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Original Research

Novel Long-Acting Progesterone Protocols Used to Successfully Synchronize Donor and Recipient Mares With Satisfactory Pregnancy and Pregnancy Loss Rates

Gabriel M. Greco [a], Eduardo G. Fioratti [a], Lorenzo G. Segabinazzi [a], José A. Dell'Aqua Jr. [a], André M. Crespilho [b], Maria Manoela B. Castro-Chaves [a], Marco Antonio Alvarenga [a,*]

[a] Department of Animal Reproduction and Veterinary Radiology, São Paulo State University, UNESP, Botucatu, Brazil
[b] UNISA, São Paulo, Brazil

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ABSTRACT

The present study aimed to evaluate pregnancy and pregnancy loss rates of recipients treated with alternative long-acting progesterone protocols, designed to synchronize acyclic and cyclic mares, regardless of their cycle phase. A total of 150 Campolina breed mares were used as recipients. Recipient mares were assigned to six different groups with 25 animals each. Groups 1 to 5 were treated with progesterone at some point. Group 1 (acyclic recipients); group 2 (cyclic estrous recipients with one ≥35 mm follicle); group 3 (cyclic estrous recipients with an anovulatory follicle); group 4 (early estrous cyclic recipients); group 5 (diestrous cyclic recipients), and group 6 (cyclic recipients—control). Embryos (day 8) were transferred 4 days after ovulation or 4 days after progesterone injection. Pregnant diagnosis was performed by transrectal ultrasonography 1 week after embryo transfer. Pregnant recipients were evaluated for possible losses and mares treated every 14 days with 3 g (intramuscular) of long-acting progesterone, until 120 days of pregnancy. Pregnancy at 15 days and pregnancy loss rates were recorded and statistically evaluated through multivariate regression ($P < .05$). Pregnancy and pregnancy loss rates were similar within groups (G1: 76%–10.5%; G2: 76%–5.9%; G3: 56%–0%; G4: 80%–10%; G5: 60.9%–0%; and G6: 60%–13.3%). In conclusion, the novel long-acting progesterone protocols proposed in this study allowed successfully the utilization of mares with asynchronous cyclic as embryo recipients, serving as an alternative specially when few recipients are available and usual synchronization is not possible.

1. Introduction

Equine embryo transfer (ET) has been routinely conducted especially in Brazil and Argentina, which are responsible for 50% of all transferred embryos around the world [1]. It is known that the selection and management of recipient mares are the most important factors affecting pregnancy and embryonic loss rates in ET programs [2]. Therefore, better results are usually achieved when high numbers of recipients are available.

Synchrony between donor and recipient is needed for pregnancy establishment after an ET procedure [3]. However, at the beginning and the end of the breeding season, the percentage of recipients showing normal estrous cycles is lower than donors. During these periods, it is not uncommon to evaluate recipients in anestrous/transitional phase or with ovulatory failure, developing hemorrhagic anovulatory follicles.

* Corresponding author at: Marco Antonio Alvarenga, Departamento de Reprodução Animal e Radiologia Veterinária, FMVZ, UNESP, Distrito de Rubião Junior, s/n°, 18618-970 Botucatu, São Paulo, Brazil.
E-mail address: malvarenga@fmvz.unesp.br (M.A. Alvarenga).

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Progestin treatment allows the use of ovariectomized or acyclic recipients with satisfactory pregnancy and embryonic loss rates [4,5]. In the past few years, long-acting progesterone compounds have been widely used in Brazil [6,7] and Argentina [8]. Acyclic recipient mares are treated every 7 or 14 days, respectively, with either 1.5 g of progesterone or 3.0 g of progesterone of a long-acting progesterone formulation containing 300 mg/mL of progesterone (P4LA300) until pregnancies reach about 120 days of age [9].

The optimal recipient-to-donor ratio is rarely available with the ongoing increase of equine ET in Brazil, even during the breeding season, impairing proper synchronization, and lowering pregnancy rates. The present study aimed to evaluate pregnancy and pregnancy loss rates of recipient mares treated with alternative long-acting progesterone protocols, designed to successfully synchronization between acyclic and cyclic mares, regardless of their estrous phase.

