REFEREED

ORIGINAL RESEARCH

Estradiol Interactions with Dopamine Antagonists in Mares: Prolactin Secretion and Reproductive Traits

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ABSTRACT

Two experiments studied the effects of pretreatment with estradiol benzoate before treatment with a dopamine antagonist on prolactin secretion and reproductive traits in mares during (1) the seasonal anovulatory period and (2) the normal breeding season. Experiment 1 was performed in winter with 17 mares selected for low follicular activity. Nine mares received estradiol benzoate injections every other day for a total of 10 injections; 8 mares received similar injections of vehicle. Ten days after onset of injections, all mares were placed on daily injections of sulpiride (250 mg) for 35 days or until ovulation. Plasma prolactin concentrations were higher ($P < .001$) in mares receiving estradiol than in controls for all assessments from days 12 through 36. Plasma luteinizing hormone (LH) concentrations were also increased ($P < .05$) by estradiol treatment from days 14 to 23. Mean day of first ovulation was 73.6 for control mares and 29.0 for estradiol-treated mares ($P = .016$). Estradiol treatment greatly enhanced prolactin secretion in response to sulpiride and increased LH secretion in seasonally anovulatory mares, which together hastened the date of first ovulation by an average of 45 days. Experiment 2 was designed to assess the efficacy of a long-acting, single-injection microparticle preparation of another dopamine antagonist, domperidone, for increasing prolactin secretion in cyclic mares in the summer. The experimental design and procedures used in experiment 1 were repeated, except that a single 3-g domperidone-microparticle injection was administered on day 11 rather than 45 days of sulpiride injections. Day 0 was the first day of estrus for each mare. Prolactin concentrations were higher ($P < .05$) in mares receiving estradiol than in control mares from days 12 through 25 and after a thyrotropin-releasing hormone injection on d 21. Estrous cycle traits (time to ovulation and time of luteal regression) were not affected ($P > .1$) by treatment. Estradiol enhanced the prolactin response to a single injection of 3 g domperidone in cyclic mares in the summer in a manner similar to the estradiol enhancement of prolactin secretion in response to daily sulpiride injections in anovulatory mares in winter. Thus, the single injection of domperidone could possibly replace the daily sulpiride injections used in experiment 1 to induce ovulation in seasonally anovulatory mares; this needs to be tested in future experiments.

Keywords: Mares; Domperidone; Prolactin; Seasonal reproduction; Sulpiride

INTRODUCTION

Prolactin seems to be involved in the vernal transition of seasonally anovulatory mares. Treatment of seasonally anovulatory mares with ovine prolactin[1] or recombinant porcine prolactin[2] induced ovulation earlier in the year relative to control mares. Stimulation of prolactin with dopamine antagonists also has been reported to hasten the onset of follicular activity and ovulation, but to varying degrees of success. Using the orally active dopamine antagonist, domperidone, Brendemuehl and Cross[3] treated mares starting January 15 and hastened mean date of ovulation by 78 days. Besognet et al[4] injected mares with a readily available antagonist, sulpiride, beginning February 5, and hastened ovulation by 33 days. In contrast, Donadeu and Thompson[5] treated mares with sulpiride from January 14 through February 14,

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and no mare ovulated within that period. In a study of the prolactin response to daily injections of sulpiride, Thompson and Depew[6] reported a rapid drop in response after the first day; they concluded that sulpiride stimulated prolactin secretion, but evidently not production, in the long term.

Estradiol has been reported to increase prolactin secretion and pituitary content in ovariectomized pony mares in summer[7]; the effect of estradiol on prolactin in winter is unknown. Estradiol was also reported to increase luteinizing hormone (LH) secretion in ovariectomized mares in February.[8] Possibly the lack of estrogen in seasonally anestrous mares resulted in less than ideal prolactin and ovarian responses to sulpiride in previous experiments. Experiment 1 presented herein was designed to test whether estradiol pretreatment of seasonally anovulatory mares would enhance the prolactin and ovarian responses to daily injection of sulpiride. Based on the results of experiment 1, experiment 2 was performed to test whether estradiol pretreatment produced a similar stimulation of prolactin response to a single, 3-g injection of domperidone in long-acting, biodegradable microparticles.

