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Luteal response and follicular dynamics induced with equine chorionic gonadotropin (eCG) administration after insemination in sheep

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ABSTRACT

The objective of this study was to evaluate the ovarian response to equine chorionic gonadotropin (eCG) administrated during the luteal phase after insemination in sheep. Thirty cyclic multiparous ewes received a fixed-time intrauterine insemination (Day 0) and were assigned to three experimental groups that received 400 IU of eCG on Day 5 (n = 10) or Day 10 (n = 10), while another group remained as control group without eCG (n = 10). *Corpus luteum* area and follicular diameter were daily measured by transrectal ultrasonography and serum progesterone concentrations were daily determined by radioimmunoassay during 30 days after insemination in pregnant ewes or until the following ovulation in non pregnant ewes. The administration of eCG on Day 10, but not on Day 5, increased *corpus luteum* size and serum progesterone concentrations ($P < 0.05$). Follicular dynamics were influenced by both eCG treatments on Day 5 and Day 10, promoting the growth of the largest follicle present during treatment or increasing the length of the wave that emerged after treatment ($P < 0.05$). In conclusion, the administration of 400 IU of eCG 10 days after insemination in sheep increased *corpus luteum* size and progesterone concentrations, as well as promoted follicular development. This information could be useful to develop new pharmacological strategies to improve the establishment of pregnancy in sheep.

1. Introduction

Embryo survival and the maintenance of pregnancy is relevant in all species and is particularly important in females which reproduction is determined by the season (e.g. sheep), since the possibility of rebreeding is limited. The available evidence is that, like cattle and goats, a fertilization rate of 90–95% appears to be normal in sheep (see review by Diskin and Morris, 2008). However, pregnancy rate determined around 30 days after natural mating or insemination usually is lower than 60–70% (Diskin and Morris, 2008). Most of the pregnancy losses occur around or before the critical period of gestation, which is defined on Day 17 or 15 after ovulation in cattle and sheep respectively (Quinlivan, 1966; Humblot, 2001; Inskeep and Dailey, 2005). At this time uterine secretion of prostaglandin (PG) F2α should be inhibited to avoid luteolysis (Thatcher et al., 1994), mainly mediated by interferon tau secreted by the *conceptus*. Evidence exists that this mechanism is associated with previous luteal function and progesterone action during early pregnancy. Mann and Lamming (1999) determined, in cattle, that the presence of well developed *conceptus* on Day 16 after insemination was associated with better ability for interferon tau secretion, which was preceded by greater progesterone concentrations during early luteal phase. Furthermore, Khan et al. (2007) reported in ewes that the improvement of the luteal function before the critical period of gestation stimulates the growth and development of the *conceptus* increasing interferon tau production and pregnancy rates. Therefore, new strategies focused on the improvement of the gonadotropin luteal support during early embryo development to enhance pregnancy establishment should be investigated.

Endogenous equine chorionic gonadotropin (eCG) in mares induces accessory *corpus luteum* that improves gestation support since it is a variant of the equine LH differentially glycosylated by the trophoblast cells (Amoroso et al., 1948; Murphy, 2012). When eCG is used in non-equidae species, it has the peculiar property to provoke both FSH and LH activity (Murphy, 2012). In ruminants eCG is administered during the growing phase of the preovulatory follicle, allowing the linkage of FSH and LH follicle receptors (Soumano et al., 1996). In addition, its carbohydrate chains contain sialic acid that awards a particular long half-life compared to other glycopro-

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tein hormones (Martinuk et al., 1991), thus conferring an extended length of its effect. Consequently, administration of a single dose of eCG before ovulation in cattle induces follicle growth, increases the size of the preovulatory follicle, enhances ovulation rate and improves the luteal function after ovulation (Núñez-Olivera et al., 2014). For this reason, eCG is usually administered before ovulation in protocols for fixed-time artificial insemination in sheep and goats (Menchaca and Rubianes, 2004) and cattle (Bo et al., 2016), increasing pregnancy rate mainly in anestrous females. However, the use of eCG after ovulation to improve luteal support, looking for a similar function as it was described in mares, had not been proposed in ruminants until recently.

The *corpus luteum* requires the support of LH at least during part of its development and this effect may be achieved by eCG; thus, the use of this hormone is currently being studied in our Laboratory in sheep and cattle. Previous reports suggested a significant improvement on pregnancy rate when eCG was administered early in the pregnancy in beef (Cutaia et al., 2010) and dairy cattle (Bartolomé et al., 2012). Therefore, these results encourage the evaluation of this gonadotropin in others species to improve maternal recognition of pregnancy. We hypothesize that eCG administered within 10 days after ovulation may improve luteal function in sheep.

The objective of the current study was to determine the effect of the administration of eCG after insemination (i.e. before the critical period of gestation) on luteal function and follicular dynamics in sheep.

