Research Report

Effect of human chorionic gonadotropin treatment on the duration of oxytocin-induced prolonged corpus luteum function in mares

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Abstract

Oxytocin is used increasingly in mares to prolong corpus luteum (CL) function to suppress estrus. When mares develop prolonged CL function in response to oxytocin treatment, the CL generally remains functional for 60–90 days. However, a longer duration of CL function can make this method of estrus suppression even more clinically useful. We hypothesized that human chorionic gonadotropin (hCG) treatment during the period of oxytocin-induced prolonged CL function would extend the duration of CL function (i.e. beyond 90 days). Prolonged CL function was induced in mares (n = 14) by treating them with 60 units of intramuscular oxytocin once daily on days 7–14 after ovulation. Mares were then randomly assigned equally to a control group that received no additional treatment and an hCG-treated group that received 2,500 units of intramuscular hCG on days 30, 45, 60, 75, and 90 after ovulation. Jugular blood samples were collected for progesterone concentration determination on the day of ovulation and then 3 times (M, W, and F) weekly for 120 days. Duration of CL function was not different ($p > 0.05$) and it was 78.0 ± 7.5 and 91.4 ± 20.4 days (mean ± standard deviation [SD]) in control and hCG-treated mares, respectively. Therefore, hCG treatment (during the period of oxytocin-induced prolonged CL function) did not extend CL function, hence alternative methods should be explored.

Keywords: Mare, oxytocin, corpus luteum, hCG

Introduction

Oxytocin treatment is used increasingly to prolong corpus luteum (CL) function as a means of suppressing estrous behavior in performance mares by allowing continued secretion of progesterone to keep mares out of estrus naturally.[1] Oxytocin treatment of mares beginning in mid diestrus prolonged CL function by inhibiting the expression of endometrial cyclooxygenase-2,[2,3] the inducible form of the rate-limiting enzyme necessary for endometrial secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at luteolysis. As a result, the luteolytic process (i.e. regression of the CL) was blocked causing CL function to continue beyond its typical duration of 2 weeks. This is in sharp contrast to the stimulatory effect of oxytocin, both endogenous and exogenous, on endometrial secretion of $PGF_{2\alpha}$ during late diestrous in mares.[4–6] Importantly, therefore, oxytocin can have a proluteolytic or an antiluteolytic effect in mares, and these diametrically opposed responses occur in a tightly controlled, time-dependent manner during the estrous cycle.

The most widely used oxytocin treatment protocol for estrus suppression utilizes 60 units of intramuscular oxytocin once daily on days 7–14 after ovulation and it generally induces prolonged CL function in >70% of treated mares.[7] An alternative method that does not require detection of ovulation utilizes 60 units of intramuscular oxytocin once daily for 29 consecutive days.[8] Estrous behavior rather than ovulation as the basis for initiating oxytocin treatment has also been described.[9] Additional work is underway to develop a formulation of oxytocin that does not require daily treatment.[10]

When mares develop prolonged CL function in response to oxytocin treatment, the CL generally remains functional for 60–90 days. However, when oxytocin treatment is used for estrus suppression, a longer duration of CL function is preferable. We hypothesized that human chorionic gonadotropin (hCG) treatment during the period of oxytocin-induced prolonged CL function would further extend CL function (i.e. beyond 90 days) via two potential mechanisms: (1) a

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direct luteotrophic effect and/or (2) by inducing ovulation of a diestrous follicle(s) resulting in the formation of new CL(s) that remain functional for an additional 60–90 days. Therefore, the objective was to determine whether repeated hCG treatment during the period of oxytocin-induced prolonged CL function further extends CL function.

Materials and methods

Animals

This study was conducted in the Northern Hemisphere (41.7°N) under natural photoperiod using Quarter Horse-type mares (n = 14) between 6 and 15 years of age that weighed 300–500 kg. Animal procedures were approved and conducted following the guidelines of the Utah State University Institutional Animal Care and Use Committee (Protocol #2249).

