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Effect of Postbreeding Intramuscular Administration of Deslorelin Acetate on Plasma LH and Progesterone Concentrations and Conception Rate in Mares

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To clarify effect of postbreeding intramuscular administration of deslorelin on mares, plasma LH and progesterone concentrations and conception rate were evaluated. In Experiment 1, deslorelin, buserelin, or a placebo was administered on day 9 (day 0=day of last breeding or AI). Plasma samples were collected daily from days 6 to 15, and additional samples were taken after treatment. There was a significant increase in hormonal concentrations after deslorelin treatment. In Experiment 2, deslorelin or placebo was injected on day 9. There was no difference in conception rates at days 15 and 30 between the two groups. While a single postbreeding treatment of deslorelin increased peripheral LH and progesterone concentrations, it may not affect the conception rate in mares.

Key words: *conception rate, deslorelin, LH, progesterone*

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Early embryonic death has been reported to be one of the causes for declining reproductive performance in the mare. Nine to 14 percent of early embryonic deaths occurs between 11 and 40 days after ovulation in the mare [4]. Administration of buserelin acetate, a GnRH analog, 8 to 12 days after ovulation increased the conception rate in the mare [7, 9, 10]. The mechanism of this increase remains unknown. In the cow, administration of a GnRH analog in the postbreeding luteal phase induced ovulation, elevated peripheral progesterone concentrations, and increased conception rate [2, 8, 11]. However, buserelin treatment in the luteal phase failed to induce ovulation and did not elevate blood progesterone level in the mare [13]. A long-acting GnRH analog, deslorelin acetate, has been recently developed, and the efficacy of a single treatment of deslorelin acetate in inducing ovulation in the mare at estrus has been reported [3, 12]. Deslorelin acetate may have a stronger luteotrophic action than buserelin acetate and may be able to induce ovulation in the mare in the luteal phase since it possesses a half-life of 6 to 24 hr [3, 12]. If a higher conception rate in the mare injected with buserelin acetate was at least partly the result of an increased level of blood progesterone, a long-acting deslorelin acetate may be more effective in improving reproductive performance. The objective of this study was to clarify whether treatment with deslorelin acetate in the postbreeding luteal phase alters blood LH and progesterone concentrations (Experiment 1) and is effective in increasing conception rate (Experiment 2) in the mare.

In Experiment 1, a total of seven mares (Percherons, Bretons and their crossbreds) were randomly divided into three groups. Three out of the seven were treated

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with deslorelin (deslorelin acetate, BioRelease Deslorelin Injection, BET Pharm, Lexington, KY, USA; DES), two were treated with buserelin (buserelin acetate, Itorelin, ASKA Pharmaceutical Co., Ltd., Tokyo, Japan; BUS), and the remaining two were used as the control (treated with glycerin as a placebo drug; CON). In Experiment 2, a total of 51 mares (27 Percherons, Bretons and their crossbreds and 24 Thoroughbreds, Arabians and their crossbreds) with a total of 58 estrous cycles (28 in Percherons, Bretons and their crossbreds and 30 in Thoroughbreds, Arabians and their crossbreds) were used. The mares were randomly divided into two groups evenly based on the total number of estrous cycles: a deslorelin-treated (29 estrous cycles; DES) group and control (glycerin-treated placebo; CON) group (29 estrous cycles). Age and parity of the mares (mean ± SD) were 9.7 ± 5.4 years and 3.5 ± 2.8 in the DES group and 10.0 ± 4.8 years and 4.0 ± 3.3 in the CON group, respectively. All of the animals used in this study were clinically healthy and were handled in an ethically appropriate manner.

This study (Experiments 1 and 2) was conducted during the period between April and July 2006. The mares were either naturally bred or artificially inseminated every 2 or 3 days from the time after estrus detection to the time of confirmation of ovulation or the end of estrus. Day 0 was defined as the day of last breeding (AI). Ovulation was confirmed by disappearance of a mature follicle, which was observed by ultrasound (SSD-500, Aloka, Tokyo, Japan) with a 5 MHz linear transducer (UST-588U-5, Aloka). Either 750 μg/0.5 m*l* of deslorelin (DES), 40 μg/10 m*l* of buserelin (BUS) or 0.5 m*l* of glycerin (CON) was intramuscularly injected nine days after the last breeding (AI). We performed a randomized, double-blind trial, that is, both the veterinarians who injected drugs and the owners of the mares were unaware of which drug was used during the experimental period. Pregnancy diagnosis was carried out by ultrasound at 15 and 30 days following breeding (AI). In addition, the number of corpora lutea was recorded at drug administration on day 9 and at first pregnancy diagnosis to determine whether ovulation at the luteal phase was induced after drug administration in Experiment 1.

