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ORIGINAL RESEARCH

Evaluation of Injectable Sustained Release Progestin Formulations for Suppression of Estrus and Ovulation in Mares

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ABSTRACT

Thirty-one mares were used in an experiment to evaluate the effectiveness of three sustained-release injectable formulations of altrenogest and one formulation of medroxyprogesterone acetate (MPA) for long-term suppression of estrus and ovulation. Luteolysis was induced by injection of prostaglandin-$F_{2\alpha}$ (Lutalyse) on day 0 (6th day after the previous ovulation) and was immediately followed by treatment with 1) no injection (controls; n = 7), 2) 1.5 mL of an altrenogest solution in sustained-release vehicle (LA 150, 1.5 mL; 225 mg altrenogest; n = 6), 3) 3 mL (450 mg altrenogest) of the same solution (n = 6), 4) 500 mg altrenogest in lactide-glycolide microparticles suspended in 7-mL vehicle (MP 500; n = 6), or 5) 1.0 g MPA as a 5-mL suspension. Mares were checked for estrus daily, and their ovaries scanned every other day until a 25-mm or greater follicle was detected, after which they were scanned daily. Control mares returned to estrus an average of 3.9 days after Lutalyse administration; all the single-injection altrenogest formulations increased ($P <$ .05) the days to return to estrus, with the greatest increase occurring in mares receiving MP 500. Return to estrus was not affected by MPA treatment. Time of ovulation was determined by serial ultrasound scans and confirmed by daily plasma luteinizing hormone (LH) and progesterone concentrations. Control mares ovulated an average of 8.8 days after Lutalyse administration. Treatment with 1.5 or 3 mL of LA 150 increased ($P <$ .05) the mean days to ovulation to 16.5 and 21.2 days, respectively; MP 500 increased ($P <$ .05) the days to ovulation to 33.5 days. Administration of MPA did not affect ($P >$ .1) days to ovulation relative to control mares. The MP 500 treatment provided long-term suppression of estrus and ovulation and could prove useful for that purpose. Treatment with the LA 150 solutions provided shorter-term suppression, and a relatively tight grouping of the individual mares around the mean days to ovulation; these one-shot formulations could be useful for synchronizing ovulation in cyclic mares and inducing normal estrous cyclicity in vernal transitional mares exhibiting erratic, anovulatory estrous periods.

Keywords: Altrenogest; Estrus; Medroxyprogesterone acetate; Ovulation; Progestins

INTRODUCTION

Progestins are frequently used to prevent the expression of estrus in race, show, and broodmares for periods of weeks and up to months or longer. Daily administration can be an impractical method for administration of altrenogest or progesterone to mares, being inconvenient and time consuming. Recent advances in biodegradable, controlled-release drug delivery systems offer the potential for single-administration products to replace prolonged daily treatment protocols, resulting in reduced labor, less handling stress, and greater flexibility for veterinarians in maintaining effective compliance rates on farms and show barns with wide varieties of management systems. The primary objective of the current experiment was to evaluate the effectiveness of three sustained-release injectable formulations of altrenogest, a progestin that along with progesterone is known for its efficacy for pregnancy maintenance and inhibition of estrus behavior and ovulation in the mare.[1-9] Medroxyprogesterone acetate, a synthetic progestin that is widely prescribed but not documented in controlled experiments to affect estrus or ovulation, was included for comparison.

MATERIALS AND METHODS

Thirty-one mares of light horse breeds, maintained on native grass pasture at the Louisiana State University Agricultural Center horse farm in Baton Rouge, LA, were used in this study. They were in good body condition (scores of >6[10])

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and ranged in age from 5 to 16 years. Verification of normal reproductive cyclicity was determined by transrectal ultrasound scanning of the ovaries, estrus detection with a vigorous stallion, and serial radioimmunoassay of plasma progesterone concentrations.

The goal for treatments was to start all mares in mid-diestrus simultaneously with induction of luteolysis using prostaglandin $F_{2\alpha}$. To achieve this goal, when estrus detection was initiated (March 27), those mares displaying estrus and having plasma progesterone concentrations below 1 ng/mL were examined by ultrasound until a follicle 30 mm or greater was detected. When a follicle reached 30 mm, 2,000 international units (IU) human chorionic gonadotropin (Chorulon, Intervet, www.intervet.com) was administered (intramuscularly) to induce ovulation. Those mares were subsequently examined by ultrasound until ovulation and corpus luteum formation were verified. Six days after ovulation (day 0), they were injected with 10 mg dinoprost tromethamine (Lutalyse; Pfizer Animal Health, www.lutalyse.com), randomly assigned to treatment, and administered the appropriate treatment injection.

