A326E Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and "omics"

Spindle configuration of *in vitro* matured bovine oocytes vitrified and warmed in media supplemented with a biopolymer produced by an Antarctic bacterium

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Biological molecules isolated from organisms that live under subfreezing conditions could be used to protect oocytes from cryoinjuries suffered during cryopreservation. Bacterial exopolysaccharides (EPS) constitute a common class of molecules that interact with ice in nature either by triggering ice nucleation or by inhibition of ice nucleation and growth. The aim of this work was to evaluate the spindle configuration of in vitro matured bovine oocytes vitrified/warmed in media supplemented with exopolysaccharide (M1) produced by *Pseudomonas* sp ID1 (Carrión et al., Carbohydr Polym 117:1028. 2015). After 22 h of *in vitro* maturation, a total of 546 oocytes form prepubertal (3 replicates) and 405 oocytes from adult cows (4 replicates) were vitrified/warmed in media supplemented with various concentrations of EPS M1 (0, 0.001, 0.01, 0.1 and 1 mg/ml). After warming, oocytes were allowed to recover for 2 additional hours in IVM medium. Fresh, non-vitrified oocytes were used as a control. At 24 h of IVM, oocytes from all treatments were fixed and immunostained with the Alexa-fluor 488 antibody and DAPI. Microtubule and chromosome distribution was analyzed by immunocytochemistry under a fluorescent microscope. ANOVA was performed to analyze differences in meiotic spindle configuration ($P < 0.05$). When cow oocytes were vitrified, similar percentages of normal spindle configuration were observed when compared to fresh control oocytes, except for the 0.1 mg/ml EPS M1 group that showed significantly lower rates compared to the fresh control group. Significantly higher rates of prepubertal oocytes exhibiting a normal spindle configuration were recorded in the non-vitrified group compared to all vitrified/warmed groups, regardless of the EPS M1 supplementation. However, the addition of EPS M1 to the vitrification/warming media decreased the ratio of decondensation or absence of chromosomes and microtubules in prepubertal oocytes. Although percentages of normal spindle configuration after vitrification were lower for prepubertal than for cow oocytes, no significant differences were observed when oocytes were vitrified with 0.001, 0.1 and 1 mg/ml EPS M1. In conclusion, supplementation with EPS M1 concentrations during vitrification and warming did not induce adverse changes in the spindle of bovine oocytes, regardless of the concentration used. Although a more severe damage on spindle configuration could be observed after vitrification of prepubertal oocytes, EPS supplementation during vitrification and warming seems to have a greater benefit during vitrification of prepubertal than adult bovine oocytes. Further experiments are required to investigate if *in vitro*-matured oocytes vitrified/warmed in presence EPS M1 can improve their development competence after being vitrified/warmed. This study was supported by the Spanish Ministry of Science and Innovation (Project AGL2016-79802-P and grant CTQ2014-59632-R).