

Efficacy of Medroxyprogesterone Acetate in Suppression of Estrus in Cycling Mares

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ABSTRACT

The effects of compounded medroxyprogesterone acetate (MPA) on follicular activity and estrous behavior were evaluated. Eighteen cycling mares were assigned to one of three treatment groups. Mares in the MPA group ($n = 6$) were injected intramuscularly with 1,600 mg MPA (week 1), then 400 mg weekly for the next 5 weeks. Saline mares ($n = 6$) were injected intramuscularly weekly for 6 weeks. Altrenogest mares ($n = 6$) received 10 mL orally daily for 7 weeks. Mares were teased daily for 60 days and categorized as displaying estrous, diestrous, or neutral behavior. Transrectal ultrasound examinations were performed three times weekly, or daily when a 30-mm follicle was identified, until ovulation. Blood samples were harvested weekly for analysis of progesterone concentration and daily from days 14 to 23 for analysis of luteinizing hormone (LH) concentration. Mares treated with saline or MPA showed normal intervals of diestrus and estrus during the study. All altrenogest mares showed behavioral diestrus during treatment. All mares in the saline and MPA groups showed normal follicular development and ovulations. No altrenogest mares ovulated during treatment; four mares returned to estrus and resumed normal follicular development after treatment ceased. Progesterone analyses agreed with transrectal ultrasonographic ovarian activity for all mares. LH levels were lower for altrenogest-treated mares compared with MPA-treated and saline-treated mares during the treatment period. In conclusion, compounded MPA at dose rates and intervals used in this study was not effective in suppression of estrus, follicular development, or LH secretion in mares.

Keywords: Equine; Estrous behavior; Estrous suppression; Medroxyprogesterone acetate; Altrenogest

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INTRODUCTION

The physiologic breeding season of mares extends from March to October in the northern hemisphere, and typically during this time mares have a 21-day cycle length including 5 to 7 days of estrus.¹ The behavioral signs associated with estrus can potentially have detrimental effects on the performance and temperament of mares in work (including racehorses, performance horses, and show mares) and make handling more difficult. These estrous behaviors may include posturing, tail elevation and swishing, urinating and clitoral winking, squealing, kicking, aggressiveness and responsiveness to other horses, and sensitivity over the flanks, hindquarter, and abdomen.

Suppression of estrus may be advantageous for mares with estrous cycle-related behavior and performance problems, and less commonly for mares that show signs of pain or colic during estrus.² Most treatments described for reducing or eliminating estrous behavior in performance mares involve suppression of estrus through the use of hormones or other therapies to prolong the diestrous period.

Nonpharmacologic suppression of estrus can be achieved by manually reducing a pregnancy between days 16 and 22 postovulation, effectively resulting in a "pseudopregnancy" for more than 64 days postovulation in 11 of 11 mares,³ or by reducing a pregnancy after formation of endometrial cups. However, termination of pregnancy may be considered unethical by owners. The insertion of sterile glass balls into the uterus resulted in a prolonged diestrus for up to 90 days postovulation in 39% of mares,⁴ but the low efficacy may not be acceptable by owners. Ovariectomy may be a last resort option for mares to eliminate estrous behavior or colic signs associated with estrus.² Recently, it was reported that owners perceived behavioral improvement in 19 of 23 mares after ovariectomy.⁵

Pharmacologic suppression of estrus typically involves the use of progesterone or progestins to mimic endogenous progesterone associated with diestrus. Other pharmacologic agents used include repeated oxytocin injections to prolong diestrus⁶ and human chorionic gonadotropin to induce diestral ovulation.⁷ Gonadotropin-releasing hormone (GnRH) vaccines for mares are available commercially in some countries (Equity, Pfizer Animal Health, Australia) and can inhibit estrous behavior for at least 3 months.⁸ Synthetic

progestins such as daily oral supplementation with altrenogest (0.044 mg/kg; Regu-Mate)⁹ or daily injections of progesterone in oil (0.2 mg/kg)¹⁰ are effective at suppressing estrus. Both methods can be time consuming, requiring daily treatment, administration can be difficult, and injectable progesterone-in-oil can result in injection aversion and localized muscle pain and swelling, which is especially undesirable in the competition horse. A compounded long-acting formulation of progesterone can maintain blood progesterone concentrations greater than 1 ng/mL for up to 10 days,¹¹ which should be sufficient to suppress estrous behavior. However, long-acting injectable progesterone can be associated with significant muscle pain and swelling, which is undesirable in a performance horse. In late transitional mares, norgestomet failed to suppress estrous behavior;¹² progesterone and estradiol subcutaneous implants (Synovex-S), failed to suppress estrus in cycling mares.¹³ A number of progestins have failed to maintain pregnancy in the mare, including megestrol acetate, hydroxyprogesterone caproate, hydroxyprogesterone hexanoate, and norgestomet,^{14,15} presumably because of their inability to bind the equine progesterone receptor.