Experimental protocol

Beginning in May, the reproductive tract of each mare was examined via transrectal palpation and ultrasonography, and when mares developed an ovarian follicle ≥35 mm in diameter in conjunction with prominent endometrial edema, they were examined daily until ovulation was detected (day 0). Jugular blood samples were collected on day 0 and then every M, W, and F through day 120. Blood samples were allowed to clot at room temperature, then serum was recovered and kept frozen at –20°C until progesterone concentrations were determined. After ovulation, prolonged CL function was induced in mares (n = 14) by treating with 60 units of intramuscular oxytocin (Bimeda – MTC Animal Health, Inc., Cambridge, Ontario, Canada) once daily on days 7–14. Mares were then randomly assigned equally to a control group that received no additional treatment and an hCG-treated group that received 2,500 units of intramuscular hCG (Chorulon®, Merck Animal Health, Summit, NJ) on days 30, 45, 60, 75, and 90 after ovulation. After reconstituting each vial of lyophilized hCG with the sterile diluent provided by the manufacturer, individual doses were drawn into syringes and kept frozen at –20°C until use. Mares that received hCG treatment were examined (via transrectal palpation and ultrasonography) on days of treatment to assess their ovarian follicular status. Mares that had ovarian follicle(s) ≥ 30 mm in diameter were reexamined 3 days later to determine whether ovulation(s) had occurred after hCG treatment.

Progesterone assay

Progesterone was measured using a commercially available kit (Immulite Progesterone, Siemens, Malvern, PA) designed for an enzyme-amplified chemiluminescence assay system (Immulite 1000, Diagnostic Products Corporation, Los Angeles, CA) and performed according to the manufacturer's protocol. Intraassay coefficient of variation was 3.9% and the interassay coefficient of variation was 5.8%. The sensitivity of the assay was 0.2 ng/ml, and values below the assay sensitivity were assigned a value equal to the sensitivity.

Data analyses

Duration of CL function (number of days the progesterone concentration was > 1.0 ng/ml), a continuous outcome variable, was compared between control and hCG-treated mares, an input categorical variable, with a two-tailed t-test (GraphPad, San Diego, CA). Means of all daily blood progesterone concentrations (continuous outcome variable with repeated measures) from day 0 through the day progesterone concentrations dropped below 1.0 ng/ml (or until day 120) were compared between control and hCG-treated groups using a two-tailed t-test. Alpha value for determination of significance was set at $p < 0.05$.

Results

Control and hCG-treated mares' progesterone profiles are depicted (Figure 1). The duration of CL function was not different ($p > 0.05$) and it was 78.0 ± 7.5 and 91.4 ± 20.4 days (mean ± standard deviation [SD]) in control and hCG-treated mares, respectively. Two hCG-treated mares had blood progesterone concentrations >1.0 ng/ml through day 120; however, in the other 5 mares progesterone concentrations returned to baseline between days 74 and 91. Although there was no difference in the duration of CL function, daily blood progesterone concentrations were higher ($p < 0.0001$) in hCG-treated mares compared to control mares (Figure 1; 7.8 ± 4.1 vs. 5.1 ± 2.7 ng/ml [mean ± SD] respectively). Six diestrous ovulations occurred within 3 days of hCG treatment in 5 mares. Three ovulations occurred after hCG treatment on day 30, two ovulations occurred after treatment on day 60 and 1 ovulation occurred after treatment on day 75. Diestrous ovulations occurred in two mares that maintained CL function through day 120 (after hCG treatment on day 30 in 1 mare and day 75 in the other mare), but in the other 3 mares with diestrous ovulations progesterone concentrations dropped to baseline between days 74 and 91 (ovulation occurred after hCG treatment on day 30 in 1 mare; day 60 in 1 mare; and day 30 and 60 in 1 mare). Quite notably in 1 mare, following a diestrous ovulation after hCG treatment on day 60 (i.e. ovulation detected on day 63), blood progesterone dropped precipitously to basal concentrations within 2 weeks despite the presence of the newly formed CL (Figure 2).

