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Effect of short-term artificial light and transvaginal progesterone device on first ovulation in late transitional mares

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In study I, plasma progesterone concentrations were evaluated in anoestrous mares that received an intravaginal progesterone release device (IPRD) for 10 days. Mares were divided into 3 groups based on the dosage of progesterone (0 g, n=3; 1.38 g, n=5; and 1.9 g, n=5). No statistical differences were found in plasma progesterone concentrations between the two doses tested. In study II, the effects of a protocol based on a short program of artificial light combined with an IPRD containing 1.38 g of progesterone on oestrous behaviour and onset of ovulation were evaluated. IPRDs were inserted into 31 late transitional mares (10 days of treatment). The mares were divided into a control group (n=9, IPRD with 0 g of progesterone) and two treatment groups (T1, n=10, IPRD with 0 g of progesterone and artificial light; T2, n=12, IPRD with 1.38 g of progesterone and artificial light). The percentages of mares in heat within the first 14 days after treatment were 100%, 70%, and 100% in the control, T1, and T2 groups, respectively (P=0.097), and their ovulation rates were 44%, 60%, and 100%, respectively (P≤0.01). In conclusion, a protocol based on artificial light and an IPRD containing 1.38 g of progesterone for 10 days could be considered to advance the first ovulation of the year in late transitional mares, as it ensures a higher rate of ovulation within the first 14 days after treatment.

Key words: *late transitional mares, light, ovulation, progesterone*

J. Equine Sci.
Vol. 33, No. 1
pp. 1–6, 2022

The mare is seasonally polyoestrous, with regular ovulatory cycles occurring during the longer days of spring and summer. At the beginning of the breeding season in early spring, the mare enters into a transition period between the anovulatory season and the first ovulation of the year. This period is characterized by long, erratic oestrous behaviour, with growth and regression of multiple small follicles that fail to ovulate [11].

In the equine industry, there is a desire to breed mares as early as possible in the breeding season to ensure that foaling will occur earlier than it does for other breeders. Several protocols have been utilized to try to advance the first ovulation of the year. Light control has been used for years to extend the day length, hastening the onset of the breeding season [6, 10, 22]. More recently, different hormonal protocols have been used to optimize reproduction during the transition period: implant of a GnRH agonist [2, 18], purified equine follicle-stimulating hormone [21], melengestrol acetate [19], oral progestogens [1, 28], long-acting progesterone [25], and different intravaginal

Received: October 23, 2021
Accepted: February 8, 2022
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progesterone release devices (IPRDs) combined with the administration of $PGF_{2\alpha}$ and/or inductors of ovulation (hCG or GnRH agonist) [7, 17, 20, 31].

There have been conflicting reports on the efficacy of progesterone in shortening the transitional period and advancing the first ovulation of the year. The differences between reports seem to be due to the stage of the transitional period when treatment begins. Thus, some authors have demonstrated that progesterone therapy does not advance the first ovulation of the year when administered to mares in deep seasonal anoestrous or the early transitional period [25, 28], while others have indicated that progesterone allows synchronization and advancement of the first ovulation in anoestrous/transitional mares [3, 20]. The latter authors have demonstrated that the administration of progesterone to mares in the transition phase with follicles bigger than 10 mm in diameter at the beginning of treatment stimulates follicular growth.

Several kinds of intravaginal devices containing different amounts of progesterone for use mainly in cattle have been developed by the pharmaceutical industry, and their development was soon followed by extra-label use in horses. The amount of progesterone contained in the intravaginal device may influence the circulating progesterone levels [30], and some studies, especially in cows, have demonstrated that plasma progesterone concentrations affect the LH pulse frequency and ovarian activity [26, 32].

Although there are some reports that have evaluated the efficacy of the increasing natural day length during a two-month period in conjunction with the administration of progestogens at the end of the treatment [13], to our knowledge, there are no reports about the use of a short regimen of increased daily light in combination with progesterone administration.

