

Ovulation synchronization following commercial application of ultrasound-guided follicle ablation during the estrous cycle in mares

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Abstract

A regimen of progesterone plus estradiol (P&E) was used as a standard for ovarian synchronization to test the efficacy and evaluate the commercial application of ultrasound-guided follicle ablation as a non-steroidal alternative for ovulation synchronization in mares. Recipient mares at a private embryo transfer facility were at unknown stages of the estrous cycle at the start of the experiment on Day 1 when they were randomly assigned to an ablation group ($n = 18\text{--}21$ mares) or to a P&E group ($n = 20\text{--}21$ mares). In the ablation group, mares were lightly sedated and all follicles ≥ 10 mm were removed by transvaginal ultrasound-guided follicle aspiration. In the P&E group, a combination of progesterone (150 mg) plus estradiol (10 mg) prepared in safflower oil was given daily (im) for 10 d. Two doses of prostaglandin $F_{2\alpha}$ (PGF, 10 mg/dose, im) were given 12 h apart on Day 5 in the ablation group, or a single dose on Day 10 in the P&E group. Human chorionic gonadotropin (hCG, 2500 IU/mare, im) was given at a fixed time, 6 and 10 d after PGF treatment in the ablation and P&E groups, respectively, with the expectation of a follicle ≥ 30 mm at the time of treatment. In both the ablation and P&E groups, transrectal ultrasonography was done at the start of the study (Day 1) and again on the day of hCG treatment and daily thereafter to determine the presence of a CL, measure diameter of the largest follicle and detect ovulation. The mean interval from the start of the study and from PGF treatment to ovulation was shorter ($P < 0.0001$) in the ablation group (13.7 and 9.7 d, respectively) compared to the P&E group (22.3 and 13.2 d, respectively). Following fixed-day treatment with hCG after PGF treatment, the degree of ovulation synchronization was not different ($P > 0.05$) between the ablation and P&E groups within a 2-d (56 and 70%) or 4-d (83% and 90%) period. Although ultrasound-guided follicle ablation may not be practical in all circumstances, it excluded the conventional 10-d regimen of progesterone and estradiol and was considered an efficacious and feasible, non-steroidal alternative for ovulation synchronization in mares during the estrous cycle.

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1. Introduction

Various combinations of reproductive steroids (progestogens and estrogens), prostaglandin $F_{2\alpha}$ (PGF, native and analogues), human chorionic gonadotropin

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(hCG) and gonadotropin-releasing hormone (GnRH, native and analogues) have been used to control follicular development and the time of ovulation for basic and applied purposes during the spring transition, estrous cycle and postpartum period in mares [reviews 1–6]. From an applied perspective, the most common objectives are to coordinate the expected time of ovulation with insemination and align ovulations in recipient mares with donor mares in an embryo transfer program. Regimens of progestogens (injectable, oral and intravaginal) and PGF used alone or in combination have limited control on follicular development and, therefore, are primarily used to inhibit or delay ovulation [1–6]. The hormonal regimen used most often to control both follicular development and ovulation is a combination of progesterone plus estradiol (P&E). Typically, the regimen involves intramuscular or subcutaneous administration of P&E for 10 d beginning at unknown or random stages of the estrous cycle, PGF on the last day of steroid treatment, and hCG or GnRH when the largest follicle reaches >35 mm [1–6]. According to seminal studies done in the 1980s, this steroidal regimen without hCG treatment resulted in ovulation synchrony among mares ranging from 54 to 68% within a 2-d period and from 72 to 94% within a 4-d period [7,8]. With hCG treatment, ovulations were synchronous within a 2-d period in 70–73% of the animals [9]. In the latter study [9], the time to ovulations ranged from 8 to 17 d after the last steroid treatment or 18–27 d after the first steroid treatment; hence, it is apparent that a large portion (approximately 45%) of the interval from the first steroid treatment to the average time of ovulation (approximately 22 d) involves the daily handling of animals and steroid hormones. Although effective, daily steroidal treatments are time consuming and labor intensive. In addition, repeated intramuscular or subcutaneous treatments increase the risk of injection-site inflammation and, as a consequence, some mares will become intolerant to immediate and future injection of hormones or other products.

Transvaginal ultrasound-guided aspiration of antral follicles was initially developed as a non-surgical and repeatable procedure to collect oocytes from horses [10] and cattle [11] for basic and applied purposes, and has since been adopted for use in other domestic [12] and non-domestic [12–16] species for similar purposes. Ultrasound-guided follicle aspiration/ablation has been used extensively as a non-steroidal approach to study the nature of follicular dynamics in horses and cattle, without the potential confounding effects of exogenous steroids [17–19], as a tool to induce follicular wave

emergence at the start of gonadotropin treatment within animals [20–23], and to enhance ovulation synchronization among animals [24,25].

Synchronization of follicular wave emergence and ovulations within and among animals using transvaginal ultrasound-guided follicle aspiration/ablation was first documented in a controlled study in cattle [24]. In a comparable study done in two controlled experiments in mares [25], follicle aspiration/ablation at random stages of the estrous cycle resulted in renewed follicular growth within 3 d in 90% of the animals. Subsequent to PGF treatment, ovulations were detected in 81% of the mares within a 4-d period following hCG treatment when the largest follicle reached ≥ 30 mm and in 88% of the mares within a 1-d period following fixed-day treatment with hCG when the largest follicle was expected to reach ≥ 30 mm. Combined over both experiments, the mean interval from follicle ablation to ovulation was 13 d and ranged from 7 to 17 d. Potentially, therefore, ultrasound-guided follicle ablation represents an approximate 41% reduction in the time from the start of the ablation synchronization regimen to the average time of ovulation compared to the conventional P&E synchronization regimen. Although ovarian synchronization using a follicle ablation regimen seemed to require less time and the degree of ovulation synchrony appeared comparable to a P&E ovulation synchronization regimen, critical comparison of the two regimens has not been done.

In the present study, the conventional practice of using a combination of P&E to suppress follicular development during treatment and synchronize renewed growth and ovulations after treatment was used as a standard to test the hypotheses that: (1) ultrasound-guided follicle ablation can markedly reduce the interval from the start of the synchronization regimen to ovulation compared to the P&E regimen, and (2) the degree of ovulation synchrony is comparable between the follicle ablation and P&E regimens. Secondly, the study was designed to examine the feasibility of using ultrasound-guided follicle ablation in a commercial situation with recipient mares at a private embryo transfer facility.

2. Materials and methods

The study was conducted during the ovulatory season (December to January) in São João da Boa Vista, São Paulo, Brazil with pure bred Mangalarga and other cross-bred horse mares that served as recipients at a commercial embryo transfer facility (Fleury Equine Reproduction); donor mares were not involved in this

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