Medroxyprogesterone acetate (MPA) is a long-acting injectable progestin marketed as a contraceptive for human females (Depo-Provera, Pfizer), given every 3 months to prevent pregnancy through inhibition of gonadotropin secretion. Depo-Provera is reported to be an effective contraceptive in ungulates including hippos and giraffes but not in equids.¹⁶ MPA has been used successfully to suppress estrus in bitches and queens, although its use is associated with a high incidence of undesirable side effects.¹⁷ It is reported that MPA is used by some veterinarians in the United States for the purpose of suppressing estrus in mares, with dose rates and frequency varying from 200 to 250 mg every 8 to 14 days,^{18,19} 250 to 500 mg given once during the luteal phase,¹⁰ and up to 1,800 mg monthly for 450- to 550-kg mares.¹⁹ However, the efficacy of such dosing rates and intervals are not known, with reports ranging from no effect,^{19,20} to suppressing estrus for 2 to 3 months,¹⁰ and 6 weeks to 6 months.¹⁹ Researchers showed that 1,000 mg MPA given every 7 days failed to maintain pregnancies in mares after induced luteolysis.¹⁵ No studies have been undertaken to evaluate the effectiveness of MPA in suppressing cyclicity in mares, nor have any short-term or long-term safety studies been performed.

The aim of our study was to determine the effectiveness of compounded MPA in suppression of estrous behavior and the effects on follicular activity in comparison with altrenogest. We hypothesized that compounded MPA injections given to cycling mares would have no effect on ovarian follicular dynamics, progesterone, or luteinizing hormone (LH) concentrations, or teasing behavior, whereas altrenogest would suppress LH concentrations and suppress estrous behavior.

MATERIALS AND METHODS

Animals

Eighteen Quarter Horse mares aged 5 to 13 years were selected for the study after displaying normal behavior to a stallion during estrus and displaying normal follicular dynamics as assessed by daily transrectal ultrasonography during estrus. Procedures regarding the care and use of animals were approved by the Colorado State University Animal Care and Use Committee. The study was conducted from July through October 2007.

Treatments

Mares were randomly assigned to one of three treatment groups, and all treatments began day 7 after ovulation. MPA-treated mares were given 1,600 mg MPA (Wedgewood Pharmacy, Swedesboro, NJ) intramuscularly on day 7, then 400 mg on days 14, 21, 28, 35, and 42. Control mares received a placebo injection of saline on days 7, 14, 21, 28, 35, and 42. Altrenogest-treated mares were given 10 mL (22 mg) altrenogest orally (Regu-Mate, Intervet, Millsboro, DE) daily from day 7 through day 48.

Teasing Behavior

Mares were teased in a chute/rail using an experienced stallion daily from days 0 to 60. A total of seven stallions were used; the same stallion was not used on consecutive days. Teasing behavior was recorded as estrus when two or more receptive signs toward the stallion were observed (eg, ears forward, leaning, clitoral winking, urination), and diestrus when two or more negative signs were observed (eg, attempting to kick, bite, or move away from the stallion) and neutral when the mare displayed passive behavior toward the stallion. Assessors of teasing behavior were blinded to mare treatment group.

Ovarian Follicular Changes

Mares were palpated per rectum and examined by transrectal ultrasonography three times weekly, and daily when a follicle greater than 30 mm diameter was identified until ovulation. Assessors of follicular activity were blinded to mare treatment group.

