Induction of Ovulation and LH Response in Cyclic Mares Treated with Gonadorelin Diacetate Tetrahydrate

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Summary and Implications

The objective of this study was to test the effectiveness of Cystorelin™ (gonadorelin diacetate tetrahydrate) in inducing ovulation in cyclic mares. Mares were treated with either three 75 µg (1.5 mL) i.m. injections of Cystorelin™ or three 1.5 mL i.m. injections of sterile saline (control) given two hours apart. Blood samples were collected every 30 minutes to monitor LH concentrations. No difference was observed in LH secretion patterns of control and treated mares. Mares treated with Cystorelin™ ovulated 2.25 ± .25 days after treatment which was one day earlier (P<.05) than control mares (3.25 ± .41 days). Of the treatment mares, 71% (12/17) ovulated within 48 hours after treatment compared with 14% (1/7) of control mares. Treatment and control mares were not different (P>.81), however, for variability in days to ovulation. Treatment with Cystorelin™ effectively hastened ovulation in mares, which could prove useful for timed artificial insemination. Further research is necessary, however, to develop a protocol that can also reduce variability in the time of ovulation.

Introduction

The mare is a domestic animal species with many reproductive challenges. Mares are seasonal breeders, their estrous cycle lengths can be erratic, the length of the period of estrus is highly variable, and the timing of ovulation within the period of estrus is highly unpredictable. Collectively, these factors pose obstacles that can be difficult to overcome.

Having the ability to regulate the time of ovulation in the mare would be beneficial for many reasons, but especially for use with artificial insemination. Precise control of the time of ovulation would increase management and labor efficiency, optimize the time of breeding when using transported semen, and reduce the number of times a mare is bred during a given estrous cycle.

Various exogenous hormones have been used in an attempt to regulate cyclicity in mares. Although progesterone analogues and prostaglandin F2α (PGF) have been used to synchronize estrus, the response to these hormones has been variable and control of the time of ovulation has been inconsistent. Two other exogenous hormones, human chorionic gonadotropin (hCG) and the GnRH analogue deslorelin, have been used to induce ovulation in the mare, but use of each has resulted in negative side effects.

The initial use of hCG to control the time of ovulation in mares can be successful; however, due to the antigenic nature of hCG, mares often become refractory to repeated treatments, rendering the product ineffective. The GnRH analogue deslorelin, when administered via an implant (Ovuplant™), has reportedly caused desensitization of the anterior pituitary gland to GnRH, resulting in suppressed gonadotropin secretion and an extended interval between induced ovulation and the subsequent ovulation if the mare does not become pregnant. (This side effect is most prevalent when Ovuplant™ is left in place for over 48 hours.) Ovuplant™ was withdrawn from the market and is no longer available. The unpredictability and/or market unavailability of these ovulation-inducing hormones has led to demand for a reliable and readily available treatment for inducing ovulation in mares.

The cattle industry has many different GnRH products available to induce rupture of cystic ovarian follicles. These products have also been used in an extra-label manner to control the time of ovulation in timed artificial insemination protocols such as OvSynch. To our knowledge, no one has tested the effectiveness of Cystorelin™ in controlling the time of ovulation in mares, and this experiment was designed to do so.

Materials and Methods

A total of 24 Iowa State University mares was used in this study. Mares were of Thoroughbred (n=9) and stock type (Paint and Quarter Horse; n=15) breeding. The first trial (Trial A) was performed during the 2006 breeding season (May-June) using 6 mares, and the second trial (Trial B) was performed during the 2007 breeding season (February-May) using 18 mares. In both trials mares possessing an ovarian follicle 3.5 cm to 4.0 cm in diameter were randomly assigned to either treatment with three 75 µg (1.5 mL) i.m. injections of Cystorelin™ or three 1.5 mL i.m. injections of sterile saline (control) given two hours apart. After the start of treatment, ultrasonography was performed daily until ovulation was confirmed.

Blood samples were collected from treatment and control mares via an indwelling jugular catheter. Blood was drawn 30 minutes prior to, immediately prior to, and at 30-minute intervals after the initial injection (GnRH or saline) for six (Trial A) or 10 (Trial B) hours to measure luteinizing hormone (LH). In trial B, an additional blood sample was collected

daily starting the day after treatment until ovulation was confirmed. Luteinizing hormone was measured by radioimmunoassay. Intra-and inter-assay coefficients of variation and assay sensitivities were 6%, 9%, and 0.2 ng/mL, respectively.