Discussion

Despite the fact that hCG treatment of mares with oxytocin-induced prolonged CL function resulted in diestrous ovulations and higher concentrations of progesterone, it did not successfully extend the duration of CL function. As evidenced by the precipitous fall in progesterone concentration that occurred within 2 weeks after an hCG-induced diestrous ovulation on day 63 in 1 mare (and a similarly precipitous drop in progesterone concentrations in mares in control and treated groups), it appeared that it was the return of the endogenous luteolytic process in that period that was responsible for the inability of hCG treatment to extend the duration of CL function. The postulate[11] that a return of the luteolytic mechanism was responsible for the cessation of luteal function after ~60 days was supported in the present study. Return of the luteolytic mechanism between days 60 and 70 of oxytocin-induced prolonged CL function has now been demonstrated based upon the ability of exogenous oxytocin to stimulate endometrial secretion of $PGF_{2\alpha}$ in the same manner that it does at the expected time of luteolysis in cycling mares (i.e. through its proluteolytic effect).[12]

In light of the return of the luteolytic process after ~day 60 during the period of prolonged CL function, it is plausible that retreatment with oxytocin before the luteolytic process is

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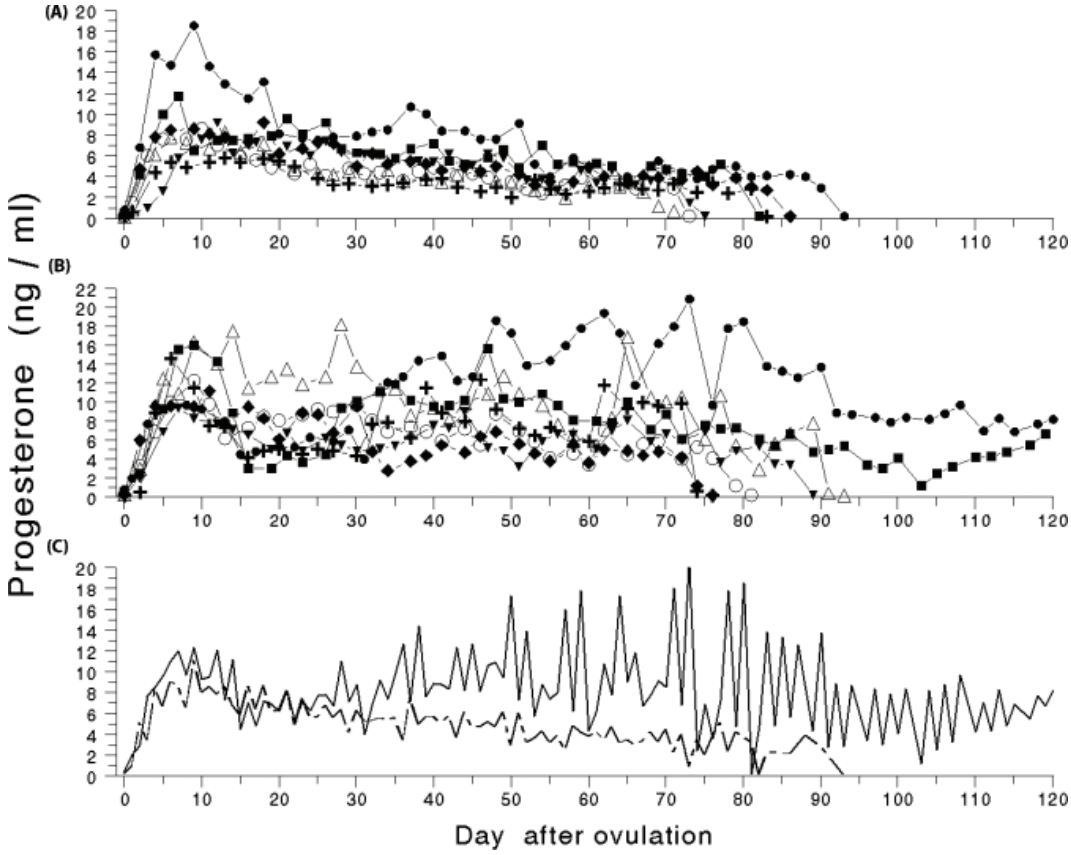

Figure 1. (A) Serum progesterone concentrations from day of ovulation (day 0) through day 120 in 7 control mares with oxytocin-induced prolonged CL function. For clarity, lines are truncated once progesterone concentrations dropped below 1.0 ng/ml. (B) Serum progesterone concentrations from day of ovulation through day 120 in 7 mares with oxytocin-induced prolonged CL function that were treated with 2,500 units of hCG IM on days 30, 45, 60, 75, and 90 after ovulation. For clarity, lines are truncated once progesterone concentrations dropped below 1.0 ng/ml. (C) Mean serum progesterone concentrations from day of ovulation through day 120 in 7 control mares with oxytocin-induced prolonged CL function (broken line) and 7 mares with oxytocin-induced prolonged CL function that were treated with 2,500 units of intramuscular hCG on days 30, 45, 60, 75, and 90 after ovulation (solid line). Daily blood progesterone concentrations were higher in hCG-treated mares compared to control mares ($p < 0.0001$). For clarity, lines are truncated once progesterone concentrations dropped below 1.0 ng/ml. Variability in the mean values was due to the M, W, F bleeding schedule that resulted in a sub-set of mares being represented on any given day after ovulation.

