Original Research

Effect of a Single Injection of Long-acting Progesterone on the First Ovulation in Early and Late Spring Transitional Mares

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ABSTRACT

Since 1966, exogenous progestins have been used in equine practice for pregnancy maintenance, estrous suppression, and control of erratic sexual behavior. This study was designed to investigate the use of a new compounded controlled-release progesterone preparation (BioRelease P4 LA 300) in early and late spring transitional mares. In the first experiment, the pharmacodynamic properties of the preparation were studied in five geldings. In the second experiment, the use of a single intramuscular injection (600 mg) was tested in 68 embryo-recipient mares maintained under natural photoperiod in the Southern Hemisphere. Experiment 1 demonstrated elevated serum concentrations of progesterone (>1 ng/mL) for 7.6 ± 2.2 days. In experiment 2, there was no effect of treatment in mares that were treated on September 18, independent of their follicular status at day of treatment (10 to 15 mm; 20 to 25 mm, respectively). When mares with a follicular size of 20 to 25 mm were treated on October 14, significantly more progestin-treated mares (10/12; 83%) ovulated between 10 and 24 days after treatment than untreated controls (3/12; 25%) ($P < .05$). Additionally, there was a trend in mares treated on October 14 for a shorter treatment to ovulation interval (mean $\pm$ SD, 18.6 ± 8.7 days) compared with untreated controls (mean $\pm$ SD, 26.7 ± 14.7 days) ($P = .07$). Administration of one single injection of long-acting progesterone is a simple and effective method of controlling the first ovulation of the season in late transitional mares.

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1. Introduction

Mares entering a seasonal anestrus phase during the winter of temperate latitudes do not normally commence ovulatory cycles until April or early May in the Northern Hemisphere, or October or early November in the Southern Hemisphere [1]. As spring approaches, the mare's ovarian activity changes gradually from a quiescent inactive state of winter anestrus to a multifollicular state associated with prolonged and often erratic estrous behavior [2]. During the transitional period, there is a hormonal imbalance, with high follicle-stimulating hormone (FSH) secretion and low luteinizing hormone (LH) secretion [3]. During winter, pituitary stores of LH and hypothalamic stores of gonadotropin-releasing hormone (GnRH) are significantly reduced. After the winter solstice, concentrations of GnRH in the hypothalamus are readily replenished, whereas pituitary stores of LH increase more slowly. This hormonal environment results in follicular growth without ovulation [4]. Typically, during February, March, and early April in the Northern Hemisphere, and during August,

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September, and October in the Southern Hemisphere, several (3.7 ± 0.9) preovulatory size (>30 mm) follicles arise and regress without ovulating, until a competent follicle develops, which results in the first ovulation of the season [5]. Thus, the goal of any hormonal treatment during the transitional period is to hasten the initial ovulation of the breeding season and to suppress the long, erratic estrous periods.

Several methods have been reported to hasten vernal transition, including artificial lighting [6], multiple injections of GnRH analogs [7], equine FSH and pituitary extracts [8], or stimulation of prolactin secretion through the use of dopamine D_2-receptor antagonists [9-11]. There is a controversy as to whether exogenous progesterone during the transition period actually hastens ovulation or possibly just synchronizes the first ovulation of the year [12].

The use of progestins in transitional mares was first reported in 1973 by van Niekerk et al., where injection of 100 mg of progesterone in oil administered daily for 7 days to mares in the late transitional phase terminated estrous signs within 2 days, and mares ovulated within 6 to 7 days after cessation of therapy [13]. In 1982, Webel and Squires reported that oral administration of altrenogest (0.044 mg/kg s.i.d.) for 12 to 15 days had a similar effect on the late transitional mares, with the treatment being most effective when mares with at least moderate-sized follicles (>20 mm) were treated after mid-March [14]. In both studies, the treatment was found to be ineffective when administered to mares during deep winter anestrus [13,14]. A combination of artificial photoperiod and 10 to 15 days of exogenous progesterone exposure has been used to synchronize the first ovulation of the year [15].

It is inconvenient and time-consuming to administer altrenogest or progesterone to mares daily. In 1997, Newcombe and Wilson reported the use of progesterone-releasing intravaginal devices in transitional mares, resulting in >90% of mares ovulating in a mean of 18.3 days after insertion of the device [16]. An equine intravaginal progesterone delivery device (Cue-Mare, Duirs PfarmAg, Hamilton, New Zealand) was shown to be effective in transitional mares with a history of estrous behavior of >10 days and follicles measuring <25 mm [17]. The authors reported 87% ovulation response rate within 7 days of removal of device, with a 52.9% conception rate for mares being bred that cycle.

