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Synchronization of cyclic and acyclic embryo recipient mares with donor mares

Ivan V. Oliveira Neto[a], Igor F. Canisso[b], Lorenzo G. Segabinazzi[a], Camila P.F. Dell'Aqua[a], Marco A. Alvarenga[a], Frederico O. Papa[a], Jose A. Dell'Aqua Jr.[a,*]

[a] Department of Animal Reproduction and Veterinary Radiology, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, Brazil
[b] Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana IL 61802, USA

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ABSTRACT

This study compared hormone treatments given to mares during anestrus, spring transition, and different stages of the estrous cycle, by assessing uterine features and pregnancy rates after embryo transfer (ET). Embryo recipient mares (n = 160) were equally arranged as follows: G1-spontaneous ovulation (control), G2-anestrus, G3-spring transition, G4-early estrus, G5-estrus, G6-diestrus, G7-early diestrus treated with a dose of dinoprost, and G8-early diestrus treated with two doses of dinoprost. At treatment initiation (Day-4), G2-7 were given dinoprost and estradiol-17β, thereafter, estradiol-17β was repeated on Days-3,-2, and -1. On Day0, mares received long-acting altrenogest. Then, each mare had one ET performed from Day + 3 to Day + 8 after altrenogest. Immediately before the ET, mares received a boost of altrenogest and had uterine features assessed. Pregnant mares on each of the checks (by 7, 30, 60, and 120d after ET) were maintained on weekly injections of LA-P4 until 120d. G8 received similar management, but dinoprost was repeated on Day-3. G1-G6 and G8 displayed uterine edema and satisfactory pregnancy rates ≥65%. Repeating dinoprost to G8 likely ensured proper luteolysis and response to estrogen as determined by higher uterine edema scores and pregnancy rates than G7 ($p < .05$). Our results were consistent with previous studies and other successful commercial ET programs (except G7), thus, demonstrating the usefulness of the hormone treatments described herein to synchronize embryo recipient mares with donor mares. Thus, we foresee that other groups may use the strategies described herein for the management of embryo recipient mares.

1. Introduction

Embryo transfer is an assisted reproductive technique used to maximize the number of offspring from mares with a desired phenotype, or as a tool to circumvent health or reproductive dysfunction (e.g., chronic orthopedic issues, recurrent embryonic loss, or severely fibrotic endometrium), or to obtain foals from fillies or mares with an active athletic career (Squires et al., 1999; Panzani et al., 2007; Campbell, 2014; McCue and Squires, 2015). The success of an embryo transfer program is affected by multiple factors, such as the management and fertility of dams and sires, experience of the professional(s) collecting and transferring embryos, and quality and management of recipient mares (Squires et al., 1999; McKinnon et al., 1988; McCue and Squires, 2015).

* Corresponding author.
E-mail address: dellaquajunior@uol.com.br (J.A. Dell'Aqua).

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The viability of a commercial embryo transfer program relies heavily on maintaining and managing surrogate mares, as caring for these animals represents the most costly component of a program. Successful programs typically have high utilization rates of recipient mares with satisfactory pregnancy rates after the embryo transfer (e.g., ≥70%) (McCue and Squires, 2015). Synchronization of embryo recipients with donor mares is one of the limiting factors for high usage of recipient mares with good pregnancy rates (McCue and Squires, 2015). Though, while a large number of equine embryos are produced and transferred worldwide, the literature is particularly scarce concerning the onset of synchronization protocols of an embryo recipient mare and a donor mare during different stages of the estrous cycle (i.e., early vs. late estrus or diestrus).

Reported synchronization protocols may involve the use of prostaglandin F2α (i.e., dinoprost tromethamine) or its analogs (e.g., sodium cloprostenol), steroid hormones (progestins and estrogens), and ovulation induction agents such as human chorionic gonadotropin and GnRH agonists (e.g., deslorelin acetate, histrelin) (Carnevale et al., 2000; McCue et al., 2002; Rocha Filho et al., 2004; Carnevale et al., 2005; Caiado et al., 2007; Fleury et al., 2007; Greco et al., 2008; Greco et al., 2012; Kaercher et al., 2013; McCue and Squires, 2015; Greco et al., 2016; Pinto et al., 2017). Prostaglandin F2α alone is commonly used in cyclic mares to bring them back into estrus when necessary (Cuervo-Arango et al., 2017). Whereas, a combination of estrogen (e.g., estradiol 17β, estradiol benzoate, or estradiol cypionate) and progestin (e.g., altrenogest, or progesterone) is typically used in acyclic and transitional recipient mares or when there is a shortage of recipient mares, demanding a tight-synchrony between one embryo donor and one or two of its recipients (Rocha Filho et al., 2004; Greco et al., 2012; McCue and Squires, 2015; Silva et al., 2017; Pinto et al., 2017).

