Efficacy of lyophilized deslorelin on induction of ovulation in mares

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Abstract

Options for induction of ovulation in mares include human chorionic gonadotropin (hCG) and potent agonists of gonadotropin-releasing hormone (GnRH) such as deslorelin acetate. When no commercially produced GnRH agonists are available, GnRH agonists can be acquired through compounding pharmacies. The objective of this project was to evaluate the efficacy of lyophilized deslorelin acetate to induce ovulation and to determine if the efficacy was adversely affected after 12 months of storage. Mares in estrus were administered 1.5 mg of deslorelin (lyophilized- 2009, lyophilized- 2010, or aqueous deslorelin) as an intramuscular injection once a follicle ≥ 35 mm was detected in the presence of uterine edema. Mares were subsequently examined once per day to determine the day of ovulation. A total of 66 mares were administered lyophilized deslorelin in 2009 and 135 mares were treated in 2010 with lyophilized deslorelin from the same initial lot stored for 12 months. Thirty mares were administered liquid suspension deslorelin in 2010. There was no significant difference in mean days to ovulation among treatment groups with majority of treated mares (89.6%) ovulating within 48 hours after deslorelin administration. Results of this study indicate that lyophilized deslorelin stored for 12 months, reconstituted in sterile saline, is equally as effective at inducing a timed ovulation in estrual mares as deslorelin in a liquid suspension. In conclusion, non-reconstituted vials of lyophilized deslorelin may be stored for at least 12 months without a significant loss in efficacy.

Keywords: Ovulation, induction, mare, deslorelin, GnRH agonist

Introduction

Options for induction of ovulation in mares include hCG and potent agonists of GnRH such as deslorelin acetate.[1-3] Deslorelin and hCG are equally effective in inducing a timed ovulation when administered to mares in estrus with a follicle ≥ 35 mm in diameter.[4] The interval from administration to ovulation has been reported to be approximately 36 hours for hCG and 40 hours for deslorelin.[5] One advantage of deslorelin is that efficacy in inducing a timed ovulation is not reduced after multiple doses are administered during a single breeding season.[6] In contrast, efficacy of hCG has been reported to decrease if used multiple times in a single breeding season.[2,3]

The implant version of deslorelin acetate (Ovuplant™, Fort Dodge Animal Health, Fort Dodge, IA) is no longer commercially available in the United States. However, the GnRH agonist can be acquired through a variety of compounding pharmacies. This project was designed and completed prior to the release of a FDA approved deslorelin product. The goals of the current study were to: 1) evaluate the efficacy of a lyophilized deslorelin preparation at inducing ovulation and 2) determine if lyophilized deslorelin could be stored at room temperature for 12 months and still retain clinical efficacy.

Materials and methods

Deslorelin

An allotment of lyophilized deslorelin acetate produced in a single lot was acquired from a compounding pharmacy (Applied Pharmacy Services, Mobile, AL) in April 2009. The allotment consisted of multiple glass vials each containing 15 mg of lyophilized deslorelin acetate. The shipment was divided into two batches, one batch was used beginning in April 2009 for the 2009 breeding season and the other batch was stored at room temperature and used beginning in April 2010 for the 2010 breeding season. Individual vials of deslorelin were reconstituted with 10 ml of sterile 0.9 % saline as needed to yield 10 doses of 1.5 mg each.

A compounded preparation of deslorelin acetate in aqueous suspension (Franck's Pharmacy, Ocala, FL) was acquired in April 2010 for comparison to the lyophilized form. The dose of liquid suspension deslorelin was also 1.5 mg.

Mares

Mares utilized in this study were 2-12 years of age and were of light stock-type breeds. Mares were examined by transrectal ultrasound to monitor follicular development and the quantity and degree of uterine edema. The diameter of the largest follicle was measured with electronic calipers and the edema score evaluated on a scale of 0 to 3, with 0 being no edema and 3 being maximal edema.[7] Once a follicle ≥ 35 mm was detected in the presence of uterine edema, mares were randomly assigned to receive 1.5 mg of deslorelin as an intramuscular injection. Mares were subsequently examined once per day to determine the day of ovulation.

Statistical analysis

Comparison of follicular size at the time of treatment, uterine edema at the time of treatment, interval to ovulation and the percentage of mares ovulating within 48 hours were made by use of a one-way ANOVA with post-hoc analysis by Tukey's test. Values were considered to be statistically different at $p < 0.05$. All data are presented as the mean ± standard deviation.

Results

A total of 66 mares received lyophilized deslorelin in 2009 and 135 mares were treated with lyophilized deslorelin in 2010 from the same initial lot and stored for 12 months. Thirty mares were administered liquid suspension deslorelin in 2010.