Thus, the aims of this study were twofold: first, to evaluate progesterone profiles after insertion of a IPRD containing two different doses of a hormone (study I); and second, to evaluate the effects of a short program of artificial light exposure plus an IPRD for 10 days on oestrous behaviour and the onset of ovulation in late transitional mares (study II).

Materials and Methods

Field studies were performed in compliance with animal welfare regulations established by the Faculty of Veterinary Sciences, UNCPBA.

StudyI

Experiments were carried out in August at the facilities of Syntex S.A. in Ayacucho city, Province of Buenos Aires, Argentina. Thirteen mares of the light Criollo cross-type breed, ranging from 3 to 10 years old and having an average body weight of 450 ± 30 kg, were used in this study. Mares were in seasonal anoestrous and were examined by transrectal ultrasonography (real-time, B-mode scanner, Pie Medical 480) to confirm the absence of ovarian activity (follicles ≤20 mm). They were randomly divided into three groups receiving progesterone releasing devices (DIB®, Syntex S.A., Buenos Aires, Argentina) that contained 0 (n=3), 1.38 (n=5), or 1.9 g (n=5) of the hormone and were inserted into the vagina. On Day 11, the devices were removed.

Blood samples were daily collected for 13 days, starting 10 min before device insertion. Plasma progesterone concentrations were measured using an RIA kit (Coat-A-Count®, Siemens Medical Solutions Diagnostics, Los Angeles, CA, U.S.A.) previously validated for use with equine plasma [12]. The sensitivity of the assay was 0.1 ng/m*l*, and the intra- and inter-assay coefficients of variation were below 13% for concentrations between 0.1 and 40 ng/m*l*.

The area under the concentration-time curve (AUC) from Day 0 to Day 12 after the beginning of treatment was calculated by the trapezoidal rule [4]. The AUC values obtained after the insertion of the intravaginal devices containing progesterone were statistically compared between the groups by an unpaired t-test. Statistical analysis was carried out using Statistical Analysis System V9.1 (SAS, Institute Inc., Cary, NC, U.S.A.). Data are presented as the mean ± SEM, and differences were considered to be significant when $P<0.05$.

StudyII

Animals and location: Experiments were carried out at the stud farm General Lavalle, a property of the Argentine Army, in Tandil, Province of Buenos Aires, Argentina (37°25'S, 56°16'W). Mares of the Silla Argentino breed, ranging from 3 to 10 years old and having body weights between 480 and 520 kg, were used. Management conditions and nutritional status were similar for all animals. The mares were maintained on cultivated and natural pastures with free access to water. This study was carried out from September to the beginning of October, a period considered to be a transitional phase, although great variability exists.

Ultrasonographic assessment: The mares were examined by transrectal ultrasonography (real-time, B-mode scanner, Pie Medical 480) at the beginning of the study and at the moment of intravaginal device withdrawal. Afterwards, those that showed oestrous behaviour were examined daily until ovulation occurred or until the end of the study (14 days after device removal) in the case of mares that did not ovulate. The diameter of the largest follicle was recorded, and ovulation was assessed based on the disappearance of the previously observed ovulatory follicle, which was defined as a follicle with a follicular diameter ≥40 mm,

loss of the follicular spherical shape, and uterine oedema. Furthermore, the mares were teased daily from the day after device removal until the end of the study.