Serum Progesterone and Luteinizing Hormone

Blood samples were harvested via jugular venipuncture, samples allowed to clot at room temperature, centrifuged at 400g for 15 minutes, and serum harvested and frozen at -20°C until hormone analyses were performed. Samples for progesterone concentration were collected weekly; samples for LH concentration were collected daily from days 14 through 23 postovulation (ie, sample collection began after the initiation of treatments). Serum samples were analyzed by radioimmunoassays for progesterone²¹ and LH²² as previously described. Assay detection limits

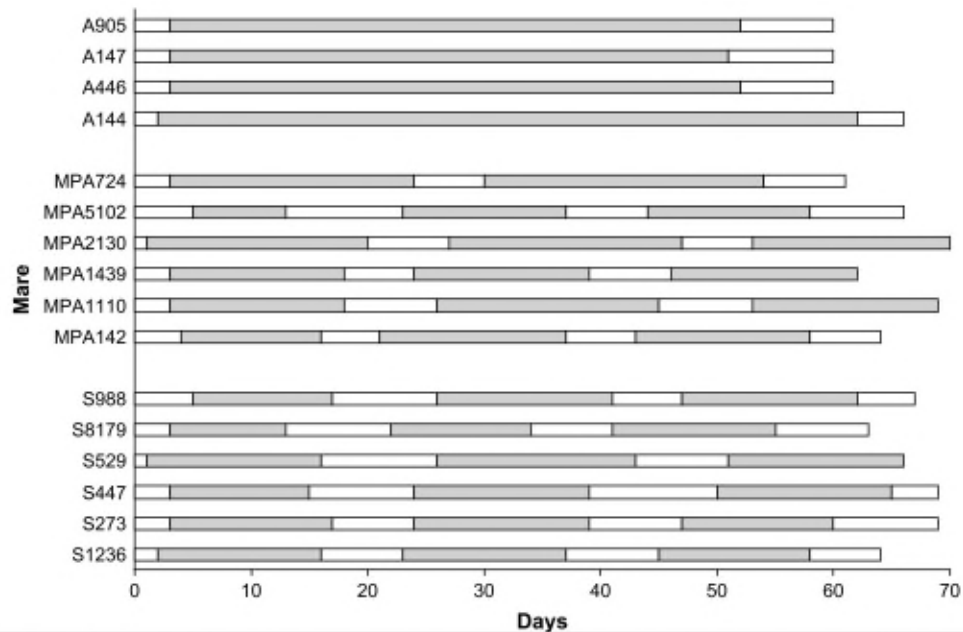


Figure 1. Teasing behavior of individual mares grouped by treatment (A = altrenogest administered daily from days 7 through 48; MPA = medroxyprogesterone acetate administered weekly from days 7 through 42; S = saline administered weekly from days 7 through 42). Open bars indicate when mares showed estrous behavior, gray bars indicate when mares displayed diestrus behavior. Data from two altrenogest-treated mares that became anestrus during the treatment period are not included. All mares ovulated on day 0. One mare (A144) had a persistent corpus luteum from days 0 through 56 and returned to estrus at day 62.

for progesterone and LH were 0.01 and 0.1 ng/mL, respectively. Intraassay and interassay coefficients of variation were 7% and 10%, respectively, for progesterone, and 9% and 10%, respectively, for LH.

Statistics

Statistical analysis was performed using SAS v9.1.3 (SAS Institute Inc., Cary, NC) and NCSS 2007 (Kaysville, UT). Mare teasing behaviors were analyzed by chi-square statistics to detect group differences. Progesterone and LH data were analyzed by repeated-measures analysis of variance with the GLIMMIX procedure of SAS. The models contained time, treatment, and time by treatment interactions as fixed effects. Mare within treatment was a random effect. Post hoc comparisons were done using *t*-tests to compare between treatments at each time and between times for each treatment. Area under the curve (AUC) analysis was used to evaluate group differences in LH attributable to treatment using the trapezoidal technique. Values were considered to be statistically different at $P < .05$.

RESULTS

All saline- and MPA-treated mares showed regular estrous and diestrus behavior during and after the treatment

period (Fig. 1), whereas no altrenogest-treated mares displayed estrous behavior during the treatment. The number of mares displaying behavioral estrus was not significantly different between the saline and MPA treatment groups but were both significantly different for the altrenogest-treated group ($P < .00001$). Mares treated with saline or MPA showed no differences in duration of estrous behavior (mean \pm SD; 8 ± 2 and 7 ± 1 days, respectively) or diestrus behavior (14 ± 2 and 16 ± 4 days, respectively). Four altrenogest-treated mares displayed estrus behavior after cessation of treatment (range, 6–18 days). One altrenogest-treated mare had a persistent corpus luteum evident on ultrasound examination throughout the treatment period. This was confirmed by serum progesterone levels greater than 2 ng/mL from days 0 to 56. This mare was excluded from further analyses. During the treatment period, two altrenogest-treated mares exhibited reduced follicular activity consistent with anestrus; these mares were excluded from further analyses.