2. Material and Methods

This experiment was conducted in the 2013/2014 breeding season. A total of 148 ETs were performed (87 in farm A and 61 in farm B) in two stud farms located in Conselheiro Lafaiete (latitude 20°40′S and longitude 43°48′W), Minas Gerais state, Brazil. One hundred fifty Campolina breed mares, aged 3 to 10 years (±7.4 years), weighing 450 to 600 kg, remaining in similar management and pastures, receiving 2.0 kg of concentrated balanced grain, water, and mineral salt ad libitum, and showing a body condition score from 6.0 to 8.0 (±7.0) according to Henneke et al [10], were used as recipients. Most of these mares produced a foal 1 year before the experimental breeding season. These animals were evaluated at 7-day intervals by rectal palpation and ultrasonography, using a 5.0-MHz linear transducer (Chison V8300—China), and classified as acyclic or cyclic according to their reproductive status by only one veterinarian. Acyclic mares were palpates at least two times before the start of the treatment at 7-day intervals with no corpus luteum being observed. Mares used in this study did not show any reproductive abnormalities through ultrasonography and were negative for endometrial inflammation after cytologic evaluation [11].

Donor mares aged 8 to 18 years were used and were evaluated three times for a week. When one ≥35 mm follicle and at least grade 2 endometrial edema [12] were found, ovulation induction was performed with intramuscular (IM) injection of 750 μg using deslorelin acetate (Sincrorelin, Ouro Fino, Brazil). Inseminations were processed 24 hours after induction of the ovulation with fresh semen (doses containing 500×10^6 viability spermatozoa) from two stallions of known fertility. Eight-day embryos were recovered by the nonsurgical technique using transcervically catheter (Bullet tip 28FR, Pets-Inc, TX, USA) connected to 0.75-μm embryo filter (WTA, Brazil). Flushes were conducted with lactated Ringer solution prewarmed to 36°C. Embryos were washed in 10 drops of a holding media (Botu-Embryo, Botupharma, Brazil) and maintained at room temperature until 1 hour after flushing. Embryos were classified as development stage and viability according to McKinnon and Squires [13]. Embryos in appropriate development stage and good viability (grades 1 and 2) were transferred to recipients using nonsurgical technique 4 days after ovulation (D4) [5,14–17] in control group or 4 days after treatment with P4LA300 (BioRelease LAP4300, BetLabs, Lexington, KY, USA) in all the other groups. Embryos were transferred by only one veterinarian. Progesterone-treated recipients received another 1.5 g (IM) of P4LA300 on day of ET. Pregnant diagnosis was performed by ultrasonography 1 week after transfer and monitoring at 15-day interval up to 120 days of pregnancy. Pregnant recipients under progesterone treatment were treated every 14 days with 3 g (IM) of P4LA300 until fetuses reached 120 days of age.

2.1. Groups

Mares were divided into the six following groups, containing 25 animals each, according to the description given in the following paragraphs.

2.1.1. Group 1: Acyclic Recipients

Acyclic recipients that showed at least grade 2 endometrial edema [12] after 2 days of treatment with 5.0 mg (IM) of estradiol benzoate (Estrogin, Farmavet, São Paulo, Brazil) were treated with 1.5 g (IM) of P4LA300. Mares that not show edema after estradiol benzoate were not used.

2.1.2. Group 2: Cyclic Estrous Recipients With One ≥35 mm Follicle

Cyclic estrous recipients that showed one ≥35 mm follicle and at least grade 2 endometrial edema [12] were simultaneously treated with 2.000 IU (IV) of human chorionic gonadotropin (hCG) (Chorulon, Intervet International B.V., Boxmeer, the Netherlands) and 1.5 g (IM) of P4LA300, aiming induce ovulation. In this case, ET was performed 4 days after P4LA300 injection. Progesterone injections were discontinued if corpus luteum was observed by ultrasonography on the day at ET.

2.1.3. Group 3: Cyclic Estrous Recipients With an Anovulatory Follicle

Cyclic estrous recipients that developed a hemorrhagic anovulatory follicle 48 hours after ovulation induction with hCG and still showing a minimal degree of endometrial edema received 1.5 g (IM) of P4LA300. Hemorrhagic follicle was characterized by the increase in the follicular size which fails to rupture in association with the observation of echogenic specks that float freely in the follicular fluid, with subsequent luteinization of the follicular wall in most occasions [18–20].

2.1.4. Group 4: Early Estrous Cyclic Recipients

Cyclic mares in early estrous and showing at least grade 2 endometrial edema and only follicles ≤35 mm were treated with 1.5 g (IM) of P4LA300.

2.1.5. Group 5: Diestrous Cyclic Recipients

Cyclic diestrous recipients from 5 (D5) to 14 days after ovulation (D14), showing no endometrial edema, good uterine tone, and a visible corpus luteum in one of their