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Induction of the presence of corpus luteum during superovulatory treatments enhances in vivo and in vitro blastocysts output in sheep

A. Gonzalez-Bulnes [a,*], F. Berlinguer [b], M.J. Cocero [a], R.M. Garcia-Garcia [a], G. Leoni [b], S. Naitana [b], I. Rosati [b], S. Succu [b], A. Veiga-Lopez [a]

[a] *Departmento de Reproduccion Animal INIA, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain*
[b] *Dipartimento di Biologia Animale, Universita degli Studi di Sassari, Via Vienna, 2, 07100 Sassari, Italy*

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Abstract

This report offers the results of two experiments developed to test possible benefitial effects of the presence of corpus luteum (CL) on in vivo and in vitro sheep embryo production; using two different breeds treated with two different protocols by two different teams at two different centres. In the first trial, estrus was synchronized in 11 ewes with two doses of cloprostenol, 10 days apart. On day 1 after estimated ovulation, sheep were treated with progestagen sponges and superovulated with eight decreasing doses (26.4 units NIH-FSH-S1 × 3, 22.0 units × 2, and 17.6 units × 3) of ovine FSH injected twice daily. Ovulation rate and number of embryos obtained in vivo were compared to those from 12 control ewes without cloprostenol treatment. Presence of a CL improves the number of transferable embryos (7.4 ± 0.6 versus 4.1 ± 0.6 in control ewes, $P < 0.05$). The second trial investigated the effects of the presence of CL on embryos produced in vitro from six ewes bearing CL and six ewes without CL at start a superovulatory treatment consisting of 96 units of ovine FSH administered in four equal doses given every 12 h. There were not detected effects of the CL on the number and size of follicles or in the number, morphology and ability to resume meiosis of their oocytes. However, oocytes from ewes with CL showed higher rates of fertilization (73.5 versus

* Corresponding author. Tel.: +34 91 347 4046; fax: +34 91 347 4014.
E-mail address: bulnes@inia.es (A. Gonzalez-Bulnes).

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45.5%, $P < 0.005$), higher development to blastocyst (35.8 versus 19.3%, $P < 0.01$) and higher hatching rates after vitrification (80.0 versus 25.0%, $P < 0.05$).

Keywords: Corpora lutea; Embryo; Ewes; Oocyte; Superovulation

1. Introduction

There are several emerging evidences indicating that embryo yields in superovulated sheep are deeply affected by the ovarian status at start of the FSH treatment. Former studies [1], confirmed later by ultrasonography [2], found that ovarian response to exogenous FSH supply was positively related to the number of small gonadotrophin-responsive follicles (2–3 mm) in the first day of the superovulatory FSH regimen. However, a recent study has found that the number of total embryos and its viability rate are related to the more limited category of follicles with 3 mm in size [3]; conversely, follicles of 2 mm in size are related to a higher degeneration rate, which might indicate a compromised developmental competence. On the other hand, the presence of large dominant follicles ($\geq$6 mm in size) decreases both number and viability of recovered embryos [4].

The number and quality of embryos are also affected by the presence or absence of a corpus luteum (CL) at the start of FSH treatment [5]. The absence of a CL exerts a negative effect on viability of embryos by increasing the degeneration rate. However, there is also an interaction with the presence of large follicles; the dominance effect on recovery and viability rates being higher in absence of CL. Effects from CL can be related to alterations in oocyte/embryo developmental competence by itself – abnormalities in the developmental competence of the oocytes or disturbances in the processes of fertilization and early embryo development [6] or to changes in the uterine environment, which expose the embryos to a hostile or non-supportive environment [7]. The data found in the study cited above [3] suggests that influence from CL is mainly related to changes in the uterine environment, although follicular population and oocytes were also affected.

The hypothesis that arose from these premises was that number and viability of embryos obtained either in vivo or in vitro would be increased by starting the superovulatory treatment during the early luteal phase of the estrous cycle, allowing the presence of a CL during the progestagen treatment. Present study reports results from two different experiments conducted to evaluate the usefulness of the induction of a CL prior to the administration of two different and previously validated protocols for in vivo and in vitro embryo production ([4] and [8], respectively); using two different breeds treated by two different teams at two different centres. The in vitro study, developed later, also looked for increasing the knowledge of mechanism underlying the effects derived from presence or absence of CL. In this way, we evaluated influence of CL on the development of follicles in response to FSH, on the developmental capacity of their oocytes and on the cryotolerance to vitrification procedures of embryos derived from in vitro maturation (IVM), fertilization (IVF) and culture (IVC) of these oocytes.