Recently, long-acting progesterone compounds have been made available, which allow the administration of progestins only once [18]. The long-acting compounds are designed to release progesterone at a controlled rate for a period of 7 to 10 days.

The goal of this study was to investigate the bioavailability of a new compounded controlled-release progesterone preparation (BioRelease P4 LA300: BET PHARM LLC, Lexington, KY; available at: www.betpharm.com), and subsequently to test in a clinical stetting whether a single injection of long-acting progesterone was able to control follicular growth and ovulation in mares in early and late spring transition. Additionally, it was investigated whether progesterone-treated mares would ovulate earlier than untreated controls.

2. Materials and Methods

2.1. Experiment 1

Five geldings of various light horse breeds received a single intramuscular injection of 600 mg (2 mL) of BioRelease P4 LA300. Serum samples were collected on days −2, 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 after treatment and stored at −20°C until assayed for progesterone, using commercially available reagents (Diagnostic Systems Laboratories, Webster, TX).

2.2. Experiment 2

Experiment 2 was carried out at one reproduction facility in central Victoria (Australia) during the 2008 breeding season. Sixty-eight light breed mares from a commercial embryo transfer recipient herd were included in the study. The breed distributions were as follows: 52 Standardbreds, 15 Thoroughbreds, and 1 Quarter Horse. The average age was 7.6 ± 2.37 years (mean ± SD). All mares were maintained at pasture throughout the year, and were supplemented with lucerne hay and grain as necessary. Throughout the study period (September 2008 to November 2008), all the mares were maintained in good body condition. Ultrasonographic examination of the mares was performed every Monday, Wednesday, and Friday, from the beginning of September until the end of the study (November 2008). The largest follicle on each ovary, as seen on the ultrasound, was recorded at each examination, as were endometrial edema (grades: 0 to 4), cervical tone (1 to 3), and intrauterine fluid volume and echogenicity [19]. Transitional mares included in this study were identified through multiple ultrasonographic evaluations that did not reveal luteal tissue or significant follicular activity over at least a 30-day period.

On the basis of size of the largest follicle measured by ultrasonography, mares were divided into three groups (A, B, and C) at two different times. Group A consisted of 20 mares in deep anestrus with little or no ovarian activity (largest follicle: 10 to 15 mm) as measured on September 18; group B (n = 24) consisted of 24 mares in with some follicular activity (largest follicle: 20 to 25 mm) on September 18; and group C consisted of 24 mares in late transition phase (largest follicle: 20 to 25 mm) on October 14.

Half of the mares in each group (n = 10, group A; n = 12, groups B and C) were randomly assigned to receive an intramuscular injection of 600 mg of long-acting progesterone (BioRelease P4 LA 300 (2 mL), and half of the mares served as untreated controls. Follicular status of mares was recorded using ultrasonography three times weekly by a clinician who was unaware of the treatment status of the mares. Estrus was identified on the basis of ultrasonographic findings including endometrial edema (≥ grade 2), a soft relaxed cervix (grade 3), and presence of a dominant follicle (>35 mm). Mares were induced to ovulate with deslorelin (BioRelease Deslorelin LA; BET PHARM LLC, Lexington, KY; available at: www.betpharm.com) (2.25 mg i.m.) when a dominant follicle (>35 mm) was recorded. Ovulation was diagnosed by the absence of a previously identified preovulatory follicle and visualization of the characteristic echogenic structure formed by the collapse of
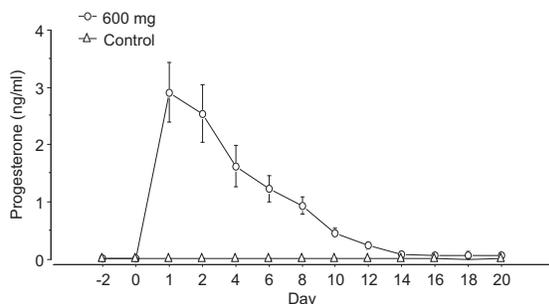
Fig. 1. Serum progesterone level of treatment group and untreated controls.

the follicular walls. The duration from progesterone treatment to 35-mm follicle and treatment to first ovulation was calculated for each individual mare.

2.3. Statistical Analysis

2.3.1. Experiment 1
Progesterone concentrations were analyzed by factorial ANOVA (SAS 9.2, Cary, NC), with gelding and day as the main effects, which were tested with the gelding × day interaction.

2.3.2. Experiment 2
Days from treatment to ovulation were expressed as mean ± SD, single-point variables were analyzed by ANOVA testing groups (A, B, C), treatment (controls, treatment), and the group x treatment interaction. To compare response with treatment, success was defined as ovulation within 10 and 24 days after treatment, and an ANOVA was run of the coded data (success = 1; no success = 0) [20], testing group, treatment, and group × treatment interaction. For all comparisons, significance was set at $P < .05$.

