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Hormonal therapeutic strategy on the induction of accessory corpora lutea in relation to follicle size and on the increase of progesterone in sheep

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ABSTRACT

We determined the effect of GnRH or hCG treatment on day 4 post-time artificial insemination (FTAI) on the formation of accessory corpora lutea (acc-CL) and on the concentration of serum progesterone (P_4) in sheep. Multiparous adult Merino ewes (n = 36) were synchronized for estrus using double injection of PGF2α agonist (125 μg Cloprostenol) with an interval of 14 days. At 53–56 h after the second PG application, FTAI was performed. On day 4 post FTAI, ewes were either treated with analogue of GnRH (4 μg buserelin; n = 12) or hCG (300 IU, hCG; n = 12) or saline solution (1 ml; Control; n = 12). Two laparoscopic ovarian examinations were performed on days 4 and 10 post FTAI. In the first observation, we determined the number of post ovulation corpora lutea (po-CL) and the site, number and diameter of follicles present in both ovaries. In the second laparoscopy, we observed the number of po-CL and acc-CL. The sizes of the follicles that generated the acc-CL were determined according to the position of the follicles observed in the first laparoscopy. Serum P_4 concentration was determined on days 4, 7, 10, 13, 17 and 21 post FTAI by chemiluminescence. A similar follicular population in size and number was observed in the three experimental groups prior to the beginning of treatments (Follicles 2 mm: 6.4 ± 3.7, 3 mm: 3.0 ± 2.3, 4 mm: 1.1 ± 0.5, 5 mm: 1.4 ± 0.8; $P > 0.05$). The formation of 1.0 ± 0.4 and 1.1 ± 0.3 acc-CL was observed in the GnRH and hCG groups, respectively ($P > 0.05$), but was not observed in the Control group ($P < 0.05$). Follicle sizes from which acc-CL generated were 3, 4 and 5 mm and did not differ between hormonal treatments ($P > 0.05$). The hCG group had higher mean concentrations of P_4 on days 7, 10, 13 and 17 post FTAI compared with the GnRH group and the Control group ($P < 0.05$), while no differences were observed between these two latter groups ($P > 0.05$). Mean P_4 concentrations in ewes treated with hCG showed no differences according to the size of the follicle from which acc-CL were generated ($P > 0.05$). In conclusion, administration of hCG or GnRH on day 4 post FTAI induced the formation of one acc-CL from follicles of 3, 4 or 5 mm, indistinctly. However, serum P_4 concentration increased significantly only in the hCG group. The serum P_4 concentrations of acc-CL that originated from different follicle sizes did not differ.

1. Introduction

The main losses during gestation in sheep occur in the embryonic period and constitute a multicausal reproductive problem. Inadequate luteal function is one of the most relevant causes of pregnancy failure in sheep [1]. Insufficient progesterone (P_4) production by the corpus luteum (CL), such as suboptimal P_4 synthesis, is indicative of maternal inability to maintain pregnancy [2]. The P_4 is required for the establishment and maintenance of pregnancy in all mammals. Also growth and development of the conceptus (embryo/fetus and associated extraembryonic membranes) needs P_4 signaling to regulate endometrial functions critical for implantation and placentation [3].

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Different therapeutic strategies have been used with the objective of increasing the concentration of P_4 and improving luteal function. Hormonal treatments in sheep were carried out in different breeds, animal categories and seasons of the year, using luteotrophic hormones, such as gonadotrophin releasing hormone (GnRH) or human chorionic gonadotrophin (hCG) in the early luteal phase [4–8] as in the late luteal phase [9–12]. The hCG has an activity similar to luteinizing hormone (LH), while the GnRH acts on pituitary gland, stimulates the release of LH and follicle-stimulating hormone (FSH) and subsequently the secretion of steroid hormones from the gonads. These hormones when administrated in the luteal phase act indirectly or directly on the ovary generating the formation of an accessory corpus luteum (acc-CL) and increasing the serum P_4 concentration [6,12–14]. Increased P_4 is known to improve embryonic survival and to reduce embryonic losses in ruminants [4,15–17]. However, results of hormonal treatments performed under different experimental conditions are variable, contradictory and often inconclusive.

Besides in ewes, the relationship between follicle size and subsequent CL size has not been studied, as has been in cows; data in this species suggest that a large CL generates from a large follicle, which would provide high P_4 concentration to favor embryo development [18]. Therefore, the objective of this study was to evaluate the effect of the administration of hCG or GnRH on day 4 post fixed-time artificial insemination (FTAI) on the formation of acc-CL in relation to follicle size and on the concentration of P_4 in Merino sheep during the reproductive season in Northern Patagonia, Argentina.

