Journal of Equine Veterinary Science 64 (2018) 69–76

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Effects of Combined Estradiol-Sulpiride Treatment and Follicle Ablation on Vernal Transition in Mares: Evaluation of Plasma and Follicular Fluid Hormones and Luteinizing Hormone Receptor Gene Expression

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ARTICLE INFO

Article history:
Received 1 December 2017
Received in revised form
16 February 2018
Accepted 16 February 2018
Available online 27 February 2018

Keywords:
Mare
Season
Sulpiride
Ovary
Hormonal production
LH receptor

ABSTRACT

This experiment assessed the hormonal production, secretory aspects, and changes in luteinizing hormone (LH) receptor gene expression of early induced ovulatory-sized follicles relative to the first ovulatory-sized follicles occurring naturally in the spring. Anovulatory mares were treated on January 21 with (1) 50 mg of estradiol cypionate (ECP, n = 8) alone or (2) with ECP followed by two 3-g sulpiride injections (n = 8), 5 and 12 days later. Half of each group also received complete follicle ablation via transvaginal aspiration before ECP treatment. Ovaries were scanned regularly until detection of a 32–35 mm follicle; follicular fluid was recovered via aspiration for analysis of hormonal concentrations. Blood was collected regularly to characterize plasma prolactin, LH, follicle stimulating hormone, progesterone, and estradiol concentrations. Mean date to first 35-mm follicle was earlier ($P < .05$) in sulpiride-treated mares: five of eight (63%) responded within 28 days of the first sulpiride injection. Ablation did not affect ovarian response. Plasma prolactin was stimulated ($P < .0001$) in ECP-sulpiride–treated mares for 16 days but did not dictate ovarian response. Estradiol stimulated plasma LH ($P < .05$), which was higher ($P < .05$) in treated mares that responded. There was no effect of treatment or ablation on follicular fluid concentrations of estradiol, progesterone, leptin, or insulin-like growth factor 1 or on LH receptor gene expression. These latter similarities indicate that ECP-sulpiride early induced follicles have apparently reached a degree of maturity equivalent to naturally occurring ovulatory-sized follicles later in the spring.

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1. Introduction

Stimulation of prolactin has been shown to stimulate ovarian follicular growth and hasten the date to first ovulation in seasonally anovulatory mares [1–5]. Nequin et al. [1] hastened follicular growth in seasonally anestrous mares with one treatment of ovine prolactin. Similarly, Thompson et al. [2] administered recombinant porcine prolactin to winter anestrous pony mares and advanced the first ovulation of the year. Sulpiride and domperidone have been used most frequently to stimulate prolactin secretion, a response greatly enhanced by pretreatment with estradiol [6–11], which was also shown to stimulate luteinizing hormone (LH) in seasonally anovulatory mares [6,11].

The exact mechanism by which prolactin stimulates follicular growth in seasonally anovulatory mares has yet to be identified. Receptors for prolactin have been localized on equine ovarian follicular cells [12] and luteal cells [13], which is an indication that prolactin can exert its action directly on the ovarian follicle. Prolactin has an obligatory role in ovarian function in several species, such as formation and maintenance of the corpus luteum (CL; [14,15]). It has also been suggested that prolactin is responsible for inducing functional LH receptors on granulosa cells and CL of rats as well as sustaining progesterone secretion from the CL [16–18].

Animal welfare/ethical statement: Procedures used in these experiments were approved by the Institutional Animal Care and Use Committee of the Louisiana State University Agricultural Center.

Conflict of interest statement: The authors declare no conflicts of interest.

Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 2017-230-31530.

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https://doi.org/10.1016/j.jevs.2018.02.020
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Richards and Williams [16] and Holt et al. [17] observed an enhancive effect of prolactin on LH receptor content and progesterone production, but not a direct effect of prolactin alone. Conversely, Bjurulf et al. [18] observed a 10-fold increase in LH receptor messenger ribonucleic acid (RNA) in luteal cells of prolactin-treated rats and an increase in circulating progesterone concentrations when compared to those of controls. Furthermore, a marked decrease in LH receptor mRNA was detected in prolactin receptor null mutant mice [19]. This effect of prolactin on LH receptors and receptor mRNA in females is directly analogous to the complete requirement for prolactin for spermatogenesis in the male hamster [20,21], which was shown to be mediated by prolactin's necessity for LH receptors on hamster Leydig cells [22].

In the mare, a local role for prolactin in the ovary has been proposed because of the rapid growth of ovarian follicles in response to either exogenous prolactin or indirectly through treatment with dopaminergic antagonists in the winter. The aim of the present study was to induce early follicular growth in seasonally anovulatory mares by treatment with estradiol cypionate (ECP) and subsequently sulpiride as described by Mitcham [10] and Oberhaus et al. [11] and then assess changes in ovarian follicle hormone production and LH receptor content. The effect of complete follicle ablation before treatment was superimposed on both treatments to determine whether the induction of a new follicular wave had an effect on the ovarian response to treatment.

2. Materials and Methods

Procedures used in these experiments were approved by the Institutional Animal Care and Use Committee of the Louisiana State University Agricultural Center.