3. Results

3.1. Experiment 1

Analysis of the data indicated significant day effect ($P < .05$). Geldings receiving 2 mL of BioRelease P4 LA 300 experienced a mean peak progesterone concentration the day after injection (2.91 ± 1.16 ng/mL), followed by elevated serum progesterone levels (>1 ng/mL) for 7.6 ± 2.19 days (Fig. 1).

3.2. Experiment 2

Mean time in days from treatment to ovulation in the three groups (A, B, and C) is shown in Table 1. There was no

significant effect of treatment in days to ovulation, but there was a significant effect of group ($P = .044$) in mares treated on September 18 (groups A and B) versus those that were treated on October 14 (group C). There was no significant treatment group interaction in the days to ovulation. Additionally, a trend for a shorter treatment to ovulation interval was observed in late transition-treated mares (mean ± SD, 18.6 ± 8.7) compared with controls (mean ± SD, 26.7 ± 14.7) ($P = .07$).

The percentage of mares ovulating between 10 and 24 days after treatment in each treatment group (A, B, C) is shown in table 2. On the basis of the criterion that success was ovulation between 10 and 24 days of treatment, treatment was highly significant (mean = 0.283 for controls, 0.578 for treated mares; $P = .011$), and there was a tendency ($P = .0996$) for an interaction with group. The greatest treatment effect was in group C (Fig. 2; 0.25 vs. 0.833; $P = .003$ by least significant difference test of individual comparisons of treatment vs. control for each group).

Response to ovulation induction with deslorelin within 48 hours was similar among treatment groups (Table 3). No side effects of the treatment were observed in this study. The injection site in all 34 horses treated with P4 LA 300 was monitored daily for 1 week after injection, and no adverse reactions such as swelling, pain, or abscess formation were observed in the treated animals.

4. Discussion

The objective of this study was to investigate whether priming of mares with a single injection of a long-acting progesterone preparation was able to control follicular growth and ovulation in early and late spring transition phase. Previous studies have shown that only mares in middle to late transition period respond favorably to progestin treatment [13,14,16,21]. This is generally defined as mares having ≥20 mm follicles and being treated after March 15 (September 15 in the Southern Hemisphere) [21]. In the present study, there was no significant difference between mares treated on September 18 and controls (groups A and B), independent of their follicular status (10 to 15 mm or 20 to 25 mm, respectively). Based on our data, the mid-September time point may be too early for progestin treatment alone for mares kept under natural photoperiod, and therefore breeders should adjust their management of such mares more effectively by supplying supplemental photoperiod [15] or using a therapy designed to work earlier in year as eFSH [8] or dopamine D2-antagonists [9].

Table 1
Mean time between treatment and ovulation

Group	Number of Mares	Treatment	Date of Treatment	Time to 35 mm Follicle	Time to First Ovulation (Days)
Treatment on September 18					
A (10 to 15 mm follicle)	10	Yes	September 18	33.6 ± 21.3	36.1 ± 21.6
	10	No	September 18	30.7 ± 11.1	34.6 ± 12.0
B (20-25 mm follicle)	12	Yes	September 18	31.1 ± 24.2	34.3 ± 24.1
	12	No	September 18	23.9 ± 14.4	26.75 ± 14.3
Treatment on October 14					
C (20 to 25 mm follicle)	12	Yes	October 14	15.3 ± 7.8	18.6 ± 8.7
	12	No	October 14	23.5 ± 14.6	26.7 ± 14.7

Table 2
Percentage of mares ovulating 10 to 24 days after treatment

	Group A	Group B	Group C	Total
Treatment	4/10 (40%)	6/12 (50%)	10/12 (83%)[*]	20/34 (59%)
Control	3/10 (30%)	5/12 (42%)	3/12 (25%)[*]	11/34 (32%)

[*]$P < .05$.

For mares in the late transition period, exogenous progesterone effectively controlled the first ovulation of the year. Ten of 12 mares treated on October 14 (group C) ovulated between 10 and 24 days (mean, 18.6 ± 8.7 days) after treatment, compared with 3 of 12 (25%) of the controls (mean, 26.7 ± 14.7 days) ($P < .05$). Additionally, there was a tendency for hastening the initial ovulation in the treated mares (mean, 18.6 ± 8.7 days) compared with the controls (mean, 26.7 ± 14.7 days) ($P = 0.07$). The mean interval from treatment to ovulation (18.6 ± 8.7 days) observed in our study is very similar to the results of a study by Newcombe and Wilson, who reported that >90% of transitional mares ovulated with a mean of 18.3 days after insertion of a progesterone-releasing intravaginal devices [16].