Treatments

A total of thirty-one mares considered to be in the late transition period based on ovarian ultrasonographic assessments (multiple follicles ≥20 mm and ≤35 mm, and absence of a corpus luteum) were selected. They were randomly divided into three groups: the control (n=9), T1 (n=10), and T2 (n=12) groups. The control and T1 groups received an IPRD with 0 g of progesterone, and the T2 group received an IPRD containing 1.38 g of progesterone (1.38 g; DIB®, Syntex S.A., Buenos Aires, Argentina; selected based on the results of study I). The IPRDs were inserted into the vagina on Day 0 of the study in all mares and were withdrawn on Day 10. Furthermore, the T1 and T2 groups were exposed to artificial light from 6:00 p.m. to 11:00 p.m. in order to ensure a day length of 16 hr using 50 W reflectors located in their paddocks. The mares were exposed to artificial light from the day of insertion of the IPRD (Day 0) until the day on which the device was withdrawn (Day 10). In all groups, retainment of the device was checked daily. At the time of device removal, those mares with a corpus luteum detected by ultrasonography were treated with an injection of d-cloprostenol, a synthetic analogue of prostaglandin $F_{2\alpha}$ (75 μg, Ciclase®, Syntex S.A., Buenos Aires, Argentina).

Statisticalanalysis

The percentages of mares with follicles ≥30 mm on the day of device removal, percentages of animals displaying oestrous, and ovulation rates were compared between groups by a Fisher's exact test using the FREQ procedure. Mean follicular diameters at the beginning of the experiment and intervals between device withdrawal and oestrous or ovulation and between the first signs of oestrous and ovulation were analysed by ANOVA followed by Fisher's LSD test to detect differences between groups. All statistical analyses were carried out using Statistical Analysis System V9.1 (SAS, Institute Inc., Cary, NC, U.S.A.). Data are presented as the mean ± SEM, and differences were considered to be significant when $P<0.05$.

Results

StudyI

None of the IPRDs were lost during the treatment period.

In all groups, the plasma progesterone concentrations were below 1 ng/m*l* before device insertion, and the control group maintained this low level throughout the experiment. In the treatment groups (1.38 and 1.9 g of progesterone), the mean plasma progesterone concentrations reached their maximum levels on Day 1 (around 17 ng/m*l* for both groups) and declined to their minimum levels on Day 11 (1.38 group, mean 4.26 ± 0.27 ng/m*l*; 1.9 group, mean 5.79 ± 0.90 ng/m*l*). The plasma progesterone concentrations were below 1 ng/m*l* at 12 hr after device withdrawal (Fig. 1). The AUC values after device insertion were similar between the treatment groups (79.70 ± 4.68 and 88.48 ± 8.51 ng·day/m*l* in the 1.38 and 1.9 groups, respectively; $P=0.39$).

Due to the absence of differences between the treatments, the device with the lower progesterone content (1.38 g) was selected to be used in the second study.

StudyII

Intravaginal devices were well tolerated by the mares, which did not show vaginitis or discomfort during the treatment period, and all devices remained in the vagina until they were removed.

On the day of device insertion, none of the mares had follicles greater than 35 mm in diameter. The mean follicular diameter was similar between groups at the beginning of the experiment (control, 21.6 ± 7.2 mm; T1, 21.0 ± 1.6 mm; T2, 23.6 ± 1.6; $P=0.87$). The numbers of mares that ovulated while the devices were inserted into the vagina were 1, 2, and 0 in the control, T1, and T2 groups, respectively. These mares were treated with a d-cloprostenol injection when devices were withdrawn.

A dominant follicle, which had a diameter of greater than 30 mm and was characterized by an anechoic structure, was detected by ultrasonography in 44% (4/9), 40% (4/10), and 50% (6/12) of the mares in the control, T1, and T2 groups, respectively, on the day of device removal ($P=0.91$).

Overall, 90% of the mares entered into oestrus within the first 14 days after treatment. The percentage of mares in heat did not significantly differ between groups ($P=0.097$). The intervals between device withdrawal and oestrus were 4.67 ± 1.92 days (range 1 to 14), 2.14 ± 0.16 days (range 1 to 6), and 2.00 ± 0.48 days (range 1 to 6) in the control, T1, and T2 groups, respectively ($P=0.18$; Table 1). Three mares in the T1 group failed to show oestrus behaviour during the study.