In large-scale commercial programs, embryo recipient mares are kept in large groups and frequently checked by transrectal palpation and ultrasonography to determine the optimal ovulation synchrony with embryo donor mares (Squires et al., 1999; McCue and Squires, 2015). Checking recipient mares is a costly and time-consuming activity that requires well-trained personnel to carry out large number of scans and proper determination of ovulation synchrony with embryo donor mares. Therefore, hormone treatments allowing synchronization of embryo recipients with donor mares could prove useful to maximize the use of recipient mares and to minimize the number of ultrasonography examinations.

The overall objective of the present study was to optimize the synchronization of embryo recipient mares with donor mares. Specifically, we compared outcomes of hormone treatments, given to mares during anestrus, spring transition, and different stages of the estrous cycle, by assessing uterine features (edema, tone, and echotexture) and pregnancy rates of cooled-transported embryos.

2. Materials and methods

The present study was revised and approved by the Ethics Committee of the São Paulo State University (UNESP), Botucatu, São Paulo, Brazil under the protocol # 130/2014-CEUA. This experiment was carried out during the breeding season of the Southern Hemisphere (from August 2016 to January 2017).

2.1. Embryo recipient mares and management

Light breed type embryo recipient mares (n = 160) aging from 5 to 15 years old (9.5 ± 3.6) were enrolled in the present study. The animals were kept in a commercial embryo recipient herd in Botucatu, São Paulo Brazil. The mares were kept on pasture of Tifton 85, with free choice water and trace minerals. Mares were regularly dewormed with ivermectin at 3-mo intervals and yearly vaccinated (including a 28-day boost) with a 5-way vaccine (Tri-Equi, Hetape-Calier®, Juataba, Minas Gerais, Brazil). The recipients were brought to the farm, 6–8 weeks before enrolment in the embryo transfer program. All recipients remained in apparently good health and had good body condition scores during the study.

Embryo recipient mares (n = 160) were equally arranged into eight groups as follows: G1 spontaneous ovulation (control group); G2 anestrus, i.e., mares showing ovarian follicles < 15 mm in diameter and no detectable corpus luteum in 3 wks; G3 spring transition, i.e., mares presenting 25–30 mm follicles with no corpus luteum in 3 wks; G4 early estrus (follicles < 35 mm); G5 estrus (follicles ≥35 mm); G6 diestrus (ovulation ≥5 days); G7 early diestrus (ovulation < 5 days) treated with a single dose of prostaglandin; and G8 early diestrus (ovulation < 5 days) treated with two doses of prostaglandin one day apart (Fig. 1). All mares assigned to G1 and G4-8 had at least one ovulation confirmed before enrollment in the present study.

At treatment initiation (Day −4), animals in groups 2–7 were given dinoprost tromethamine (Lutalyse®, 10 mg, IM, Zoetis, São Paulo, Brazil) and estradiol 17β (17 βeta®, 10 mg, IM, Botupharma, Botucatu, São Paulo, Brazil). Thereafter, estradiol 17β administration was repeated on Days −3 (10 mg), −2 (20 mg) and −1 (10 mg) (Fig. 1). On Day 0, all mares received a single dose of long-acting altrenogest (Altrengest®, 300 mg, IM, Botupharma). After that, each mare had one cooled-transported (< 6 h) day-8 embryo transferred from Day +3 to Day +8 (i.e., asynchrony of five to zero days after the donor mare's ovulation) after the altrenogest injection. On the day of the transfer, each mare received a boost of long-acting altrenogest (300 mg, IM). Mares in G8 received similar hormonal treatments with the exception that a second dose of dinoprost (10 mg) was given on Day −3 (Fig. 1).

From Day −4 to Day −1, all mares had uterine edema scored (0–3 scale) via transrectal ultrasonography B-mode ultrasound coupled with a 5 MHz transducer (WellD, Pingshan, Shenzhen, China) (Fig. 2). No visible uterine edema was deemed as score 0 (Fig. 2A), and pronounced uterine edema at the uterine bifurcation was deemed as score 3 (Fig. 2D).

Mares assigned to the control group were monitored via transrectal palpation and ultrasonography every other day until a preovulatory follicle was detected and then daily until ovulation. The four days preceding the ovulation of mares in the control group (G1) were used retrospectively for comparisons of uterine edema with hormonally treated mares (G2-G8). The day of the embryo donor's mare ovulation or the immediate day after coincided with Day −4 for all embryo recipient mares.