BioRelease P4 (600 mg) resulted in elevated serum progesterone levels (>1 ng/mL) for 7.6 ± 2.19 days. This duration of estrus suppression is similar to the original study by van Niekerk et al. [13], but shorter compared with other studies, which aimed for longer progesterone treatment for 8 to 12 days [22] or 12 to 15 days [21], respectively. To the authors' knowledge, no studies have been published that aimed at determining the minimal duration of progestin treatment or blood level of progesterone required for effective control of the first ovulation of the season.

A recently published study showed an improved response in progestin primed mare to ovulation induction with human chorionic gonadotropin [23]. This finding could not be confirmed in our study, where the response rate to injectable deslorelin did not differ between mares with and without pretreatment with progesterone.

It is unclear whether endocrine changes at the ovarian level during the progestagen treatment or altered gonadotropin release after its withdrawal are responsible for the effectiveness of progestagens in the transitional mare. Squires et al. observed an increase in levels of plasma FSH at days 8 to 10 of altrenogest treatment, which the authors concluded may have triggered the events producing the first ovulation of the season [21].

Daily application of altrenogest, progesterone, or progesterone and estradiol in oil is time-consuming and can be inconvenient when broodmares are kept on pasture. The use of the long-acting progesterone compound in this study allowed administration of the drug only once, which is a significant management advantage because of the reduced labor and the associated handling stress to the mares and producers. Furthermore, this formulation can offer veterinarians an important means of maintaining effective compliance rates on farms with wide varieties of management systems.

The mares in this study served as embryo recipients; therefore, it was not possible to determine pregnancy data from transitional ovulations. However, in previously
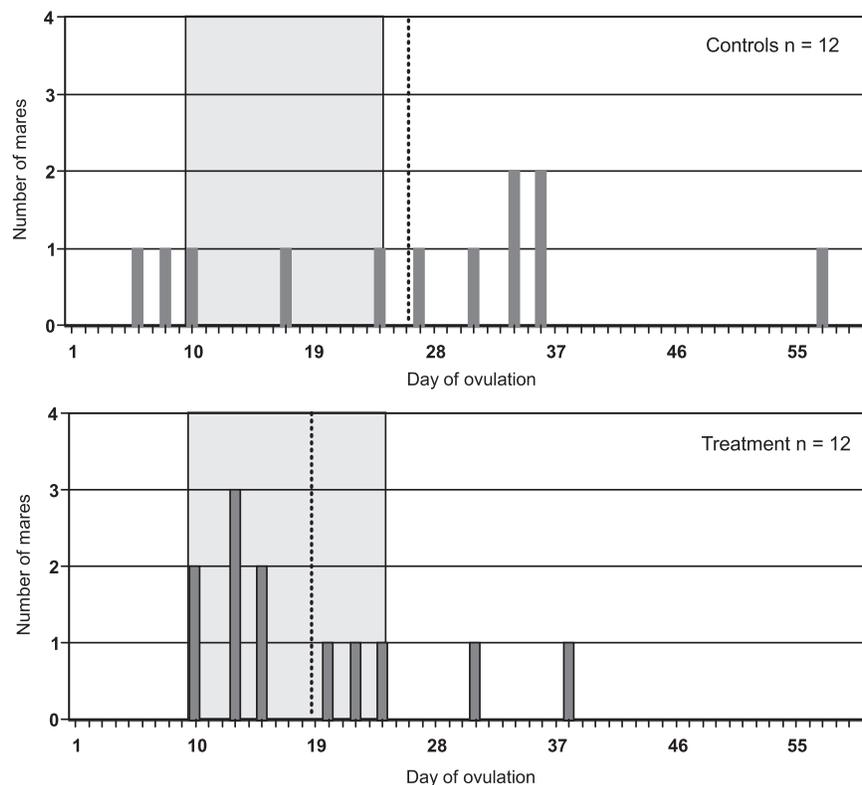

Fig. 2. Ovulation over time group C late spring transition treated on October 14.

Table 3
Response rate to ovulation induction with deslorelin

	Group A	Group B	Group C	Total
Treatment	8/10 (80%)	9/12 (75%)	9/12 (75%)	26/34 (76%)
Control	9/10 (90%)	9/12 (75%)	9/12 (75%)	27/34 (79%)

[*]$P < .05$.

published studies, pregnancy rates of progesterone-treated transitional mares were the same [13,21] or better [22] than the untreated controls.

In summary, the use of a single injection of a long-acting progesterone preparation provided a simple and inexpensive method of inducing an ovulatory estrus in the late transitional mare. More progestin-treated mares ovulated 83.3% (10/12) between 10 and 24 days (mean, 18.6 ± 8.7 days) after treatment, in comparison with 25% (3/12) of the controls (mean, 26.7 ± 14.7 days).

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