The number of mares that ovulated post-treatment was highest in the T2 group (mares exposed to artificial light plus IPRD with 1.38 g of progesterone; $P\leq0.01$). In the control group (not exposed to artificial light or progesterone), the interval from device withdrawal to ovulation was 6.25 ± 1.66 days (range 3 to 10 days), and 5/9 of the mares did not ovulate before the end of the study. In the T1 group, which was comprised of animals exposed only to an extended artificial day length, 6/10 mares ovulated, and the interval from device withdrawal to ovulation was 8.50 ± 1.36 days (range 4 to 14 days). All the mares in the T2 group ovulated within a mean of 8.25 ± 0.96 days after device removal (range 3 to 12 days; $P=0.53$; Table 1).

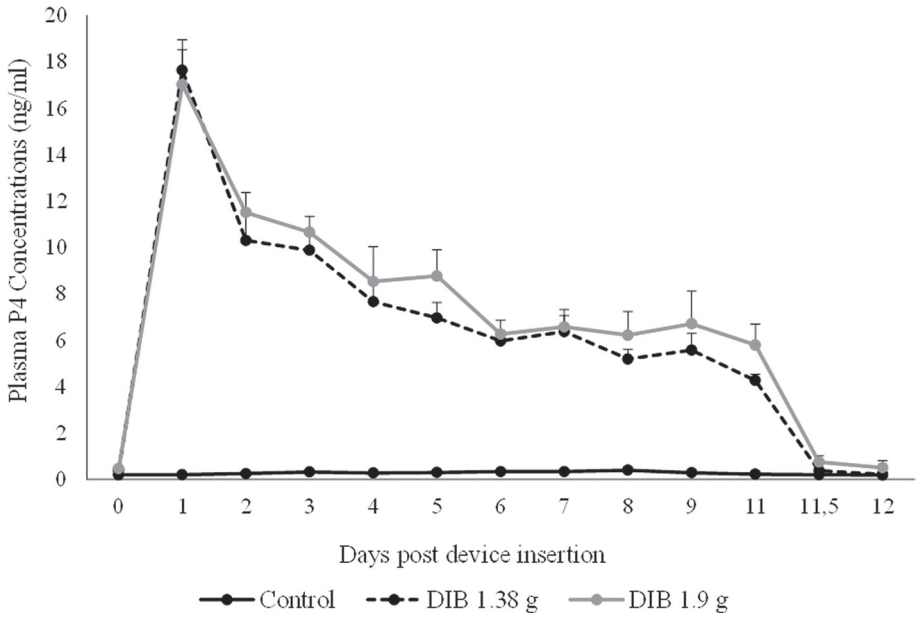

Fig. 1. Mean plasma progesterone concentrations (ng/m*l*) in anoestrous mares that received IPRDs containing 0 g (black line), 1.38 g (dotted line), or 1.9 g (grey line) of progesterone from Day 0 to Day 12.

Table 1. Reproductive performance of the control group (untreated mares), T1 group (treated with an IPRD without progesterone and with artificial light), and T2 group (treated with an IPRD with 1.38 g of progesterone and artificial light)

Treatment group	No. of animals (n)	Animals showing oestrous (%)	Interval to oestrous (days)	Animals ovulated (%)	Interval to ovulation (days)
Control	9	100 (9/9)[a]	4.67 ± 1.18[a]	44 (4/9)[a]	6.25 ± 1.66[a]
T1	10	70 (7/10)[a]	2.14 ± 1.34[a]	60 (6/10)[a]	8.50 ± 1.36[a]
T2	12	100 (12/12)[a]	1.83 ± 1.02[a]	100 (12/12)[b]	8.25 ± 0.96[a]

Values with different superscripts within a column are statistically different.

In the mares that ovulated, the intervals between heat and ovulation were 5.25 ± 1.65, 7.20 ± 1.48, and 6.42 ± 0.95 days in the control, T1, and T2 groups, respectively (P=0.68). In the T1 group, one mare ovulated without showing heat previously, and two mares exhibited oestrous behaviour but did not ovulate before the end of the study.

