Effects of a new injectable short-term release deslorelin in foal-heat mares


Department of Large Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4475, USA

Received 1 July 2003; accepted 2 December 2003

Abstract

Mares treated with subcutaneous deslorelin implants on the first postpartum estrus early in the breeding season had significant reductions in the number of large follicles at early pregnancy examinations and delayed return to estrus (in mares that failed to become pregnant); these adverse effects were attributed to a prolonged release of the drug from the implant. In 2003, an injectable short-term release (<24 h) deslorelin product became available. The objective of this study was to determine if this product would hasten ovulation in early foaling first postpartum estrus mares without reducing the number of large follicles at early pregnancy examination (14–15 days postovulation). Beginning 5–6 days postpartum, first postpartum estrus (foal-heat) mares were teased daily and examined thrice weekly (Tuesday, Thursday and Saturday) by transrectal ultrasonography. Mares in estrus with a follicle ≥34 mm diameter on Tuesdays or Thursdays were alternately assigned to: Treatment 1, n = 17; 1.5 mg injectable short-term release deslorelin, or Treatment 2, n = 16; Control (no treatment). The schedule allowed accurate determination of the number of mares ovulating within 2 days of treatment (i.e., ovulations detected on Thursday or Saturday). Mares were mated on the day of treatment and at 2-day intervals until either ovulation was confirmed or until behavioral estrus ceased. Transrectal ultrasonography was done 14–15 days after ovulation to assess ovarian follicles and pregnancy status. Fewer covers were required and more mares ovulated within 2 days of treatment in deslorelin-treated versus Control mares (P < 0.01). Pregnancy rates were normal (69%) in deslorelin-treated mares. The number of large follicles 14–15 days after ovulation did not differ between deslorelin-treated and Control mares (P > 0.10), suggesting follicular suppression did not occur with this formulation of deslorelin.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Mare; Postpartum estrus; Fertility; Deslorelin; GnRH

* Corresponding author. Tel.: +1-979-845-9137; fax: +1-979-847-8863.
E-mail address: tblanchard@cvm.tamu.edu (T.L. Blanchard).

0093-691X/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
1. Introduction

The GnRH agonist deslorelin was approved for use to hasten ovulation in mares in the USA in 1999 in the form of a controlled release subcutaneous implant (Ovuplant™, Fort Dodge Animal Health). When administered to mares with an estrous follicle ≥30 mm diameter, Ovuplant™ induced ovulation within 2 days in over 88% of cycling mares [1,2]. Two recent reports revealed that ovulation could be hastened and normal fertility could be achieved using deslorelin implants in first postpartum estrous mares [3,4]. After the first year of commercial availability in the USA, a number of veterinarians reported that some deslorelin implant-treated mares experienced a delayed return to estrus if they failed to become pregnant [5,6]. Most affected mares experienced a 3- to 7-day prolongation of the interestrous or interovulatory interval, but some experienced intervals >30 days [5,6]. The delayed return to estrus was presumably due to downregulation of pituitary gonadotrophs by the GnRH agonist [7], with associated suppression of follicular growth [8]. Treatment of foal-heat mares with deslorelin implants (placed subcutaneously in the neck and not removed) during February and March reduced the number of large follicles present at early pregnancy examination 14–15 days later, and resulted in a pronounced delay in return to estrus (e.g., 32–55 days interestrous intervals) in mares failing to become pregnant on foal-heat breeding, suggesting early foaling mares may be more susceptible to the down-regulating effects of this drug when treated on the first postpartum estrus [3]. In that study [3], mares were alternately treated with either hCG or deslorelin implants to ensure effects of treatment early in the breeding season would be equally represented between treatment groups; while subsequent interestrous intervals were not correlated with date of treatment with hCG (P > 0.10), they were highly negatively correlated with date of treatment with deslorelin (r = −0.98, P < 0.02). Therefore, treatment of mares during foal-heat with deslorelin implants earlier in the breeding season resulted in a longer delay in return to estrus if they failed to become pregnant [3]. Removal of deslorelin implants at the time ovulation was detected prevented subsequent follicular suppression and delayed return to estrus [4,9], suggesting prolonged absorption of deslorelin occurred in some mares when controlled release implants were not removed. Removal of deslorelin implants, while a relatively straightforward process, may be objectionable to some practitioners or mare owners.

