



A261 Support Biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology and “omics”

Effect of thermal stress on the maturation of bovine oocytes, *in vitro* embryo production and expression of genes related to stress response

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The sirtuins are members of the histone deacetylase (HDAC) family and are involved in the regulation of transcription activation and apoptosis. In this regard, the aim of this study was to investigate the influence of different temperatures during *in vitro* maturation (IVM) on the developmental competence of bovine oocyte, as well as mRNA expression of sirtuins. Cumulus-oocyte complexes (COCs) from Simmental cows were stained with Brilliant Cresilblue (BCB) and categorized as BCB+ or BCB-. Soon after, they were matured *in vitro* at different temperatures (37°C, 38.5°C and 40°C). After IVM, the oocytes were denuded and evaluated for the extrusion of the first polar body, and the cumulus cells were stored for mRNA expression analyses. Next, *in vitro* embryo production (IVEP) was carried out with the oocytes. Real-time PCR of oocytes and cumulus cells was performed to determine mRNA expression of sirtuins. For parametric data, ANOVA was applied and when a significant difference was found, the Tukey test with 5% probability was performed. For non-parametric data, the Kruskal Wallis statistical test was performed with 5% probability (software R). The different maturation temperatures did not significantly influence the maturation rate and the oocyte cleavage rate. However, COCs matured at 38.5°C (control) had a higher rate of blastocysts (37%), in contrast to those at matured at 37°C and 40°C (33.2% and 21.5%, respectively). At all different temperatures, the rates of blastocysts were higher for BCB+ oocytes than for BCB- oocytes. In the BCB+ oocytes the mRNA expression of SIRT1, SIRT2, SIRT3 and SIRT5 were higher after maturation than in immature oocytes, but no difference was observed in BCB- oocytes. In addition, the maturation temperature proved to have an effect on the expression of SIRT1, SIRT2, SIRT3 and SIRT5 mRNAs in BCB+ oocytes, with the control group been superior to all other treatments. In cumulus cells, only the mRNA expression of the SIRT2 gene had no effect on the different temperatures in the BCB+ oocytes, whereas the expression of the mRNA of the SIRT1 gene presented higher expression at the temperature of 38.5°C. The mRNA expression of the SIRT 3 and 5 was affected by the temperature of 40°C. Cumulus cells from BCB- oocytes had an effect only on the expression of SIRT3 mRNA, which was higher at 40°C. BCB+ oocytes are more resistant to different temperatures than BCB- oocytes. In addition, the IVM temperature influences the mRNA expression of SIRT 1, 2, 3 and 5, which are important for cell protection. The most significant effects were observed in the hottest temperature (40 °C). Therefore, findings from this study indicate that low quality oocytes, according to the BCB selection, are more susceptible to detrimental effects of unideal IVM temperature, which causes irreversible effects on the oocytes and decreases production of blastocysts.