2. Materials and methods

2.1. Animals and experimental groups

The experiment was carried out during breeding season (April) at the facilities of the IRAUy, Montevideo, Uruguay (34 °S, 56 °W). Thirty multiparous Corriedale ewes with body condition score 3.3 ± 0.5 (scale 0–5) were used. Ewes were kept outdoors in a sheltered pen and an indoor box stall (4 × 3 m) was used for ultrasonographic examinations. Animals were fed with alfalfa hay and balanced ration (2000 and 300 g/ewe/day in average, respectively); water was available *ad-libitum*. All procedures were approved by the Animal Care Committee of the Fundación IRAUy certified by the National Council of Animal Care of Uruguay.

Ewes received a short-term protocol to synchronize ovulation described by Menchaca and Rubianes (2004). Briefly, the treatment consisted of 6 day administration of an intravaginal device (0.3 g progesterone, DICO®, Syntex, Buenos Aires, Argentina), and one dose of PGF2α analogue (125 μg cloprostenol, Ciclase DL, Syntex, Buenos Aires, Argentina) plus 300 IU of eCG (Novormon, Syntex, Buenos Aires, Argentina) given at the time of device removal. Fixed-time intrauterine insemination was performed by laparoscopy (Karl Storz, Hopkins, Tuttlingen, Germany) 48–54 h after device removal. Semen was collected by artificial vagina from one ram and it was extended to use 100×10^6 spermatozoa per ewe (i.e. 500×10^6 sperm/ml) in ultra high temperature treated skim milk. Extended semen was maintained at 30 °C and used within 1 h from collection. The day of insemination was defined as Day 0 of the experiment. After insemination, ewes were stratified by body condition score to be randomly assigned for either no treatment (n = 10) or the intramuscular administration of 400 IU eCG on Day 5 (n = 10) or Day 10 (n = 10).

2.2. Ovarian ultrasonography

Daily ovarian examinations were performed by ultrasonography (B-mode, 7.5 MHz linear transducer, Well-D, China) by the same operator from intravaginal device removal to 30 days after insemination in pregnant ewes, or until the following ovulation in non pregnant ewes. Ovarian structures were measured, mapped and recorded to obtain individual data of each *corpus luteum* and follicles from 3 mm in diameter. Ovarian examination was video recorded in two archives per ewe per day (i.e. one video for each ovary) for further clarification of data.

Interpretation of follicular dynamics was performed under the criterion reported by Menchaca and Rubianes (2004). Antral follicles were classified as small (3 to <4 mm of diameter), medium (4 to <5 mm) and large follicles (≥5 mm). Follicular wave was defined as a group of small follicles that give origin to one or more follicles ≥5 mm in diameter. For each follicular wave, the day of the emergence was defined as the day when the largest follicle achieved 3 mm in diameter followed by an increase in its diameter in the next day. The growing phase of a follicle was the period from 3 mm until the day its regression begun. The growth rate (mm/day) for the largest follicle was calculated as final size (i.e. maximum diameter)—initial diameter (i.e. 3 mm)/period of evaluation expressed in days. Inter-wave interval was the period between the emergences of two successive follicular waves. Wave length was determined as the interval between the emergence of the wave and the day when the largest follicle regressed to 3 mm. The occurrence of ovulation was detected by the collapse of a large follicle, usually greater than 5 mm in diameter. Ovulation rate was defined as the number of follicles that reached ovulation per ewe that was later confirmed by the presence of the *corpus luteum*.

The effect of eCG on follicular development was evaluated regarding to the status of the largest follicle of each follicular wave at the moment of eCG administration. Thus, the terms wave in regression phase or wave in growing phase were referred to follicular status at time of eCG treatment. The new wave emerging soon after eCG administration was named as emerging wave after treatment.

2.3. Serum progesterone determinations

Daily blood samples (10 ml) were collected by means of jugular venipuncture from Day 0 to Day 30 from pregnant ewes, or until estrous behavior from non pregnant ewes (e.g. until Day 16). Samples were centrifuged within 1 h after collection and serum was stored at −20 °C. Daily progesterone concentrations were determined in duplicate by a direct solid-phase radioimmunoassay (RIA) $Iodo^{125}$ using DPC kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA) in the Laboratory of Nuclear Techniques, Facultad de Veterinaria, Montevideo, Uruguay. The sensitivity of the assay was 0.1 ng/ml and the intra-assay coefficients of variation were 7.5%, 5.5% and 7.7% for low (1.0 ± 0.5 ng/ml), medium (2.2 ± 0.2 ng/ml) and high (8.2 ± 0.5 ng/ml) control values, respectively. The inter-assay coefficients were 9.9%, 12.3% and 9.8% for low, medium and high control values, respectively.

2.4. Statistical analysis

Daily follicular diameter, luteal area and serum progesterone concentrations were compared using mixed procedures (StataCorp, 2013), including the animals as random effect, eCG treatment and day, as the fixed effects. The mixed model included treatment, day and its interaction. The effect of eCG on follicular development early in the luteal phase was determined by comparing eCG treated *vs.* eCG untreated ewes on Day 5, and the effect on follicular development in the late luteal phase was analyzed separately by comparing eCG treated *vs.* eCG untreated ewes on Day 10. Fisher exact test was used to compare number of ewes with one, two or three CLs, pregnancy rate and number of ewes with one or two fetuses. The non parametric Wilcoxon rank-sum test was used to compare inter-wave interval, length of the wave, growing phase length, the day