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Effect of astaxanthin on post-thaw viability of bovine vitrified oocytes: preliminary results

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Although various techniques for cryopreservation of bovine oocytes are known, their developmental potency after thawing is still unsatisfactory. Astaxanthin (AX) is a powerfull antioxidant, which improved post-thaw viability of pig oocytes but its effects on bovine vitrified oocytes are to be examined. Our goal was to examine whether AX, added to the post-thaw medium, can improve the viability of bovine vitrified oocytes. Oocytes, recovered from cow´s ovarian follicles at slaughtering, were *in vitro* matured (IVM) and then vitrified in minimum volume on the nickel electron microscopy grids by ultra-rapid cooling technique. Following several months the oocytes were warmed and incubated 3 hours for post-thaw recovery in the maturation medium (TCM199, 10% FCS, 0.25mmol.l^{-1} sodium pyruvate, 50µg/ml gentamicin, 1/1 I.U FSH/LH (Pluset)) either without AX (Sigma Aldrich, Missouri, USA; 0µM; vitrified group; n=186) or with 2.5µM (the dose was chosen according to the previous reports) of AX (vitrified+AX group; n=179). Fresh IVM oocytes (n=157) served as a control. Afterwards, oocytes of all groups were fertilized *in vitro* using frozen bull semen and cultured in B2 medium (prepared according to Menezo) with 10% FCS, 10mg/ml BSA, 31.25mM sodium bicarbonate and 50µg/ml gentamicin on a monolayer of BRL-1 (Rat epithelial cells; ECACC, UK) cells at 38.5°C and 5% CO_2 until the blastocyst stage (6-8 days). Experiments were performed in four replicates. Total blastocyst rate (D6-D8) was significantly less in vitrified (12.90%) and vitrified+AX (13.41%) groups compared to control group (25.48%), whilst cleavage rate was different only in vitrified group (53.26%), but not in vitrified+AX (55.87%) compared to control (64.33%). However, AX significantly ($p<0.05$) increased (Chi-square test) the proportion of embryos that reached the blastocyst stage earlier (Day 6; 20.83%), compared to the vitrified group without addition of AX (8.33%), thus approaching this value to those in the control group (25.00%). Vitrification led to slight decrease ($p>0.05$; Student´s t-test) in the blastocyst cell number from 103.03±4.42 (control group) to 92.24±6.20 (vitrified), whilst AX reversed this suppressive effect (102.87±6.00). Apoptotic occurrence (TUNEL-index) did not differ significantly among control (10.33±1.84%), vitrified (13.02±3.24%) and vitrified+AX (13.93±3.35%) groups (Student´s t-test). AX indicated a trend to improve quality of actin cytoskeleton by increasing the proportion of embryos with excellent actin quality (grade 1) in vitrified+AX oocytes (82.61%) in comparison to the fresh (64.87%) or vitrified (61.90%) groups, although differences were not significant (Mann-Whitney U-test). In conclusion, astaxanthin, added to vitrified/warmed oocytes during post-thaw recovery period, accelerated development to the blastocyst stage, what was reflected in increased rate of faster developing blastocysts with no effect on the total blastocyst yield.

Keywords: astaxanthin, oocytes, vitrification

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