There was no significant difference ($p > 0.05$) between 2009 and 2010 mares treated with lyophilized deslorelin in diameter of the largest follicle or uterine edema score at the time of deslorelin treatment (Table). The 2009 lyophilized deslorelin treated mares had smaller follicles ($p < 0.05$) at the time of treatment as compared to the mares administered liquid deslorelin. The 2010 lypholilized deslorelin treated mares had less uterine edema ($p < 0.05$) at the time of treatment as compared to the mares administered liquid deslorelin. There was no significant difference in the interval to ovulation between mares treated with lyophilized deslorelin in 2009, mares treated with the same lot of lyophilized deslorelin in 2010, and mares treated with deslorelin in liquid suspension.

There was also no difference in the percentage of mares ovulating within 48 hours after deslorelin administration between deslorelin treatment groups. Fifteen of the 24 mares that failed to ovulate within 48 hours after administration of deslorelin did go on to ovulate within the subsequent 24 hours (within 72 hours after deslorelin treatment). These mares had significantly ($p < 0.05$) smaller follicles (37.7 ± 3.2 mm) at the time of deslorelin treatment than mares that ovulated by 48 hours (40.9 ± 4.6 mm). There was no difference ($p > 0.05$) in uterine edema or month of year between mares that ovulated by 72 hours versus mares that ovulated by 48 hours post-treatment with deslorelin.

Combining all three deslorelin treatment groups, a total of 35 of 231 (15.2 %) treated mares ovulated within 24 hours while the majority of treated mares (172 of 231; 74.5 %) ovulated between 24 and 48 hours after deslorelin administration. Mares that ovulated within 24 hours after deslorelin administration had significantly ($p<0.05$) larger follicles (45.3 ± 4.6 mm) at the time of treatment as compared to mares that ovulated within 48 hours (40.9 ± 4.6 mm). There was no difference in edema scores or month of year at the time of treatment between mares that ovulated by 24 or 48 hours after administration of deslorelin (data not shown).

When the three deslorelin treatment groups were combined, 207 of 231 mares (89.6 %) ovulated within 48 hours of GnRH agonist administration (Figure). Of the remaining 24 mares, 15 (6.5 %) ovulated 3 days after treatment, 5 (2.2 %) developed hemorrhagic follicles, 3 (1.3 %) ovulated after hCG was administered (2 days after deslorelin treatment), and the dominant follicle of 1 (0.4 %) mare regressed.

Discussion

Deslorelin stimulates a prolonged release of luteinizing hormone (LH) from the anterior pituitary that induces ovulation of the dominant follicle in mares in estrus.[8] Mares may be administered deslorelin when a timed ovulation is desired, such as when stallion or semen availability is limited or in the use of cooled-transported semen or frozen semen.

Timing of deslorelin administration during the estrous cycle is critical for successful outcome. In light horse breeds, it is recommended that deslorelin be administered when the dominant follicle first develops to a diameter of 35 mm or greater and edema is present in the endometrium.[4] With this treatment protocol, a majority of mares will ovulate within 48 hours after treatment. In the current study, the average interval from deslorelin treatment to ovulation, based on ultrasound examinations once per day, was 1.9 ± 0.5 days.

Consistency in the interval to ovulation is an important attribute of an ovulation inducing agent. In the case of the lyophilized deslorelin, 90.0 % of treated mares ovulated within 48 hours after administration. Mares that ovulated within 24 hours after treatment may have either responded exceptionally fast to the GnRH agonist or the dominant follicle may have already been under the influence of endogenous LH. The percentage of mares that ovulated within 24 hours after deslorelin treatment (15.2 %) was similar to the percentage of mares that ovulated within 24 hours after hCG administration (18.5 %) in a previous retrospective study.[3]

The larger follicle diameter in mares that ovulated within 24 hours (45.3 ± 4.6 mm) as compared to mares that ovulated between 24 and 48 hours of the deslorelin administration (40.9 ± 4.6 mm) suggests that mares that ovulated within 24 hours of treatment may have already been approaching ovulation secondary to a rise in endogenous LH. Unfortunately, endogenous LH concentrations were not evaluated in this study. The obvious clinical implication of this observation is that the administration of deslorelin does not preclude a mare in estrus from ovulating within 24 hours.

Five of the 231 mares (2.2 %) treated with deslorelin developed hemorrhagic anovulatory follicles. This is lower than the percentage of anovulatory follicles reported in other studies[9-11] and suggests that there is no causative relationship between deslorelin administration and formation of anovulatory follicles.