Data were analyzed using the GLM procedure of SAS for a completely randomized design (SAS Inst. Inc., Cary, NC) to test the effect of treatment and breed on days to ovulation and plasma LH levels. Levene's test was used to analyze the variability in days to ovulation between treatment and control mares.

Results

The effect of year was tested and found to be non significant (P>.57), so data were pooled across years. The diameter of the largest follicle at the time of treatment was not different (P>.42) between treatment (3.8 ± .03 cm) and control (3.8 ± .06) mares. Mares treated with Cystorelin™ ovulated 2.25 ± .25 days after treatment which was one day earlier (P<.05) than control mares (3.25 ± .41). Of the treatment mares, 71% (12/17) ovulated within 48 hours after treatment compared with 14% (1/7) of control mares (Table 1). Treatment and control mares were not different (P>.81) for variability in days to ovulation.

Mean plasma LH levels were calculated over four time periods (-30 to 0 min [Period 1 = baseline], 30-120 min [Period 2], 150-240 min [Period 3], and 270-360 min [Period 4]). Luteinizing hormone response was analyzed by comparing the mean LH value of Periods 2, 3, and 4 with the baseline mean (Figure 1). Although the concentration of LH in Period 2 [2.60 ng/ml] tended (P<.07) to be higher than baseline [1.97 ng/ml] in Cystorelin™-treated mares, elevations in plasma LH concentration in Periods 3 and 4 were not different in treatment versus control mares (P>.15 and P>.18 respectively).

Discussion

The reproductive status of treatment and control mares was not different, as evidenced by similar (3.8 cm) ovarian follicle diameters at the initiation of treatment. Our three-shot Cystorelin™ protocol was effective in hastening ovulation in mares by one full day. The 71% of mares that ovulated within 48 hours after treatment with Cystorelin™ is comparable to results reported by others who investigated the use of hCG or deslorelin BioRelease™ for control of the time of ovulation in mares.

Variability in days to ovulation was similar between Cystorelin™ treated and control mares (P>.81), despite the average earlier post-treatment onset of ovulation. This result was surprising but may be due to the relatively small number of experimental animals available for use in this study. Clearly, a refinement in protocol is needed to obtain a more consistent and predictable interval from

Cystorelin™ treatment to ovulation if this protocol is to be universally adopted in the equine breeding industry.

Luteinizing hormone secretion patterns were similar between control and treatment mares. Although the LH release in response to treatment was not statistically higher than LH levels observed in control mares, there was a tendency (P<.07) for elevated LH level after the first Cystorelin™ treatment. This may explain why a higher proportion of Cystorelin™-treated mares ovulated within 48 hours of treatment. We were somewhat surprised at the lack of a significant and sustained increase in plasma LH level in response to Cystorelin™ treatment, but our results may be partially explained by the low dose of gonadorelin we administered, individual variation among mares, or the relatively small number of experimental animals used in our study. Interestingly, inconsistencies in LH response have been previously reported by other researchers.

Data from this experiment suggest that Cystorelin™ could effectively be used in routine equine breeding management to hasten ovulation in mares. Although we did not specifically test this in our study, Cystorelin™ treatment potentially could be used to overcome problematic hCG antibody formation (as well as availability issues with deslorelin products). This treatment protocol is practical for horse breeders to use and only requires a veterinary prescription for Cystorelin™ use. This protocol is much more convenient that that for Ovuplant™ which typically required the services of a veterinarian to insert and remove the implant. Further studies with increased mare numbers need to be conducted to further refine this protocol, however, before recommending the widespread use of Cystorelin™ to control the time of ovulation in cyclic mares.

Disclaimer

Cystorelin™ is not approved by the U.S. Food and Drug Administration (FDA) for use in horses, and FDA has not determined that the product is safe and effective in horses.

Table 1. Ovarian status and response to treatment with either Cystorelin™ or saline.

Variable	Cystorelin™ mares	Control mares
Follicle size (cm) at initiation of treatment*	3.8 ± .03	3.8 ± .06
Days from initiation of treatment to ovulation*	2.25 ± .25[a]	3.25 ± .41[b]
Hours to ovulation (post treatment)	<u>Percent ovulated</u>	
24	6% (1/17)	0% (0/7)
48	65% (11/17)	14% (1/7)
72	6% (1/17)	43% (3/7)
≥96	12% (2/17)	43% (3/7)
No ovulation	12% (2/17)	0% (0/7)

* Mean ± standard error of the mean (SEM)
[a,b] Means with unlike superscripts differ (P<.05)

Figure 1. Mean plasma LH increase over time between treatment and control mares.