

Evaluation of Sustained Release Progestin Formulations in Mares

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ABSTRACT

Introduction

Progestins are frequently used to prevent the expression of estrus in race, show, and broodmares for periods of a week to a month or longer. Unfortunately, daily treatments are an impractical method to administer altrenogest or progesterone to mares, being inconvenient and time consuming. Recent advances in controlled release drug delivery systems offer the potential for single administration products to replace prolonged daily treatment protocols. Such formulations reduce labor and the associated handling stress to the animals and producers and offer veterinarians a means of maintaining effective compliance rates on farms and show barns with wide varieties of management systems.

Although altrenogest and progesterone have unequivocally been demonstrated to suppress estrus and ovulation in mares (Loy and Swan 1966, Squires et al. 1983, 1992), other widely prescribed synthetic progestins, including medroxyprogesterone acetate (MAP), have been shown not to affect estrus, ovulation or pregnancy maintenance (McKinnon et. al., 2000). The present study was designed to evaluate the effectiveness of four sustained release progestin formulations on inhibition of estrus behavior and ovulation in the mare.

Materials and Methods

Thirty one randomly selected light-horse mares were maintained on native pasture and ad libitum hay at the Louisiana State University horse unit for this study. Verification of reproductive cyclicity was determined by follicular ultrasound, teasing with a vigorous stallion, and radioimmunoassay of plasma progesterone concentrations. Mares displaying estrus (Estrus mare group) with plasma progesterone levels below 1 ng/mL were examined by ultrasound until a follicle 30 mm or greater was determined. When a follicle reached 30 mm, 2 mL containing 2000 IU human chorionic gonadotropin (Chorulon®, Intervet, www.intervet.com) was administered to induce ovulation. Mares in the estrus group were subsequently examined by ultrasound until ovulation and corpus luteum formation were verified. Six days after ovulation was determined, mares in this group were given a 2 mL (10mg) injection of the prostaglandin dinoprost tromethamine (Lutalyse®, Pfizer Animal Health, www.lutalyse.com) and randomly assigned to treatment. Mares not displaying estrus (Non-estrus mare group) with progesterone concentrations above 1 ng/mL and the presence of a corpus luteum verified by ultrasound were given 2 mL Lutalyse®. Once estrus was detected by teasing with a stallion, these mares were treated as the mares assigned to the estrus mare group. Day of treatment (d 0) was simultaneous with the final, mid-diestrus injection of Lutalyse (5 or 6 d after ovulation). Mares were randomly allotted to one of 5 treatments (n=6) listed in table 1.

Table 1. Experimental Sustained Release Progestin Treatments			
Treatments	Total Dose	Dose/day	Dose Volume
All treatments were given immediately following 2 mL Lutalyse®*			
1) Controls (2mL Lutalyse only)*	----	---	---
2) Medroxyprogesterone acetate as a 5 mL solution.**	1000 mg	----	5mL
3)Altrenogest LA150 as a 1.5 mL solution. *** 10 day formulation	225 mg	22.5 mg/day for ten days	1.5 mL
4) Altrenogest LA150 as a 3 mL solution*** 15 day formulation	450 mg	30 mg/day for 15 days	3 mL
5) Altrenogest MP 500 as lactide-glycolide microparticles suspended in 7mL)**** 30 day formulation	500 mg	16.6 mg/day for 30 days	7 mL
*Lutalyse®, Pfizer Animal Health, www.lutalyse.com			
** Hagyard Pharmacy LLC, Lexington, KY 40511, www.hagyardpharmacy.com			
*** BET Pharm LLC, Lexington, KY 40511, www.betpharm.com			
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Mares were examined via ultrasound every other day after treatment until a follicle 25 mm or greater was detected. Once a follicle exceeded 25 mm, the mare was scanned daily until the follicle ovulated or regressed to < 25 mm. Blood samples were collected before each ultrasound examination for later hormone assays. Once a mare returned to estrus and ovulated, or ovulated a large, dominant follicle without showing estrus she was considered finished. Concentrations of progesterone were measured with commercially available reagents (Diagnostic Systems Laboratories, Webster, TX). Intra- and interassay CV and assay sensitivities were 5%, 8%, and 0.1 ng/mL. Data were analyzed by the Proc Mixed procedure of SAS (SAS Institute Inc., Cary, NC). Single point variables were analyzed via a one way ANOVA.