Mean serum progesterone concentrations for each treatment group at 7-day intervals are shown in Figure 2. Altrenogest-treated mares had serum progesterone concentrations less than 2 ng/mL from days 21 through 56. There were significant time and time \times treatment interactions ($P < .0001$ and $P = .006$, respectively).

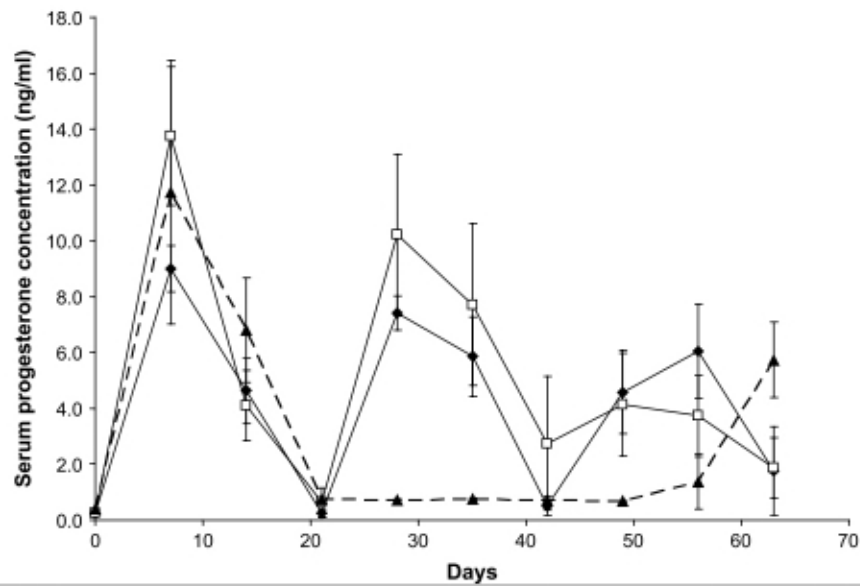


Figure 2. Mean serum progesterone concentrations (ng/mL ± standard error) of saline-treated mares (n = 6, —◆—, administered weekly from days 7 through 42), medroxyprogesterone acetate-treated mares (n = 6, —□—, administered weekly from days 7 through 42), and altrenogest-treated mares (n = 3, - -▲- -, administered daily from days 7 through 48). Data from two mares that became anestrus during the treatment period are not included, and data from one mare with a spontaneous persistent corpus luteum after the initial ovulation are not included). All mares ovulated on day 0.