MATERIALS AND METHODS

Experiment 1

Mares and Treatments. Eighteen mares were identified as seasonally anovulatory by ultrasonographic (Aloka 550V with 5-MHz linear array transducer; Aloka Science and Humanity, Wallingford, CT) scanning of the ovaries in late December and early January; progesterone concentrations were used after-the-fact to confirm the lack of ovulation or luteal tissue. All mares were Quarter Horse or Thoroughbred type ranging in age from 7 to 17 years, with body condition scores (BCS) of 5.5 to 8 (as described by Henneke et al.[9]). Mares were maintained as a group on winter rye grass pasture with supplemental native grass hay as needed to maintain body condition.

On January 11 (day 0), mares were randomly allotted to two groups: estradiol treated (n = 9) and controls (n = 9). Beginning on day 0, treated mares received intramuscular injections of 11 mg estradiol benzoate (Sigma Chemical Co., St. Louis, MO) in vegetable oil (2 ml) every other day for a total of 10 injections. Control mares received equivalent injections of the vehicle. Beginning on day 11 (halfway through the estradiol and vehicle treatments), all mares were started on a daily subcutaneous injection regimen of 250 mg sulpiride in 1 ml vegetable shortening. Sulpiride injections were continued until a mare ovulated or through day 45, whichever occurred first.

Assessments and Blood Sampling. Samples of blood were collected by jugular venipuncture from each mare on the mornings of days—1, 0, 1, 2, 5, and 11 (before treatment injections) and then every 3 days thereafter. Once a mare displayed an ovarian follicle of at least 25 mm, blood samples were collected daily until 5 days after ovulation.

In addition to the routine morning blood samples, frequent blood samples (0, 1, 2, 4, 6, 8, and 12 hours relative to injection of sulpiride) were also collected on days 11, 13, 17, and 25 for characterization of the prolactin response to injections. Beginning on day 20, all mares had their ovaries scanned every 3 days to assess follicular sizes; once a mare displayed an ovarian follicle of at least 25 mm, she was scanned daily until ovulation.

On the morning of day 24, all mares were catheterized in one jugular vein in preparation for frequent blood sampling. Approximately 1 hour later, two samples of blood were drawn from the catheter 15 minutes apart (—15 and 0 minutes), and then thyrotropin-releasing hormone (TRH; Sigma) was administered (5 mg in 2 mL saline). Blood samples were drawn at 15, 30, 45, 60, 90, 120, 150, 180, and 240 minutes relative to TRH administration. On that day, sulpiride injections were delayed until all 240 minute samples were collected.

Blood Sample Collection and Analysis. Routine morning blood samples were collected into sodium heparin-coated tubes (Vacutainer, Becton and Dickinson, Franklin Lakes, NJ). Frequent samples collected through catheters were placed in 12 × 75 glass tubes containing 20 IU sodium heparin (Sigma). All blood samples were routinely centrifuged within 30 minutes of collection; plasma was harvested and stored at –15°C.

Prolactin and LH concentrations were estimated in plasma by RIA previously validated for horse tissues.[10,11] Plasma concentrations of progesterone were measured with commercially available reagents (Diagnostic Systems Laboratories, Webster, TX). Intra and interassay CV and assay sensitivities were 7%, 12%, and 0.2 ng/ml for prolactin; 6%, 9%, and 0.2 ng/ml for LH; and 5%, 8%, and 0.1 ng/ml for progesterone.

Experiment 2

Mares and Treatments. Fourteen seasonally cyclic mares ranging in age from 7 to 17 years with body condition scores of 6.5 to 8.5 were randomly allotted to two groups of seven mares each. Nine of these mares had been used in experiment 1; they were assigned to treatment in experiment 2 such that previous treatments were represented as evenly as possible in the treatment groups in experiment 2. Beginning July 25, all mares were checked for estrus with a vigorous stallion every other day. Mares in diestrus for at least 6 days on August 2 were administered $PGF_{2\alpha}$ (Lutalyse, 10 mg in 2 mL; Pharmacia & Upjohn Co., New York, NY) to induce luteolysis; mares in estrus