A short-term release GnRH agonist preparation that effectively induced predictable ovulation (within 2 days of treatment) without suppressing subsequent follicular development would be a desirable product to have available to the equine breeding industry. Recent research demonstrated that deslorelin was just as effective in inducing ovulation it was in a short-term biodegradable liquid vehicle and injected i.m. as when given in implant form [10]. In 2003, a short-term release (<24 h) deslorelin product in a biocompatible liquid vehicle (BioRelease Deslorelin Injection, BET Pharm, Lexington, KY, USA) became available. This product is administered in a single dose by i.m. injection. The objective of this study was to determine if use of this injectable short-term release deslorelin would hasten ovulation in early foaling first postpartum estrus mares without reducing the number of large follicles present at early pregnancy examination (14–15 days after ovulation). Effects of the drug on various reproductive end points were also evaluated.
2. Materials and methods

Palpation and breeding records were examined for 33 first postpartum estrous (foal-heat) crossbred (Quarter Horse type) broodmares maintained on pasture in southeast Texas. Beginning 5–6 days postpartum, mares were teased daily and examined thrice weekly (Tuesday, Thursday and Saturday) by transrectal ultrasonography. Mares in estrus with a follicle ≥34 mm diameter were alternately assigned to: Treatment 1, \( n = 17 \); 1.5 mg (recommended dose) BioRelease Deslorelin Injection (BET Pharm, Lexington, KY, USA), or Treatment 2, \( n = 16 \); Control (no treatment). Mares were only assigned to treatments on Tuesday or Thursday to allow accurate determination of the percent of mares ovulating within 2 days (i.e., ovulations were detected on Thursday or Saturday). Since a 3-day interval between examinations (Saturday to Tuesday) occurred in some mares that had not ovulated by Saturday, in those mares, ovulation date was calculated as the last day of behavioral estrus if estrus ceased before Tuesday. Otherwise, if a mare that had not ovulated on Saturday and was in behavioral estrus up to and including Monday, and ovulation was confirmed on Tuesday, Tuesday was recorded as the day of ovulation. Mares were also re-examined by transrectal ultrasonography 14–15 days after ovulation was confirmed (or they ceased signs of behavioral estrus during the 3-day period between Saturday and Tuesday) to assess follicular activity and pregnancy status.

Mares were bred on the day of treatment and at 2-day intervals until either ovulation was confirmed or until they ceased to show behavioral signs of estrus. Mares were bred (natural cover) by one of nine stallions. All stallions had passed a breeding soundness examination in October 2002. Mean (±S.D.) pregnancy rate per cycle and seasonal pregnancy rate for all mares bred by these stallions during the 2003 breeding season was 67.4 ± 16.6% and 92.0 ± 6.3%, respectively. Pregnancy rates per cycle and seasonal pregnancy rates for the 2003 breeding season for all mares bred during 2003 did not differ by stallion (\( \chi^2 = 2.616; P = 0.96 \) and \( \chi^2 = 0.259; P = 1.00 \), respectively). For mares not becoming pregnant, estrous cycle length was calculated from the last day of the treated estrus to the last day of the next estrus.

Days postpartum at the time of treatment, date of treatment, diameter of the dominant follicle at time of treatment, and number of follicles 20–29 and ≥30 mm diameter at treatment and 14–15 days after ovulation were analyzed by Student’s \( t \) test. Categorical data were analyzed by Chi-square procedures. While fertility among stallions appeared to be normal, comparisons were not made between pregnancy rates achieved in treatment groups, since so many different stallions were used. Additionally, due to the low number of mares that failed to become pregnant and thus returned to estrus, data for estrous cycle lengths were not compared.

3. Results

Ovulation failure (i.e., follicular regression without ovulation, followed by a prolonged period of anestrus) occurred in one deslorelin-treated and two Control mares. Data from these three mares were excluded, leaving data from 14 Control and 16 deslorelin-treated mares for analyses. Of mares that did not become pregnant when bred on foal-heat, two
deslorelin-treated and one Control mare developed persistent luteal function (confirmed by persistence of ultrasonically detectable corpus luteum on the ovary, failure to return to estrus, and failure to develop cervical relaxation, endometrial edema or a preovulatory follicle for >3 weeks), requiring prostaglandin-F2\(_a\) treatment to induce estrus. Estrous cycle lengths from these three mares were therefore excluded from the data.

Results are presented in Table 1. Mean date at treatment, day postpartum treated, size of the dominant follicle at treatment, and date of postpartum ovulation did not differ between deslorelin-treated or Control mares (\(P > 0.10\)). Day of postpartum ovulation occurred earlier in deslorelin-treated than in Control mares (\(P < 0.05\)) due to the prolonged interval from assignment to treatment until ovulation in Control mares. More mares ovulated within 2 days of treatment, and fewer covers were required, in deslorelin-treated than in Control mares (\(P < 0.01\)). Pregnancy rates for deslorelin-treated (69%) and Control (43%) mares were within the range reported for foal-heat matings. Number of follicles 20–29 or ≥30 mm in diameter present at treatment and 14–15 days postovulation did not differ (\(P > 0.10\)) between deslorelin-treated and Control mares. Estrous cycle lengths for mares failing to become pregnant (regardless of treatment) on foal-heat breedings appeared to be normal.

