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Conferences papers

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32nd Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

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A267 Support Biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology and "omics"

Sex effect in gene expression of *in vitro*-produced bovine embryos vitrified by cryotop

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Male and female embryos are known to be different in terms of developmental kinectics, metabolism, gene expression and epigenetic patterns, as well as several stress conditions resistance. Consequently, it can be assumed that the response to cryopreservation may also be different between male and female embryos. However, there are no reports in literature evaluating the effect of gender on the response of bovine embryos to vitrification. In this study, the expression of eight genes related to apoptosis and cell damage (FOSL1, HSPB1, CASP3 and CASP8), thermal stress (HSPA5 and HSPA1A) and glucose metabolism (G6PD and PGK1) in IVP bovine embryos were analysed, by qPCR, aiming to determine the difference between gender on the response to cryopreservation. Male and female cryopreserved bovine embryos oocytes obtained from slaughterhouse ovaries were used, then were submitted to 24 hour IVM, inseminated with previously tested bull semen and presumptive zygotes were transferred to *in vitro* culture (IVC) medium, where they remained for 7 days. Cleavage on D2 and blastocysts rates on D6 and D7 were evaluated. On D7, expanded blastocyst embryos were removed from IVC and distributed into two treatments: control (C) and vitrified (V) by *Cryotop* (Cryo-Ingá: Ingamed®, Maringá, Brazil) method. After the warming process, embryos from C and V groups returned to IVC conditions for additional 24 hours. Then, hatched blastocysts were stored individually in DM-PBS solution at -80 ° C for sex determination. Each embryo was submitted to a DNA and RNA extraction process simultaneously using the AllPrep DNA/RNA Mini Kit (Qiagen®, Hilden, Germany). The extracted DNA was used for embryo sex determination, which was performed by PCR and confirmed in 1.5% agarose gel. Embryos were pooled in number of 20 according to the sex into 3 pools of male embryos C and V and 3 pools of female embryos C and V. These pools were used for gene expression quantification by qPCR using Sybr Green FAST Master Mix. ACTB and GAPDH were used as endogenous controls genes. Data were analyzed by t-test, considering $P \leq 0.05$. Among analyzed genes, female and male embryos differed between them in V treatment for HSPA1A ($P = 0.0043$), CASP3 ($P = 0.0037$) and G6PD ($P = 0.0071$) genes and in C group for G6PD ($P = 0.0526$) gene. Results indicate that gender did not affect cryopreservation response, because there was no difference between treatments. Therefore, it was evident that male and female bovine embryos are different, despite being submitted to cryopreservation process or not, and those differences are sex-related, because female embryos showed higher abundance regarding to gene expression compared to the male counterparts.

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