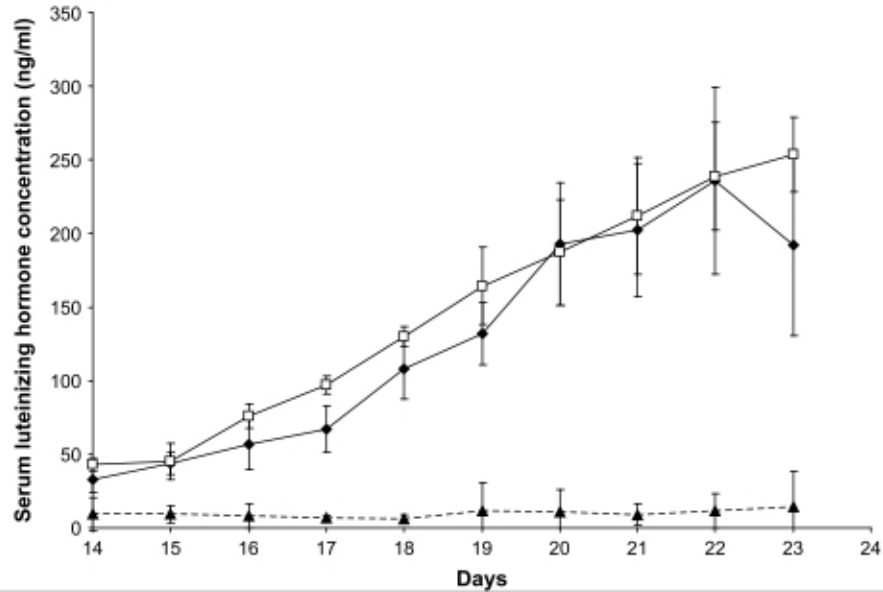


Figure 3. Mean serum LH concentration (ng/mL ± standard error) on days 14 to 23 of saline-treated mares (n = 6, —◆—, administered weekly from day 7 through 42), medroxyprogesterone acetate-treated mares (n = 6, —□—, administered weekly from days 7 through 42) and altrenogest-treated mares (n = 3, - -▲- -, administered daily from days 7 through 48). Data not shown for two altrenogest mares that became anestrus during the treatment period, and one mare with a spontaneous persistent corpus luteum following initial ovulation. All mares ovulated on day 0.

Mean serum LH concentrations for each treatment group are presented in Figure 3. There were significant differences in LH concentration attributable to time,

treatment, and treatment × time ($P < .0001$, $P = .04$, and $P < .005$, respectively). Least squares mean estimates for serum LH AUC were 495.8, 1150.6, and

1298.4 days \times pg/mL for altrenogest-, saline-, and MPA-treated mares, respectively.

DISCUSSION

Anecdotally, the use of MPA in mares to suppress estrus is widespread. In a postal survey, 2.6% of veterinarians cited MPA as their most preferred treatment regimen for performance problems associated with the estrous cycle.²³ In the same study, 65% of respondents preferred Synovex implants to treat problems associated with the estrous cycle; however, it was subsequently demonstrated that these implants were not effective in horses.¹³

The current study demonstrates that administration of compounded MPA at the chosen dose rates and intervals had no effect on estrous behavior, ultrasonographic follicular development and ovulation, or serum progesterone and LH concentrations in cycling mares. In contrast, altrenogest was effective in suppressing signs of behavioral estrus and serum progesterone and LH concentrations in cycling mares, as has been previously demonstrated.⁹

Two altrenogest-treated mares became anestrus during treatment (as evidenced by small follicles, lack of follicular development ultrasonographically, low serum progesterone, and LH concentrations), which was not unexpected given the time of year when the study was carried out.¹

One altrenogest-treated mare developed a persistent corpus luteum, a relatively common phenomenon that can occur in mares with no uterine pathology during the breeding season²⁴ and has been reported in mares treated with altrenogest.²⁵ Persistent corpora lutea also can arise from the last ovulation of the breeding season.²⁶ The affected mare in the current study returned to estrus 62 days after ovulation, a period consistent with other reports.^{24,25}

Altrenogest is effective at estrus suppression, but many owners may find this cost-prohibitive and time consuming. Alternative approaches to modifying negative behaviors associated with estrus may be considered initially. Researchers recently reported that 12 of 14 mares with a history of reduced performance associated with estrus had improved performance after having a Caslick's procedure, perhaps because of reduced referred pain from the genital region after the procedure.²⁷ Further large-scale studies are required to validate these observations. Recent studies indicate that a commercial GnRH vaccine is effective in long-term suppression of estrus in mares.^{8,28} However, anecdotal reports suggest that some mares may not cycle normally for 1 or more years after vaccination. This potential lack of ability to regulate duration of efficacy with the GnRH vaccine may limit clinical use if only short-term estrus suppression is desired. The effects of repeated GnRH vaccination (potentially in repeated breeding seasons) on future fertility need to be investigated.

The fact that a high initial dose of compounded MPA (1,600 mg), and a subsequent weekly dose (400 mg each week) did not suppress estrus, block development of a mature follicle, prevent ovulation, or suppress LH secretion suggests that this compounded synthetic progestin does not bind to the equine progesterone receptor, as has been reported for another synthetic progestin, norgestomet.¹² Compounded MPA was chosen for this study because it used by veterinarians in practice with the intent of estrus suppression. Compounded MPA is not approved for use in the horse. For further clarification on the use of compounded medications in horses, please refer to the American Association of Equine Practitioners (www.aaep.org).

In conclusion, this study clearly demonstrates that this compounded preparation of MPA, at the described dose rates and intervals, was not effective in suppression of estrous behavior; consequently, the use of this product in horses in this manner to suppress estrus cannot be justified and should be discouraged.

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