2.1. Animals and Treatments

Mares used in this experiment were housed at one of two Louisiana Agricultural Experiment Station farms: the Central Research Station Horse Unit on the Ben Hur Plantation south of the LSU campus and the Reproductive Biology Center located in St. Gabriel, Louisiana. The two farms were located approximately 9 miles apart south of the Louisiana State University main campus in Baton Rouge. All mares were maintained outdoors throughout the year. They grazed native grass pasture during the warmer months and were supplemented with hay prepared from the same native grasses for ad libitum consumption as needed in the fall and winter.

Starting on December 29, 2014, all nonpregnant mares housed at the two farms were assessed for 3 weeks by weekly ultrasonic scanning of the ovaries and sampling of jugular blood. Anovulation was defined as absence of a follicle >20 mm in diameter on either ovary, absence of any corpora lutea, and plasma progesterone concentrations consistently <1 ng/mL.

Sixteen light horse, anovulatory mares were identified (eight at each farm) and allotted into two similar groups based on age (7–25 years old) and body condition score (4–7; [23]). The groups were then randomly assigned to a combined treatment group ($n = 8$) or estradiol-only (control) group ($n = 8$). Half of each group was subjected to complete follicle ablation 5 days before sulpiride treatment.

On January 21, 2015, all mares received 50 mg of ECP (BET Pharm, LLC, Lexington, KY), intramuscularly. Five days later, on January 26, mares allotted to the combined treatment group received 3 g of sulpiride (racemic mixture; Sigma-Aldrich, St. Louis, MO) dissolved in 5 mL of vegetable shortening (Crisco; J.M. Smucker Company, Orrville, OH) subcutaneously in the girth area as previously described by Thompson et al. [24]. Control mares ($n = 8$) received 5 mL of vegetable shortening only in the girth area. All mares received a second sulpiride or vehicle treatment in the same manner 7 days later on February 2.

2.2. Blood Sampling

On January 21, 2016, just before treatment with ECP, jugular blood samples were collected from each mare into two 6-mL evacuated tubes containing sodium heparin as an anticoagulant (Vacuette; Greiner Bio-One, Monroe, NC). On January 26 (day 0), samples were drawn at 0 minutes, 1, 3, 6, 12, and 24 hours relative to treatment with sulpiride or vehicle, and this continued every four days until April 16 (day 80) to determine circulating plasma prolactin, LH, follicle stimulating hormone (FSH), and estradiol concentrations. In addition, on the day of posttreatment aspiration and for five successive days, a single blood sample was drawn to determine circulating concentrations of LH and progesterone. Plasma was harvested from all samples in the experiment by centrifugation at 1,200 × *g* for 15 minutes and stored at −20°C.

2.3. Ultrasonography and Transvaginal Follicle Aspiration

Before the start of the experiment, half of the mares in each group received a complete follicle ablation 5 days before sulpiride treatment via the aspiration procedure described below. Starting on the day of ECP treatment, ovarian activity was monitored in all mares via ultrasonography (Aloka 550V with 5-Mhz linear-array transducer; Hitachi-Aloka, Wallingford, CT) once a week until a follicle >25 mm emerged. On detection of a follicle >25 mm, the mare was scanned daily until the follicle either reached at least 35 mm or regressed to <25 mm. Once a 35-mm follicle was observed, the follicle was aspirated.

For aspiration, a mare was administered intravenous detomidine (0.01 mg/kg BW; Dormosedan; Zoetis, Parsippany-Troy Hills, NJ) for sedation and N-butylscopolammonium bromide (0.25 mg/kg BW; Buscopan; Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO) for rectal relaxation. Additional amounts of these medications were administered as needed. A 5 MHz curvilinear probe housed in hard plastic casing was used for imaging and aspiration of follicles. The follicle was aspirated with a 12-gauge double lumen needle attached to a vacuum-pump at a pressure of −150 mmHg. The probe was inserted transvaginally and placed directly against the vaginal wall. The ovary was then manipulated transrectally such that the follicle was placed next to the end of the ultrasound probe. A second technician advanced the needle through the vaginal wall and into the follicle. The follicle was immediately evacuated of follicular fluid into a sterile 50-mL conical tube; the fluid was stored for later analysis of prolactin, estradiol, progesterone, insulin-like growth factor 1 (IGF-1), and leptin. The follicle was then flushed several times with Dulbecco's phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO) supplemented with 10 U/mL of heparin and 1.5% bovine calf serum warmed to 38°C, and the flushing fluid was evacuated into a sterile bottle. Upon collection of follicle contents, cells were filtered to remove blood contamination, rinsed with PBS, and manually pipetted into cryovials. Cells were centrifuged at 700 × *g* for 15 minutes, and pellets were resuspended in RNA later (Sigma-Aldrich, St. Louis, MO) and stored at −80°C for later analysis of LH receptor expression.

2.4. Radioimmunoassay

Frozen plasma samples were thawed and analyzed for prolactin, LH, FSH, progesterone, and estradiol, as appropriate. Follicular fluid samples were analyzed for prolactin, progesterone, estradiol, IGF-1, and leptin concentrations. Prolactin, LH, FSH, IGF-I, and leptin were measured by radioimmunoassay in assays previously validated by

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