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Short Communication

Potential role for GnRH in the synchronization of follicular emergence before the superovulatory Day 0 protocol

M.F.A. Balaro [a,*], J.F. Fonseca [b], T.G.B. Barbosa [a], J.M.G. Souza-Fabjan [a], L.M. Figueira [c], T.A. Teixeira [a], L.R. Carvalheira [a], F.Z. Brandão [a]

[a] Department of Pathology and Veterinary Clinic, Veterinary School, Fluminense Federal University, Niteroi, RJ 24320-340, Brazil
[b] Brazilian Agricultural Research Corporation on Goats and Sheep, Coronel Pacheco, MG 36155-000, Brazil
[c] Department of Animal Science, Veterinary School, Fluminense Federal University, Niteroi, RJ 24320-340, Brazil

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ABSTRACT

The ability of gonadotropin-releasing hormone (GnRH) to synchronize ovulation and new follicular wave emergence before a "superovulatory Day 0" protocol was assessed in Santa Inês ewes. For estrus synchronization, a 60-mg medroxyprogesterone acetate sponge was inserted for 6 d. One day before sponge removal, 37.5-μg d-cloprostenol and 300 IU equine chorionic gonadotropin were injected intramuscularly (i.m.). After sponge removal, ewes were assigned to the following 3 groups: (1) G_C—1 mL saline at 12 h (n = 10); (2) G_{24h}—0.025-mg lecirelin (GnRH agonist) i.m. at 24 h (n = 10); or (3) G_{36h}—0.025-mg lecirelin i.m. at 36 h (n = 9). Ovarian ultrasonography was conducted to assess follicular dynamics. Blood was collected to determine plasma concentrations of progesterone and estradiol. Females from G_{36h} and G_C had a greater ($P < 0.05$) estrous response than those from the G_{24h} group (78.0 and 90.0 vs 0.0%, respectively). Ewes from G_{24h} and G_{36h} had earlier ($P < 0.05$) ovulation (48.0 ± 10.2 and 56.7 ± 5.7 h) compared with those from G_c (64.1 ± 9.7 h). The mean number of ovulations per ewe was greater ($P < 0.05$) in G_c (1.9 ± 0.6) and G_{36h} (2.0 ± 1.0) than G_{24h} (1.2 ± 0.4). Plasma concentrations of progesterone and estradiol differed over time. Follicular growth during the postovulatory day was affected ($P < 0.05$) by day of the estrus cycle as well as by the interaction ($P < 0.05$) of treatment and day of the estrus cycle. There was a larger ($P < 0.05$) population of medium follicles during the first 24 h after the ovulation in G_{24h} compared with G_c, and there was an absence of large follicles in G_{36h} between 36 and 72 h after ovulation. In conclusion, the use of GnRH agonist at 36 h more efficiently synchronized ovulation and promoted the absence of dominant follicles during early diestrus and may be used at the start of superovulatory treatment at 80 h in Santa Inês ewes.

1. Introduction

The use of multiple ovulation and embryo transfer biotechnology in sheep has contributed to rapid genetic growth [1]. However, the response of the donor ewe to gonadotropins during superovulation treatments remains a challenge because of variability in their follicular response [2]. The start of a superovulatory treatment in the absence of follicular dominance is advantageous. The "superovulatory Day 0" protocol was proposed previously as a new method to synchronize ovulation and superovulation onset at Day 0 of the estrus cycle [3]. Its use resulted in better follicular recruitment, greater ovulation numbers, reduced incidence of postovulatory abnormalities [3,4], a greater number of corpora lutea, and improved fertilization rates [5]. In combination, the use of a short-term protocol (5–6 d of progestogen treatment) reduces the time needed between

* Corresponding author. Tel.: +55 21 26299526; fax: +55 21 26299515.
E-mail address: mariobalaro@hotmail.com (M.F.A. Balaro).

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synchronization and the onset of superovulation, thereby optimizing the multiple ovulation and embryo transfer program [6]. Additionally, studies have demonstrated that the administration of gonadotropin-releasing hormone (GnRH) during mating is an option to improve the endogenous preovulatory luteinizing hormone (LH) surge and ovulatory synchrony to timed artificial insemination protocols [7]. However, the potential for GnRH in synchronizing ovulation and follicular emergence has not been assessed in tropical sheep. Therefore, the aims of this study were to evaluate the ability of a GnRH agonist (lecirelin), administered at 24 or 36 h after a short-term hormonal protocol and synchronize ovulation and follicular emergence in the absence of large follicle(s) before the onset of the superovulatory Day 0 protocol in Santa Inês ewes.