Results:

All single injection altrenogest formulations were effective at extending ($P < 0.05$) the return to estrus and interovulatory interval in mares when compared to controls (Table 2). The altrenogest MP 500 formulation had the longest effect ($P < 0.05$) at suppressing estrus and inhibiting ovulation. The mean return to estrus was 32.8 ± 4.8 days compared to 3.9 ± 0.5 days in the control mares. Mean time to ovulation after administration of Lutalyse and the altrenogest for the MP 500 formulation was 34.2 ± 3.7 days compared to 7.2 ± 1.2 days in control mares. Both doses of the liquid altrenogest LA 150 formulation effectively extended the return to estrus following treatment for 12.7 ± 0.4 days and 15.8 ± 1.5 days and resulted in a mean days of ovulation relative to treatment of 17.3 ± 0.75 and 21.5 ± 0.54 days for the 1.5 mL and 3 mL doses, respectively. Medroxyprogesterone acetate treatment was not effective at delaying estrus or ovulation

Table 2 Days to Estrus and Ovulation following Lutalyse and Sustained Release Progestin Treatment

Formulation	N	Days to estrus	Days to ovulation
LA 150 1.5-mL	6	12.7 ± 0.42^b	17.3 ± 0.75^b
LA 150 3 mL	6	15.8 ± 1.51^b	21.5 ± 0.54^b
MP 500	6	32.8 ± 4.80^c	34.2 ± 3.72^c
Medroxyprogesterone	6	6.2 ± 1.38^a	11.0 ± 2.14^a
Controls	7	3.9 ± 0.51^a	7.2 ± 1.17^a

^c Means \pm SE; means with different superscripts differ ($P < 0.05$)

Discussion:

All altrenogest formulations were effective at extending the interovulatory interval and delaying estrus, with the MP 500 formulation having the greatest inhibitory effect. This formulation could be beneficial for the performance horse by inhibiting estrus behavior for a period of 30 days and could be administered repeatedly as desired to maintain reproductive quiescence or anestrus behavior. Four of the six mares treated with MP 500 did not display estrus around their first ovulation after treatment, suggesting an additional 15 to 16 days of estrus suppression could be expected. Daels et al.(1996) reported that mares having a silent ovulation during altrenogest treatment had prolonged luteal phases of 40 to 54 days. It appears that the threshold concentration of altrenogest necessary to inhibit displays of estrus is lower than that to suppress LH secretion and ovulation which might extend estrus suppression in such mares considerably longer.

The shorter acting altrenogest LA150/1.5 mL formulation was also very effective and would be valuable when shorter periods of estrus suppression (12 to 14 days) are desired such as in transitional mares to establish normal cycles or for estrus and ovulation synchronization programs where the degree of estrus and ovulation compare favorably with daily altrenogest treatment (Squires et al. 1992) or progesterone + estradiol treatments (Burns et al. 1993). The altrenogest LA150/1.5 mL should also be suitable for pregnancy maintenance in non-cyclic or ovariectomized recipient mares. (Morrow and Burns, 2007).

Lastly, our results confirm the observations of McKinnon et. al. (2000), that medroxyprogesterone acetate treatment is not effective for the suppression of estrus or ovulation in mares. This progestin is currently widely used, although its effectiveness is based on anecdotal rather than scientific evidence. The dose used herein (1 gm in 5 mL)

was 2 to 4 times the published common dose as reported by Squires (1993). Whether this dose might be effective in a small percentage of mares is unknown; however, in the present experiment and dose, it was not.

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