reestablished could be used to extend the duration of CL function. If retreatment with oxytocin can block the return of the luteolytic process, it could plausibly be combined with the hCG treatment protocol described here. For example, had the return of the luteolytic process been blocked (by retreatment with oxytocin) in the mare that ovulated after hCG treatment on day 60, the newly formed CL should have remained functional for an additional 60 days or longer, resulting in a total duration of prolonged CL function of >120 days. Based on the results mentioned above,[12] the optimal time to readminister oxytocin would be between days 50 and 60 of prolonged CL function, because treatment of oxytocin in that interval did not appreciably stimulate endometrial secretion of $PGF_{2\alpha}$, and all of the mares maintained luteal function after treatment. In contrast, oxytocin treatment further and beyond day 60 rendered a more robust $PGF_{2\alpha}$ secretory response and CL function was more likely to decline rapidly after treatment.[12]

Considering that prolonged CL function occurs spontaneously in mares,[11] return of the luteolytic process may have developed naturally as a means of ensuring that these mares return to a cyclic state allowing them to have an opportunity for breeding and establishment of pregnancy, which is otherwise precluded during the period of prolonged CL function. Even with the return of the luteolytic process, a 60–90 day period of prolonged CL function represents a considerable portion of the physiological breeding season that is not available for breeding. Therefore, it seems likely there has been selection pressure for a process that reestablishes the luteolytic mechanism in mares with prolonged CL function, so they can return to a cyclic state.

In conclusion, although 2,500 units of intramuscular hCG treatment on days 30, 45, 60, 75, and 90 of oxytocin-induced prolonged CL function resulted in diestrous ovulations and higher concentrations of progesterone, it did not extend the duration of luteal function. The dose (2,500 units) of hCG used in this study is commonly used to induce ovulation in mares; however, it is not known whether a different dose (higher or lower) and/or different treatment schedule may

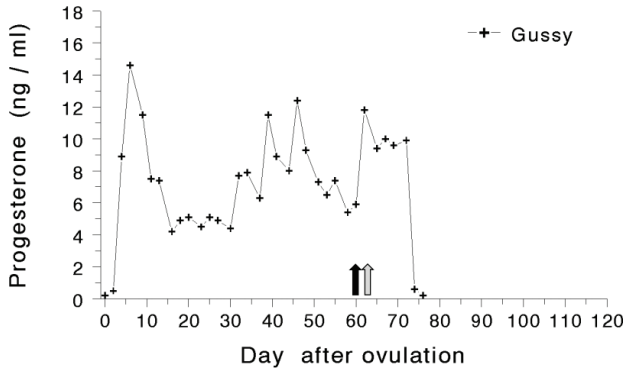

Figure 2. Serum progesterone concentrations from day of ovulation (day 0) through day 120 in 1 mare with oxytocin-induced prolonged CL function that was treated with 2,500 units of intramuscular hCG on days 30, 45, 60, 75, and 90 after ovulation. At hCG treatment on day 60 (black arrow), the mare had a 44 mm follicle on the right ovary, and on day 63 (grey arrow) that follicle had ovulated as evidenced by the presence of a new CL. Despite having a newly formed CL on day 63, mare's progesterone concentrations dropped precipitously from 9.9 ng/ml on day 72 to < 1.0 ng/ml on day 74. For clarity, the line has been truncated at the point when the progesterone concentration dropped below 1.0 ng/ml.

have impacted the outcome. Because there is now compelling evidence that it is a return of the luteolytic process after 60 days that is responsible for the cessation of prolonged CL function, it seems plausible that retreatment of oxytocin (potentially in conjunction with hCG) prior to day 60 could inhibit the return of the luteolytic process leading to an extension of the period of prolonged CL function.

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Conflict of interest and funding

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