2. Materials and methods

2.1. Location and experimental animals

The study was performed at the Farm School of the Veterinary Faculty at Universidade Federal Fluminense, located in Rio de Janeiro, Brazil (latitude 22°27″ S). This research was approved by the Animal Care Committee of the university (452/2013) and was conducted under the ethical principles of the Brazilian Society of Laboratory Animal Science. The experiment was conducted during the breeding season (autumn: May, 2013). Multiparous Santa Inês ewes were maintained in a semi-intensive system with access to pasture (*Panicum* sp., and *Brachiaria* sp.) and shelter. Chopped elephant grass (*Pennisetum purpureum*) was offered twice daily, with 300 g/animal of concentrate (17% crude protein) once per day. The animals received water and mineralized salt ad libitum.

2.2. Short-term protocol and GnRH agonist treatment

Intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Progespon; Schering Plough, SP, Brazil) were used for 6 d. One day before sponge removal, 300 IU of equine chorionic gonadotropin (Novormon 5000; MSD Animal Health, SP, Brazil) and 37.5 μg of d-cloprostenol (Prolise; Tecnopec LTDA, SP, Brazil) were administered intramuscularly (i.m.). After sponge removal, the ewes were assigned to the following 3 different groups according to their body weight and body condition score (1–5 scale; [8]): (1) G_C: 1 mL saline i.m. (n = 10) 12 h after sponge removal; (2) G_{24h}: 0.025-mg lecirelin (Gestran Plus; Tecnopec, SP, Brazil) i.m. 24 h after sponge removal (n = 10); or (3) G_{36h}: 0.025-mg lecirelin i.m. 36 h after sponge removal (n = 9). Body weights and body condition score for the 3 groups averaged 50.7 ± 7.3, 48.5 ± 5.1, and 48.5 ± 5.2 kg, and 3.1 ± 0.4, 2.8 ± 0.3, and 3.0 ± 0.3, respectively.

2.3. Ultrasonographic procedures and follicular assessment

Ovarian ultrasonography was conducted every 24 h while the sponges were in place, and every 12 h after removal until ovulation was confirmed. At ovulation (established as Day 0), ultrasonography was performed again every 24 h until Day 5 of the estrus cycle. The examinations were performed using transrectal ultrasonography (SonoScape, Shenzhen, China) equipped with a 7.5-MHz linear transducer with ewes in a standing position. The diameters, positions, and characteristics of the ovarian structures were recorded. The follicles were classified into the following 3 classes according to their size: emerging follicles (≤3.0 mm); medium follicles (3–5.0 mm); and dominant follicles (≥5.0 mm). The day of ovulation was defined as the day when the largest follicle was no longer detected. The preovulatory follicle diameter was the last measurement obtained.

2.4. Blood collection and hormonal analysis

Blood was collected by jugular venipuncture for 13 d, from sponge insertion to approximately 4 d after ovulation. Blood was collected into tubes with EDTA, and plasma was immediately separated by centrifugation at 1500 × *g* for 15 min and stored at −20°C until it was analyzed for concentrations of estradiol and progesterone using solid-phase radioimmunoassay kits (Beckman Coulter; Immunotech, Marseille, France). The assay sensitivity and intra-assay coefficients of variation were 2.2 pg/mL and 9% (estradiol), and 0.05 ng/mL and 12% (progesterone). In addition, all data were within the maximum and minimum points of the curve. The cyclicality state of the ewes was determined based on the progesterone concentrations where plasma values ≥1.0 ng/mL and <1.0 ng/mL were considered as cyclic and noncyclic ewes, respectively [9].

2.5. Statistical analysis

Descriptive statistics for the reproductive and hormonal data were calculated. In sequence, the Lilliefors test was used to verify the data normality. Parametric data were analyzed using a mixed model procedure for repeated measures, and the Tukey test ($P < 0.05$) was used to compare the means. Nonparametric data were assessed by the chi-square test ($P < 0.05$). The Pearson correlation coefficient was used to compare the hormonal and ultrasound findings. Statistical analyses were performed using GraphPad Prism 5.0a software.

3. Results

Data regarding ewe reproductive behaviors are summarized in Table 1. Females from G_{36h} and G_C had a greater ($P < 0.05$) estrous response rate than those in the G_{24h} treatment group. Ewes from the G_{24h} and G_{36h} treatment groups had earlier ovulation ($P < 0.05$) compared with G_c. For the ultrasonography evaluation, values for all variables, with the exception of ovulation rate, differed ($P < 0.05$) among the treatments (Table 1).

Progesterone and estradiol data are shown in Figure 1. Both hormones were affected by day ($P < 0.05$). Plasma progesterone concentrations at sponge insertion revealed that 26 of 29 (89.6%) ewes had a functional corpus luteum (>1 ng/mL). The largest mean progesterone values were detected during the first 3 experimental days (3.03 ± 0.49 ng/mL). The lowest mean progesterone values were