2. Materials and methods

2.1. Animals and handling

The experiment was conducted at the Farm Research Unit of the National Institute of Agricultural Technology, Bariloche, Río Negro (41° 7′ 23″ S, 70° 43′ 12″ W), during breeding season (May–June). Ethical concerns were taken into account by adhering to local animal welfare regulations and practices by the International Guiding Principles for Biomedical Research Involving Animals Committee (Comité Institucional de Cuidado y Uso de Animales de Laboratorio [CICUAL]; Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina). Protocol number for the Use of Animals of Scientific Research is 53-6-15T.

Thirty-six adult multiparous Merino sheep, mean body weight of 48.7 ± 5.7 kg and body condition of 2.5 ± 0.2 (subjective scale, 1 emaciated, 5 obese; [19]) were included in the experiment. The animals were maintained under natural grazing and with free access to water. All animals were estrus synchronized by the administration of two doses of prostaglandin F2α (PGF, 125 μg Cloprostenol, im; Cyclase, Syntex, Argentina) with an interval of 14 days. At 53–56 h after the second PG application, FTAI was performed vaginally with 0.03 ml of fresh semen with a mean concentration of 100 million spermatozoa per insemination dose [20]. Fresh semen was previously collected with an artificial vagina from one clinically healthy adult Merino ram of proven fertility (Breeding Soundness Examination and test for Brucella ovis). All the ewes were inseminated with the same ram.

On day 4 post FTAI, ewes were assigned randomly to three experimental groups: GnRH group (n = 12; 4 μg of Buserelin; im; Receptal, Intervet, Argentina), hCG group (n = 12; 300 IU of Hcg; im; Gonacor®, Ferring, Argentina) and Control group (n = 12; 1 ml of saline solution; im).

2.2. Ovarian observation

Two laparoscopic ovarian examinations were performed on days 4 and 10 post FTAI in all the animals from each experimental group. In the first laparoscopic examination, the number of post ovulation corpora lutea (po-CL) and the site, number and diameter of follicles present in both ovaries was determined. The diameter of all follicles ≥2 mm in size was measured with a manipulation probe engraved with a millimeter scale described by Cueto et al. [21]. The relative position of each follicle to other follicles and/or luteal structures was recorded in a diagram of the ovaries to evaluate the sizes of the follicles that generated the acc- CL at the second laparoscopy. In the second laparoscopic examination, the number of po-CL and acc-CL were determined. The sizes of the follicles that generated the acc-CL were determined according to the position of the follicles observed in the first laparoscopic examination.

To perform the laparoscopic examination, the ewes were deprived of food for 24 h and water for 12 h. Laparoscopic examinations were carried out by placing ewes in dorsal recumbent position on a standard cradle for laparoscopic surgery. The procedure was carried out under im general anesthetic (xylazine, 0.2 mg/kg, im; Kensol® 2%, Konig, Argentina; ketamine clorhidrate 2.5 mg/kg, Ketalar®, Parke-Davis, Argentina). In addition, a local anesthetic (lidocaine, 1 ml, im; Frankaina® 2%; Fatro Von Franken, Argentina) was administrated at the site of trocar insertion. For laparoscopic procedure, a trocar was located approximately 5 cm cranial to the udder and 5 cm to the left side of the midline. Afterwards, an endoscope (Richard Wolf, Knittlingen, Germany; 4 mm diameter; 170-mm length) was inserted into the abdominal cavity through a trocar to visualize the ovaries. The ovaries were handled by using a Veress needle placed in the midline (Endopath; Ethicon Endo-Surgery, Cincinnati, OH, USA; 2-mm diameter; 120-mm length). Finally, the trocar orifices were treated with local antibiotic cicatrizing solution (Young Plata, Quimagro, Argentina) and a preventive antibiotic therapy was carried out (1 ml/10 kg of Penicilin-Streptomycin, Over, Argentina).

2.3. Blood collection and serum progesterone assessment

On days 4, 7, 10, 13, 17 and 21 post FTAI, blood samples were collect by jugular venipuncture using vacuum tubes (BD Vacutainer®, Franklin Lakes, NJ, USA). The samples were centrifuged for 15 min at 3000 rpm and the serum obtained was stored at −20 °C until the time of analysis. Determination of serum P_4 concentrations was performed by chemiluminescence immunoassay (Elecsys®, Progesterone II, Roche, Mannheim, Germany) with a sensitivity of ≤0.1 ng/ml, a specificity 90% and an intra-assay and inter-assay coefficient of variation < 10% for samples between 0.1 and 36 ng/ml.

2.4. Statistical analysis

Analysis of data was performed using R Commander [22]. The P_4 serum concentrations in pregnant ewes were analyzed by ANOVA using a generalized linear model with time-repeated measurements. The number of acc-CL and the size of the follicles that generated the acc-CL were compared among treatment groups by one-way ANOVA and post-hoc comparison was made using Bonferroni post-tests. Statistical significance was accepted from $P < 0.05$.

3. Results

The number of 2–5 mm follicles at time of treatments was similar in all groups (Table 1; $P > 0.05$). The GnRH and hCG treated