Results of this study indicate that lyophilized deslorelin, reconstituted in sterile saline, is equally as effective in inducing a timed ovulation in estrual mares as deslorelin in a liquid suspension. In addition, storage of lyophilized deslorelin at room temperature for 12 months did not adversely affect efficacy at inducing ovulation. This information is important in clinical practice, as non-reconstituted vials of lyophilized deslorelin may be stored until the next breeding season without a loss of efficacy. However, this study only evaluated efficacy at inducing ovulation after prolonged storage and did not address any potential loss of biological activity or potency.

The dose of deslorelin used in the current study was 1.5 mg administered as an intramuscular injection. Results of a recent study suggest that intramuscular doses of 1.5 mg, 1.0 mg and 0.5 mg are equally effective in inducing ovulation in mares.[8]

In summary, a dose of 1.5 mg of lyophilized deslorelin was effective in inducing ovulation in estrual mares. Storage of lyophilized deslorelin at room temperature for 12 months was not associated with a decrease in efficacy.

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Table. Follicle diameter and edema score at time of treatment, interval to ovulation after treatment, and percentage of mares ovulating within 48 hours of deslorelin treatment.

Group	(n)	Follicle size (mm)	Edema score	Interval to ovulation (days)	% of mares ovulating within 48 hours
Lyophilized deslorelin (2009)	66	40.9 ± 4.4*	1.6 ± 0.7	1.9 ± 0.5	58/66 (87.9 %)
Lyophilized deslorelin (2010)	135	41.0 ± 5.0	1.4 ± 0.6*	1.9 ±0.5	123/135 (91.1 %)
Liquid suspension deslorelin (2010)	30	43.5 ± 4.9*	1.7 ± 0.6*	1.9 ± 0.4	28/32 (87.5 %)

* Within columns indicates a significant difference of $p < 0.05$, ** indicates a significant difference of $p < 0.01$)

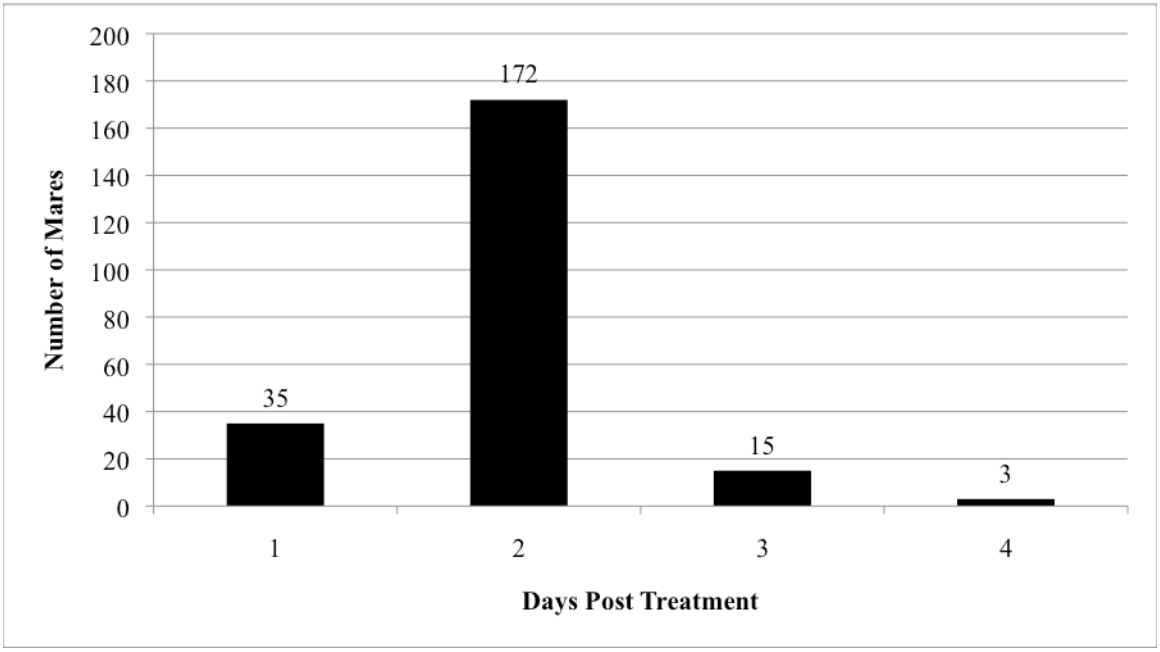

Figure. Interval from treatment to ovulation for mares administered deslorelin acetate (all treatment groups combined). 225 of the 231 mares are shown in the Figure, 5 mares did not ovulate due to a hemorrhagic follicle, and the dominant follicle regressed in 1 mare.