Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Biostimulation with the ram effect increases the embryo quality in superovulated ewes

Ana Paula Pereira Schmidt[1], Aline Matos Arrais[1], Felipe Zandonadi Brandão[2], Ribrio Ivan Tavares Pereira Batista[3], Maria Emilia Franco Oliveira[3], Joanna Maria Gonçalves Souza- Fabjan[1], Jeferson Ferreira da Fonseca[4], Rodolfo Ungerfeld[5]

[1]Universidade Federal Fluminense (*Departamento de Patologia e Clínica Veterinária - UFF*)
[2] Universidade Federal Fluminense (*Departamento de Reprodução Animal - UFF*)
[3]Universidade Federal Fluminense (*UFF*)
[4]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)
[5]Universidad de la República (*Facultad de Veterinaria - UDELAR*)

E-mail: paula_schmidt@id.uff.br

The pulsatile release of luteinizing hormone (LH) induced by the male effect may play a beneficial role in embryonic viability during superovulation. The objective of the present study was to determine if biostimulation with males for 72 h enhances the ewes' superovulatory response, including the embryo quality. Twenty ewes were used in a latin square design, so all ewes underwent both experimental treatments (CG = control group and REG = ram effect group), with an interval of 119 days between both periods. All ewes were isolated from rams 60 days before the start of the study (without visual, olfactory and auditory contact). While CG ewes remained isolated from rams throughout the study, REG ewes were joined and stimulated with rams. Estrous synchronization and superovulation were performed according to protocols previously described (Balaro *et al.*, Domest Anim Endocrinol, 54:10-14, 2016; Taira *et al*., Animal Reproduction Science, 181: 140-146, 2022). The rams were joined with the ewes simultaneously with the administration of the third dose of pFSH and had abdominal protection to prevent mating. Each ram remained with up to 7 ewes, being replaced by a new one every 12 hours, up to 36 hours after the last dose of pFSH, totaling 72 hours of biostimulation. Artificial insemination (AI) was performed at 24, 36, and 48 h after the administration of the fifth pFSH dose. The animals underwent an ultrasound exam to determine the total number of ovarian cysts and corpora lutea (CL) before the non-surgical embryo collection. Subsequently, all collected structures were examined and classified based on developmental stage and morphological characteristics indicative of quality, as described by the IETS. Only class 1 (grades I, II, and III) embryos were considered viable. Thirty grades I and II blastocysts (n=15 per group) were dry-frozen in pools of five embryos (n=3 pools per group) for analysis of differential expression of genes related to cellular stress (PRDX.1 and HSP70), embryonic quality (AQP3, CDH1, CDX2, and SIRT2) and apoptosis (BAX and BCL2), using RT-qPCR and 2−ΔΔCt method (endogenous control; ACTB and GAPDH). The data were analyzed using mixed models with Bioestat statistical software, including the treatments, time, and their interactions as the main effects. Data are presented as LS mean ± SEM. Differences were considered significant when $P \leq 0.05$. No difference was observed in the means of ovarian cysts (0.2±0.1 *vs.* 0.9±0.5), CLs (9±1 *vs.* 8±1), recovered structures (6±1 *vs.* 5±1), viable structures (4±1 *vs*. 3±1 in CG and REG treatments. However, bioestimulation increased the abundance of transcripts associated with energy metabolism (SIRT2) and cell adhesion (CDH1) and reduced the expression of those related to stress and apoptosis (PRDX.1 and BCL2). In conclusion, biostimulation promoted healthier embryo development according to gene expression, impacting positively therefore the quality of the embryos recovered.