Mares that had displayed diestrus signs at the onset of estrus detection and had progesterone concentrations above 1 ng/mL were given Lutalyse at that time; those that had not responded by entering estrus 5 days later were re-treated. Once estrus was detected in these mares, they were followed through estrus and ovulation, administered Lutalyse 6 days after ovulation, and randomly assigned to, and administered, treatment. The five treatments administered on day 0 are described in Table 1; six mares were used per treatment group except for controls ($n = 7$). Because of the diversity in treatment vehicles and forms, it was not feasible to apply an appropriate control vehicle injection to control mares on day 0; thus, no injection was administered to those mares.

Mares were assessed for estrus by daily teasing with a vigorous stallion. Once treated, they were examined via ultrasound every other day until a follicle 25 mm or greater was detected. When a follicle exceeded 25 mm, the mare was scanned daily until the follicle ovulated or regressed to smaller than 25 mm. Blood samples were collected via jugular venipuncture before each ultrasound examination for later hormonal assay. Once a mare returned to estrus and ovulated, or ovulated a large, dominant follicle without showing estrus, monitoring was discontinued.

Plasma luteinizing hormone (LH) concentrations were measured by a double-antibody radioimmunoassay previously validated for horse plasma.[11] Plasma concentrations of progesterone were measured with commercially available reagents (Diagnostic Systems Laboratories, Webster, TX). Intra-assay and interassay coefficient of variation (CV) and assay sensitivity were 6%, 9%, and 0.2 ng/mL for the LH assay and 5%, 8%, and 0.1 ng/mL for the progesterone assay. Data were analyzed by the general linear models procedure of SAS (SAS Institute Inc., Cary, NC). Single-point variables were analyzed via a one-way analysis of variance (ANOVA), and means were compared using the least-significant difference test.

RESULTS

Control mares returned to estrus an average of 3.9 days after Lutalyse administration (Table 2). All the single-injection altrenogest formulations increased ($P < .05$) the days to return to estrus, with the greatest increase occurring in mares receiving MP 500. Four of the six mares receiving the altrenogest MP 500 formulation did not display estrus around the time of their first ovulation; when removed from the experiment because of ovulation, they had gone 28, 34, 47, and 49 days without displaying estrus. For analysis, these data were used as minimum estimates and are included in the mean in Table 2. Administration of medroxyprogesterone acetate (MPA) did not affect ($P > .1$) the return to estrus relative to control mares.

Ovulation was detected by daily ultrasound scanning of the ovaries (Table 2) and was confirmed by LH and progesterone concentrations (days to LH peak minus 1 day and days to first detectable rise in progesterone minus 1 day). With a few exceptions, the estimates of days to ovulation were in agreement. Control mares ovulated an average of 6.0 (peak LH - 1), 7.2 (progesterone - 1), or 13.1 (ultrasound scanning) days after Lutalyse administration; the mean of the ultrasound data includes one control mare (recorded to have ovulated on day 23) in which both hormonal estimates indicated an ovulation on day 4.

Treatment with 1.5 or 3 mL LA 150 increased ($P < .05$) the mean days to ovulation to 16 to 21 days, and MP 500 increased ($P < .05$) the days to ovulation to 32 to 34 days. Administration of MPA did not affect ($P > 0.1$) days to ovulation relative to control mares. As a measure of how well the mares grouped around the mean time to ovulation (based on progesterone concentrations), CVs for the five groups were calculated separately; the range and CVs were 4–12 days and 37%, 15–20 days and 11%, 20–23 days and 6%, 28–48 days and 27%, and 4–20 days and 46% for the control group and those receiving LA 150 1.5 mL, LA 150 3 mL, MP 500, and MPA, respectively.

DISCUSSION

With a few exceptions, the estimation of days to first ovulation (based on ultrasound scanning, LH concentrations, and progesterone concentrations) were in good agreement, and each was highly correlated to days to first estrus ($r > 0.8$ in all cases). One exception was a control mare that evidently ovulated a mid-cycle follicle in response to the Lutalyse injection on day 0. That ovulation (4 days later) was not detected by the ultrasound technician, even though LH and progesterone indicated that it had occurred.