4. Discussion

The ovulation rate within 2 days of short-term release deslorelin treatment in this group of foal-heat mares (75%) appeared lower than that reported for other cycling mares treated with deslorelin implants in the USA \[1,2,5–7\]. An ovulation rate of 93% within 2 days of treatment with deslorelin implants was reported for 85 lactating mares \[1\]. The ovulation rate within 2 days of treatment seemed slightly lower than that reported for foal-heat mares treated with deslorelin implants placed either subcutaneously in the neck (82%) \[3\] or

<table>
<thead>
<tr>
<th>Variable</th>
<th>Deslorelin-treated (n = 16)</th>
<th>Nontreated Controls (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foaling date (days)</td>
<td>February 6 ± 15</td>
<td>(February) 11 ± 14</td>
</tr>
<tr>
<td>Day postpartum treated (days)</td>
<td>9.8 ± 2.6</td>
<td>9.9 ± 3.7</td>
</tr>
<tr>
<td>Follicle size at treatment (mm)</td>
<td>40 ± 4</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>Ovulations within 2 days</td>
<td>75% (12/16)(^a)</td>
<td>7% (1/14)(^b)</td>
</tr>
<tr>
<td>Day of postpartum ovulation (days)</td>
<td>12.8 ± 3.3(^c)</td>
<td>15.4 ± 3.2(^d)</td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>69% (11/16)</td>
<td>43% (6/14)</td>
</tr>
<tr>
<td>Numbers of covers after treatment</td>
<td>1.6 ± 1.0(^a)</td>
<td>2.9 ± 0.8(^b)</td>
</tr>
<tr>
<td>Number of 20–29 mm follicles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–15 days postovulation</td>
<td>2.1 ± 1.7</td>
<td>1.4 ± 1.5</td>
</tr>
<tr>
<td>Number of ≥30 mm follicles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–15 days postovulation</td>
<td>0.4 ± 0.5</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>Estrous cycle length in mares not pregnant(^\dagger) (days)</td>
<td>19.7 ± 4.2 (n = 3)</td>
<td>23.4 ± 2.4 (n = 7)</td>
</tr>
</tbody>
</table>

Mean values (±S.D.) for foaling date, date of treatment, day postpartum at treatment and ovulation, diameter of largest follicle at time of treatment, and outcome in 30 mares treated on the first postpartum estrus with either injection of 1.5 mg short-term release deslorelin or no treatment (Control).

Means with different superscripts (a, b, c, d) are different (for a, b: \(P < 0.01\) and c, d: \(P < 0.05\)).

\(^\dagger\) Statistical comparisons not made due to low number of mares in each group.
beneath the vulvar mucosa (84%) [4]. The slightly lower response in ovulation rate in short-term release deslorelin-treated foal-heat mares compared to that reported for deslorelin implant-treated foal-heat mares may have been due to seasonal effects. Mares in the current study foaled between January 15 and March 5, resulting in a mean foaling date that was 1–3 weeks earlier in the current study than in the studies reported above [3,4] that used deslorelin implants in foal-heat mares. The earlier foaling date for mares used in the current study may also have accounted for the three mares that did not ovulate for 7 days following deslorelin treatment, and the three mares (one deslorelin-treated and two Control) mares that experienced treatment failure (i.e., follicular regression without ovulation, followed by a prolonged period of anestrus). Previous reports indicated that mares foaling early in the year (January to March) were more likely to fail to have a foal-heat ovulation or revert to ovarian inactivity following their foal-heat ovulation than mares foaling later in the year (i.e., April or later) [12,13]. Perhaps insufficient pituitary LH was present to respond to GnRH treatment in deslorelin-treated mares that either failed to ovulate or had treatment to ovulation intervals that were 7 days. Earlier foaling (January–March) has also been reported to increase the parturition–ovulation interval in first postpartum estrus mares due to seasonal effects [11,12]. Additionally, LH concentrations were lower in the postpartum period of early foaling mares exposed only to natural photoperiod compared to those exposed to artificially increased photoperiod [14].

During a similar period in 2001, the number of large follicles present 14 days after ovulation was reduced with deslorelin treatment (subcutaneous implants left in place) compared to hCG treatment [3]. In addition, return to estrus was delayed (i.e., 32–55 days interestrous intervals) in mares failing to become pregnant when deslorelin implants were left in place [3]. However, removal of deslorelin implants after ovulation avoided follicular suppression and delayed return to estrus in foal-heat mares treated in this herd during 2002 [4]. In the current study, follicular populations at early pregnancy examination (i.e., 14–15 days postovulation) did not differ between treated and Control mares. Therefore, it appears that i.m. injection of 1.5 mg of the short-term release deslorelin did not reduce the number of large follicles present at early pregnancy examination, as reported for some mares treated with deslorelin-implants (that were not removed). While the number of mares failing to become pregnant was low and therefore precluded statistical analysis of data on estrous cycle length, a delayed return to estrus due to follicular suppression was not evident.

In summary, 1.5 mg i.m. of short-term release deslorelin reliably hastened ovulation in foal-heat mares within 2 days of treatment, with normal pregnancy rates. This form of injectable deslorelin did not reduce the number of large follicles present at early pregnancy examination, and therefore may not result in delayed return to estrus if a treated mare does not become pregnant. Further studies, with larger numbers of mares, are needed to corroborate these findings.

Acknowledgements

The authors wish to thank the Texas Department for Criminal Justice for providing the horses and facilities utilized in this study, and B.E.T. Laboratories for graciously providing deslorelin for this study.
References