In Experiment 1, blood samples were taken from the jugular vein into a 10 m*l* heparinized vacuum tube every day from day 6 to day 15 (Fig. 1). Additional samples were collected three minutes before, and 4, 8, 12, 24, 36, and 48 hr after drug administration on day 9. Plasma samples were obtained by centrifugation at 1,500 G for 15 minutes, and then were stored at –30°C until assayed.

Plasma concentrations of LH were determined by RIA with anti-ovine LH serum (YM#18; provided by Dr. Y. Mori, the University of Tokyo) and highly purified equine LH for radioiodination and as the reference standard [5].

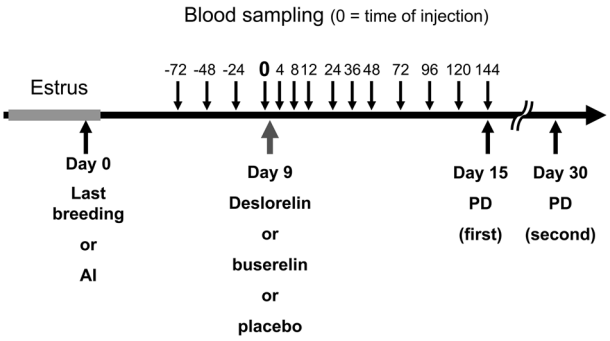

Fig. 1. Experimental design (Experiment 1). Effect of intramuscular administration of deslorelin acetate on peripheral LH and progesterone concentrations in the mare. Blood samples were collected every day from day 6 to day 15. Additional samples were collected three minutes before, and 4, 8, 12, 24, 36, and 48 hr after drug administration on day 9, and pregnancy diagnosis (PD) was performed on days 15 and 30.

Plasma concentrations of progesterone were determined by RIA with antisera to progesterone (GDN#337; provided by Dr. GD Niswender, Colorado State University) as described previously [14]. The intra- and inter-assay coefficients of variation were 5.6% and 8.7% for LH and 7.6% and 10.5% for progesterone, respectively.

In Experiment 1, statistical evaluation of data was performed by means of a repeated measures ANOVA followed by a paired Student's t-test with a modified Bonferroni procedure to control type I error rates for comparison of the mean plasma LH and progesterone concentrations before and after drug administration within the same group. In Experiment 2, chi square or Fisher's probability tests were used for comparison of the conception rates between the two groups. A probability less than 5% was considered as significant.

Plasma LH concentrations significantly increased by 4 hr after administration in the DES and BUS groups (Fig. 2). While the concentrations decreased to the pretreatment level by 8 hr after treatment and thereafter in the BUS group, a significant increase in the concentrations was observed until 12 hr after treatment in the DES group.

No mare had an induced corpus luteum at first pregnancy diagnosis on day 15 in any of the three groups.

One of the two mares in the BUS group was excluded from monitoring because it had plasma progesterone concentrations of less than 1 ng/m*l* by the time of buserelin treatment and was thus regarded as having no functional corpus luteum. Plasma progesterone concentrations significantly increased by 8 hr after treatment in the DES group (Fig. 3).