Discussion

In the literature, numerous treatments have been reported to advance the first ovulation of the breeding season and to ensure that mares conceive as early as possible. Most of these treatments are based on oral progestin administration or an extra-label use of bovine intravaginal devices containing different doses of progesterone. To our knowledge, this is the first report in which plasma progesterone concentrations have been evaluated after the insertion of the same device containing two different doses of progesterone (1.38 vs. 1.9 g) in mares. Moreover, the effects of a short program of artificial light exposure plus an intravaginal progesterone-releasing device (1.38 g) for 10 days on oestrous behaviour and the onset of ovulation in late transitional mares were evaluated.

The results of the first study using deep anoestrous mares showed no significant differences in circulating progesterone concentrations between devices containing 1.38 or 1.9 g of the hormone, which is similar to those previously reported in a study that used the same commercial brand in cyclic mares [5]. Nevertheless, the plasma progesterone concentrations recorded in this study were considerably higher than those reported by Vizuete *et al.*, who used an IPRD containing 1.38 g of progesterone from another commercial brand and reported plasma progesterone concentrations between 2.5 and 1.5 ng/m*l* [31]. Handler *et al.*, who used a device with 1.55 g of progesterone, stated that the plasma concentrations of progesterone were between 6 and 3 ng/ in anoestrus mares [15]. Previous studies in cattle indicated that the rate of diffusion of progesterone from intravaginal devices

to the bloodstream could differ due to different levels of progesterone content, differences in contact surface area, or the type of outer layer material used [30]. Differences could also be affected by the measurement method. Some of these factors could explain the differences in plasma progesterone concentrations between studies.

In the present study, none of the devices were lost during the experiments, suggesting that intravaginal devices are a good alternative for treating mares, in agreement with previous reports [14, 20, 31].

Mares exposed to a short light regimen without progesterone treatment showed erratic oestrous signs (oestrous without ovulation or ovulation without oestrous signs), and the percentage of mares that ovulated was similar to that of the control group. These observations are in agreement with the previous findings reviewed by Squires, who mentioned that mares under light regimens still experience a transition period from winter anoestrus to normal cyclicity [29]. In the present study, the combination of artificial light and progesterone administration enabled the achievement of 100% of the mares showing regular oestrous signs and ovulation between days 3 and 14 post-treatment. Earlier studies have suggested that the administration of oral progestins would be more effective for controlling the long, erratic oestrous periods at the onset of the breeding season [27] or in combination with a two-month period of increased daily lighting [23].

In this study, the percentage of mares in oestrus and the ovulation rate after treatment with light and progesterone for ten days were higher than those previously reported using only an IPRD in transitional mares [20, 31]. It has been previously suggested that, although an abrupt artificial increase in day length advances the frequency of pulsatile LH secretion [9], the administration of progesterone would probably stimulate follicular growth by either eliciting FSH release or permitting sufficient FSH secretion to maintain keep follicle dynamics [20]. Furthermore, a previous study reported that follicular development in association with increasing plasma FSH concentrations was observed during its initial treatment with progesterone followed by an increase in LH concentrations at the end of treatment, which supports the growth of the newly formed dominant follicle [16]. Earlier studies have demonstrated that, during the transitional period, there is a hormonal imbalance characterized by low LH secretion and fluctuating concentrations of FSH [8, 24]. The results of the present study suggest that exposure to artificial light combined with exogenous administration of progesterone regulates gonadotrophins secretion and, therefore, controls more efficiently the ovarian activity of late transitional mares.

In summary, the results of the present study indicate that a protocol based on artificial light supplementation plus an intravaginal device containing 1.38 g of progesterone for 10 days could be considered a good tool to advance the first ovulation of the year in late transitional mares, as it ensures a high rate of ovulation between days 3 and 14 after treatment.

Acknowledgments

We thank Syntex S.A., Buenos Aires, Argentina, for providing partial financial support for the present study. No specific funding source was involved in this study.

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