Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and "omics"

Vitrification technique improves more alterations on gene expression of in vivo-derived sheep blastocysts than slow freezing

Viviane Lopes Brair[1], Ana Lucia Rosa e Silva Maia[1], Lucas Francisco Leodido Correia[1], Lucia Prellwitz[1], Cleber Jonas Carvalho de Paula[1], Juliana Dantas Rodrigues Santos[1], Felipe Zandonadi Brandão[1], Jeferson Ferreira da Fonseca[2], Ribrio Ivan Tavares Pereira Batista[1], Joanna Maria Gonçalves de Souza-Fabjan[1]

[1]UFF - Universidade Federal Fluminense (Niterói, RJ, Brasil); [2]Embrapa Caprinos e Ovinos - Embrapa Caprinos e Ovinos (Sobral, CE, Brasil).

Transfer of fresh sheep embryos has a higher pregnancy rate compared to cryopreserved embryos, suggesting that cryopreservation compromises embryonic signaling during implantation and establishment of pregnancy. Thus, this study assessed the effect of cryopreservation techniques (slow freezing or vitrification) on the expression of genes related to implantation (*CDX2*), maintenance of pluripotency(*NANOG*), cell proliferation (*TGFB1*),mitochondrial activity(*NRF1*) and regulator apoptosis (*BAX* and *BCL2*) of *in vivo*-derived sheep embryos. After superovulation of 32 ewes, and non-surgical embryo recovery, a total of 100 grade I and II embryos were retrieved and uniformly allocated into the groups: slow freezing (SF; *n*=42), vitrification (VT; *n*=43) and control for gene expression (CTL; *n*=15; stored in liquid N_2 in cryotubes RNase/DNase free until RNA extraction). After thawing/warming, three pools of five blastocysts per group (SF: *n*=15 and VT: *n*=15) were dry frozen as described above for later RNA extraction. The remaining 55 (SF: *n*=27 and VT: *n*=28) embryos were cultured *in vitro* (24/48 h) in SOFaa medium at 38.5 °C and 5% CO_2. After RNA extraction, the relative quantification was performed in triplicate by RT-qPCR and evaluated by the comparative Ct method $(2^{-\Delta\Delta Ct})$. Survival rate was analyzed by Fisher's Exact Test. The re-expanded embryos results, after *in vitro* culture (survival rate) of SF and VT was, respectively, at 24 h: 29.6% (8/27) and 14.2% (4/28); and at 48 h: 48.1% (13/27) and 32.1% (9/28) (P>0.05). The expression of transcripts was unaffected in embryos submitted to SF compared to the CTL, except for the up-regulated *CDX2* in the SF (P<0.05). On the other hand, VT had an increased (P<0.05) expression of all genes (*CDX2, TGFB1, BAX* and *BCL2*), except for *NANOG* and *NRF1*, when compared to the CTL. Comparing SF with VT was observed difference only in *BAX* gene (P<0.05), that was up-regulated in VT group. In conclusion, *in vivo*-derived embryos submitted to either SF or VT have similar ability to survive *in vitro* and VT led to increased changes in blastocysts gene expression compared to CTL and SF. Acknowledgments: Embrapa (22.13.06.026.00.05) and CNPq (434302/2018-0).Keywords:cryopreservation, implantation, embryo, ovine.