Pregnancy diagnosis was not performed in one of the

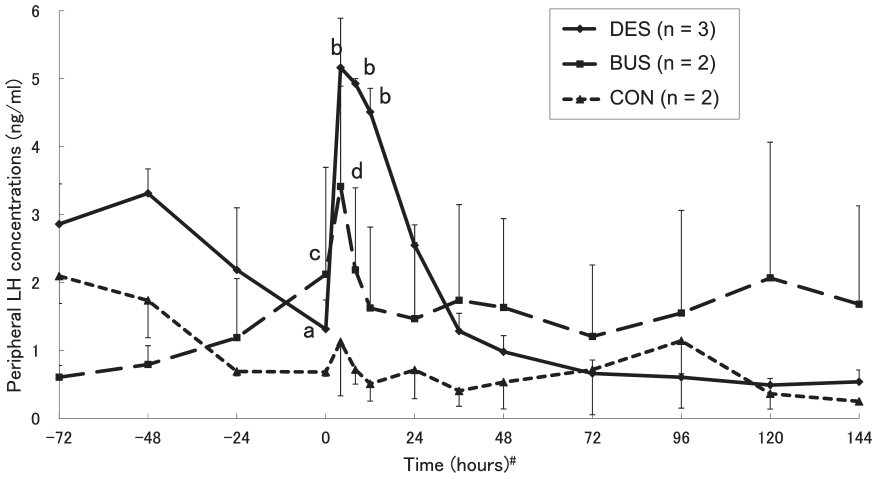

Fig. 2. Change in peripheral LH concentrations (Mean ± SE) before and after intramuscular administration of GnRH analogs or placebo (Experiment 1). DES, deslorelin-treated group; BUS, buserelin-treated group; CON, glycerin-treated control group. [ab] $P<0.05$; [cd] $P<0.05$. [#] 0=time of injection.

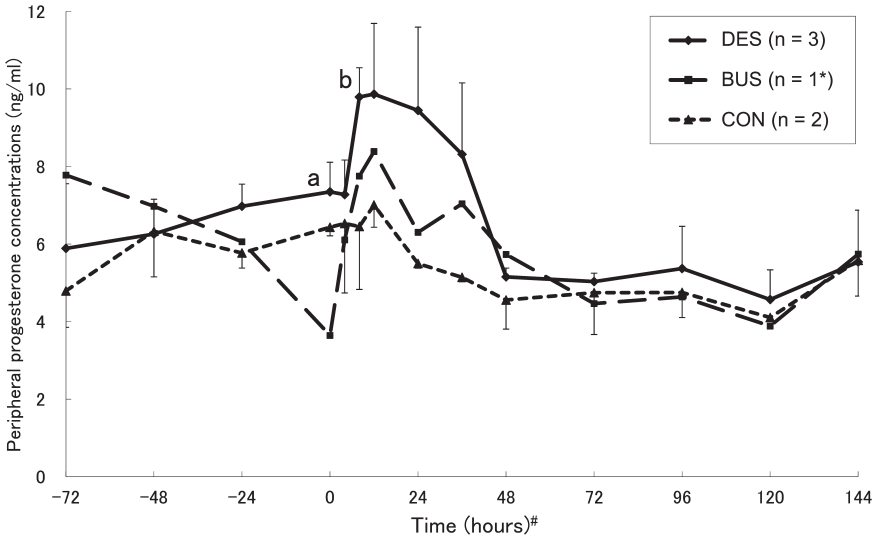

Fig. 3. Change in peripheral progesterone concentrations (mean ± SE) before and after intramuscular administration of GnRH analogs or placebo (Experiment 1). DES, deslorelin-treated group; BUS, buserelin-treated group; CON, glycerin-treated control group. * Data for one of the two animals was not available. [ab] $P<0.05$. [#] 0=time of injection.

mares because it had been sold before the time of pregnancy diagnosis. There was no significant difference in conception rates at day 15 and day 30 or in embryonic death rate between the DES and CON groups (Tables 1–3).

Administration of GnRH analog (buserelin acetate) in the luteal phase after breeding in the mare has been reported to be effective in decreasing in embryonic death and increasing the conception rate [7, 9, 10]. In the cow, GnRH administration at day 11 or 14 increased the conception rate by 10 to 12% [2, 8, 11]. Its mechanism may be explained by exogenous GnRH administration inducing ovulation in the luteal phase, leading to diestrous formation of the corpus luteum and an increase in blood progesterone level. Administration of steroids increased the diameter of the embryo at day 18 in the mare [15]. Therefore, we aimed to clarify the change in peripheral LH and progesterone concentrations

after intramuscular administration of deslorelin acetate in the luteal phase after breeding and AI (Experiment 1) and the effect of administration on conception rate in bred mares (Experiment 2).