Table 1. Injectable sustained-release progestin formulations

Treatment	Active Ingredient	Form or Vehicle	Mg Total	Volume, mL
Control	None	None		
LA 150[a] 1.5 mL	Altrenogest	BioRelease LA altrenogest solution	225	1.5
LA 150[a] 3 mL	Altrenogest	BioRelease LA altrenogest solution	450	3.0
MP 500[b]	Altrenogest	Lactide-glycolide microparticles	500	7.0
Medroxyprogesterone acetate (MPA)[c]	MPA	Aqueous suspension	1000	5.0

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Table 2. Days to first estrus and days to ovulation, based on ultrasound observations, peak LH values minus 1 day, or first high progesterone (P4) value minus 1 day

			Days to Ovulation		
Treatment	No. of Mares	Days to First Estrus	Ultrasound Scan	Peak LH -1 Day	First High P4 -1 day
Control	7	3.9[c]	13.1[a,*]	6.0[d]	7.2[c]
LA 150 1.5 mL	6	12.7[a]	16.0[ab]	16.2[ab]	17.3[a]
LA 150 3 mL	6	15.8[a]	20.2[b]	21.8[b]	21.5[a]
MP 500	6	32.8[b,†]	32.7[c]	33.7[c]	34.2[b]
MPA	6	6.2[c]	11.3[a]	10.5[ad]	11.0[c]
SEM		2.3	2.3	2.3	2.0

[a-d] Means within a column with no like superscript differ ($P < 0.05$).
* The first ovulation in one control mare was missed by the ultrasound technician, although hormonal data was consistent with ovulation on day 4; this mean reflects that missed ovulation.
† Mean of all mares; 4 of the 6 mares treated with MP 500 had silent ovulations; they did not show heat for at least 28, 34, 47, and 49 days when removed from the experiment due to ovulation; these data were used in the ANOVA and are included in the mean.

All altrenogest formulations were effective at delaying the onset of estrus and ovulation, with the MP 500 formulation having the longest lasting inhibitory effect. This formulation consists of 500 mg altrenogest encapsulated in lactide-glycolide microparticles and was designed to release approximately 16.6 mg/day over a 30-day period. This formulation did in fact inhibit onset of estrus by at least 25 days and, in some mares, considerably longer. A similar amount of altrenogest (450 mg) in a long-release vehicle (LA 150, 3 mL) inhibited the onset of estrus for approximately 15 days, indicating a greater availability (more rapid release) in the LA 150 formulation. Thus, the MP 500 formulation would be useful for longer-term suppression of estrus in performance mares, whereas the LA 150 formulation would be useful for shorter-term suppression of estrus and ovulation. Given that altrenogest can be fed to mares for months without affecting subsequent fertility after withdrawal,[12] repeated administration of MP 500 as desired to keep mares out of heat should be a useful means of achieving this goal.

Four of the six mares treated with MP 500 did not display estrus around their first ovulation after treatment. Similarly, Daels et al[4] reported that mares having a silent ovulation during altrenogest treatment had prolonged luteal phases of 40 to 54 days. Blanchard et al[9] reported a similarly increased incidence of silent ovulations in mares treated with progesterone microspheres related to residual progesterone at the end of treatment before ovulation. It appears that the threshold concentration of altrenogest necessary to inhibit displays of estrus is lower than that to suppress LH secretion and ovulation. It is assumed that any undesirable, nonreproductive behaviors of performance mares in estrus (eg, being less cooperative, less attentive to tasks, hyperexcitable, or sensitive to touch) would be suppressed by altrenogest in the same manner (and by the same concentration of altrenogest) as displays of heat; however, this needs to be confirmed directly before a final conclusion can be made.

Mares receiving LA 150 altrenogest formulation all showed normal estrus and ovulated (based on the average of all estimates) an average of 3.8 (at 1.5 mL) or 5.4 days

(at 3 mL) after onset of estrus, whereas control mares ovulated an average of 4.9 days after coming into heat. The timing of ovulations in mares receiving LA 150 was also tightly synchronized (clustered), considering that they received no ovulation-inducing drug (ie, human chorionic gonadotropin or gonadotropin-releasing hormone analog). The CVs for days to ovulation (based on progesterone concentrations) were 37% for control mares and 11% and 6% for mares treated with 1.5 and 3.0 mL LA 150, respectively. This indicates that the LA 150 treatments may be useful for synchronizing the timing of estrus in cyclic mares as well as for inducing normal estrous cyclicity in vernal transitional mares exhibiting erratic, anovulatory estrous periods, similar to altrenogest feeding[7] or estradiol plus progesterone treatments.[8]

As reported by McKinnon et al,[5] treatment with MPA did not maintain pregnancy in mares, whereas treatment with altrenogest did.[12] MPA is currently available for estrus suppression from commercial pharmacies, although it is usually stated that its effectiveness is based on anecdotal rather than scientific evidence. The dose used in the current experiment (1 g in 5 mL) was 2 to 4 times the published common dose as reported by Squires.[13] Whether this dose might be effective in a small percentage of mares is unknown; however, in the current experiment and dose, it was not.

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