovulatory intervals among treatment groups was relatively large at 3.2 days. The small number of mares in each group might account for the lack of significant differences detected. It was interesting however, that mares treated twice with a micro-dose of cloprostenol on Day 2 post-ovulation had the shortest interovulatory interval at 20.6 (±1.44) days. The luteolytic effect of cloprostenol administered in micro-doses is recognized [11], however the ecbolic effect of such small doses is not known and was not evaluated in this study.

Though differences in mean circulating progesterone concentrations were observed among treatment groups on Days 2, 3, and 4, no particular pattern indicating a greater effect of an individual treatment or day of treatment was evident. It is notable that the Clo2x-D2
group had the lowest mean concentration on Days 3–7 and was the slowest to reach concentrations of 5 ng/ml. It would appear that two doses of cloprostenol administered during the same 24-h period on Day 2 had a profound influence on luteal function, even though the dose administered was a micro-dose. This impact on luteal function was not observed with other doses, frequencies or administration days.

Gunthle et al. [6], evaluated the effect of administration day on progesterone concentrations. When administering 250 μg of cloprostenol, i.m., on Day 0, 1 or 2, they reported that the progesterone concentrations in all three groups were significantly lower than for control mares on Days 3–7 post-ovulation. In contrast, several cloprostenol treatment groups (e.g. Clo-D0, Clo1x-D0, Clo2x-D0, Clo1x-D1, Clo2x-D1, Clo1x-D2) in our study had progesterone concentrations similar to control cycles throughout the diestrous period (Figs. 1–3). In previous studies when cloprostenol was administered on consecutive post-ovulatory days progesterone concentrations were consistently reported to be lower than in controls or oxytocin treated mares for 5–7 days [7–9,12].

In a concurrent study, we found that administering 250 μg of cloprostenol, i.m., daily, starting 4 h after breeding and continuing through Day 2 post-ovulation did not decrease pregnancy outcome in mares compared with oxytocin treatment on the same days [12]. In the current study, mares treated with a second micro-dose of cloprostenol within 12 h had the lowest progesterone concentrations observed in any of the treatment groups through the middle of diestrus. Repeatedly treating a mare, even with micro-doses of cloprostenol, during a 24-h period may more effectively damage the early luteal structure than administration of single larger doses. It is yet to be determined whether multiple doses of cloprostenol administered within 24 h decrease pregnancy outcome. Regardless, repeated treatment may be a useful management approach to short-cycle mares earlier in the post-ovulatory period when not bred. The usefulness of cloprostenol for lysing immature luteal structures in the early post-ovulatory period is still to be determined.

Acknowledgements

Support was provided for this study in part through a grant from the Auburn University Competitive Research Grant Program, the Department of Clinical Sciences and the Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University.

References