In Experiment 1, an increase in LH level by 4 hr after administration and an increase in progesterone level by 8 hr after administration were observed. This result suggests a luteotrophic action of deslorelin by stimulating LH secretion. Moreover, deslorelin had a longer effect on the increase in LH secretion than buserelin in this study. The dose of deslorelin (750 μg) was determined to induce ovulation of the mature follicle 40 to 42 hr later [3, 16]. However, none of the mares had an induced ovulation or formation of a secondary corpus luteum in the luteal phase after deslorelin or buserelin treatment. Blood progesterone concentrations did not increase after buserelin treatment [7, 13]. Pulsatile administrations of buserelin acetate increased the blood progesterone level in the luteal phase in the mare [6]. A higher dose of deslorelin or pulsatile administrations of deslorelin or buserelin may be necessary to induce ovulation and to have a substantial effect causing a sufficient increase in blood progesterone level during the postbreeding period.

Table 1. Conception rate at day 15 and day 30 in the mares in the deslorelin-treated (DES) group and placebo control (CON) group

	Day 15 (%)	Day 30 (%)
DES group (n=29)	19/29 (65.5)	16/29 (55.2)
CON group (n=29)	18/29 (62.1)	15/28* (53.6)

Difference was not significant. *Pregnancy diagnosis was not performed in one of the mares.

In Experiment 2, deslorelin acetate administration nine days after breeding (AI) failed to improve the conception rate in the mare. Although conception rates varied among different methods of breeding (natural breeding, AI with fresh semen, or AI with frozen-thawed semen), there was no difference in conception rate between the two groups in each category. Therefore, method of breeding was not a confounding factor for the association between treatment and conception rate in our analysis.

The two most common pharmacologic agents used to induce ovulation are human chorionic gonadotropin (hCG) and GnRH analog. The advantage to using deslorelin over hCG is that its smaller molecular weight would reduce the chance of developing antibodies against it after using it multiple times in a season [1]. In addition, the injectable formulation of deslorelin we used in our study does not cause the prolonged interovulatory interval seen with the administration of deslorelin implants [12]. However, we did not draw the conclusion that a transient increase in blood progesterone concentration in the luteal phase after intramuscular administration of deslorelin acetate could lead to a decrease in embryonic death rate in the mare. Although intramuscular administration of deslorelin acetate did not improve conception rate in our study, it has potential for application in reproductive management of mares because of its long activity in stimulating LH secretion from the pituitary gland compared with buserelin acetate. Treatment earlier than day 9 post breeding might be more effective in improving the endocrine environment, which would lead to better reproductive performance in the mare. Further research using high-quality semen in a greater number of animals should be required to elucidate the effectiveness of

Table 2. Conception rate at day 15 and day 30 in mares in the deslorelin-treated (DES) group and placebo control (CON) group

	Percherons, Bretons and their crossbreds (n=28)		Thoroughbreds, Arabians and their crossbreds (n=30)	
	Day 15 (%)	Day 30 (%)	Day 15 (%)	Day 30 (%)
DES group (n=29)	11/14 (78.6)	8/14 (57.1)	8/15 (53.3)	8/15 (53.3)
CON group (n=29)	13/14 (92.9)	11/14 (78.6)	5/15 (33.3)	4/14* (28.6)

Difference was not significant. *Pregnancy diagnosis was not performed in one of the mares.

Table 3. Conception rate at day 15 and day 30 in mares bred by natural breeding, AI with fresh semen and AI with frozen-thawed semen in the deslorelin-treated (DES) group and placebo control (CON) group

	Natural breeding (n=7)		AI with fresh Semen (n=31)		AI with frozen-thawed Semen (n=20)	
	Day 15 (%)	Day 30 (%)	Day 15 (%)	Day 30 (%)	Day 15 (%)	Day 30 (%)
DES group (n=29)	3/3 (100)	3/3 (100)	14/16 (87.5)	11/16 (68.8)	2/10 (20)	2/10 (20)
CON group (n=29)	4/4 (100)	4/4 (100)	12/15 (80)	9/14* (64.3)	2/10 (20)	2/10 (20)

Difference was not significant. *Pregnancy diagnosis was not performed in one of the mares.

deslorelin acetate in inseminated mares. Also, the associations among progesterone level in the luteal phase in early pregnancy, development and survival of the embryo, and production of an inhibitor of luteolytic factors should be clarified in the future.

In conclusion, while a single intramuscular administration of deslorelin acetate in the luteal phase increased peripheral LH concentrations up to 12 hr after treatment and progesterone concentrations at 8 hr after treatment, it may not be effective in decreasing the embryonic death rate or increasing the conception rate in bred mares.

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