Animal Reproduction

X INTERNATIONAL SYMPOSIUM ON ANIMAL BIOLOGY OF REPRODUCTION (ISABR)

FEMALE REPRODUCTIVE BIOLOGY

Supplementation of *in vitro* maturation medium with metformin improves the development of bovine *in vitro*-fertilized oocytes toward expanded blastocyst stage

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The rising demands of recruiting *in vitro*-produced embryos for optimizing cattle husbandry encounters some limitations, including the inferior female gamete competence regarding its *in vivo* counterpart. This attributes to degenerative effects mediated by elevated reactive oxygen (ROS) generation in the artificial environment. However, oxidants neutralization attends moderately the expectations, so further antioxidant activity may remarkably enhance oocyte potential, like inhibiting free radical liberation from the respiratory chain as performed by metformin (MT). To test our hypothesis, cumulus-oocyte complexes (COCs) obtained by puncturing the visible antral follicles of abattoir ovaries were selected, basing on the surrounding cells (≥3 compacted layers) along with ooplasm appearance (opaque homogeneous) and randomly distributed in four groups with different concentrations of MT during *in vitro* maturation for 22-24h: MT0=0 (n=139), MT1=0.05 (n=142), MT2=0.1 (n=143) and MT3=0.2 mM (n=151). The *in vitro*-matured COCs were placed in *in vitro* fertilization (IVF) medium containing spermatozoa (concentration= 2X106 per ml) previously separated with mini Percoll gradient; 18 h later the presumptive zygotes were partially denuded and cultured in synthetic oviduct fluid (SOF) + 3% fetal calf serum. Cleavage and blastocyst (BL) rates from six replicates were observed at 48 and 168 h following IVF. Then, 4-5 BLs/group produced from MT0 (n= 18) and MT2 (n= 22) in five independent replications, were submitted to terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay and stained with 4',6-diamidino-2-phenylindole (DAPI) to visualize the apoptotic and viable blastomeres respectively, under a florescent microscope (EVOS M5000 microscope, Invitrogen). The qualitative variances (*in vitro* embryo production results) were analyzed using logistic regression and chi-square. Total cell number and apoptosis index (+TUNEL/blastomeres %) were analyzed by One-Way ANOVA (SAS university software, version9.1); the difference considered significant when P≤0.05. The data are expressed as mean±SEM. MT had no effect (P>0.05) on cleavage (78.8±3.2, 76±3.3, 75.5±2.9, and 76.6±3.6% for MT0, MT1, MT2, and MT3, respectively) and blastocyst (46.4±7.1, 48±4.5, 40.8±2.8, and 44.3±5% for MT0, MT1, MT2, and MT3, respectively) rates. The rate of embryos at blastocyst stage was lower (P<0.05) in MT2 (3.3±1.8%) than in MT0 (14.5±3.4%), MT1 (7.3±2.7%) and MT3 (8.8±3%), whereas rate of embryos at blastocyst expanded stage (27.5±1.8%) was higher (P<0.05) than MT0 (18.1±4.8%) with no difference (P>0.05) among MT0, MT1 (26.5±5%) and MT3 (24.6±14%). The percentage of the embryos at remaining blastocyst stages presented no difference (P>0.05) among groups (early blastocyst: 10.9±2, 12.7±2.8, 9.3±2 and 9.6±1.6%; hatching blastocyst: 1.39±1.3, 0.83±0.8, 0 and 0.72±0.7% or hatched blastocyst: 1.39±1.3, 0.57±0.5, 0.69±0.6 and 0.52±0.5%, for MT0, MT1, MT2 and MT3, respectively). Total cell number (173.77±7) and apoptosis index (19.45±2.12%) in MT2 were higher (P<0.05) than in MT0 (138.33±11.12 and 13.20±1.54%). In conclusion, 0.1 mM metformin added to the *in vitro* maturation medium favors the development of zygotes towards expanded blastocyst stage, although no effect on overall blastocyst production was found. Considering that the blastocysts at expanded stage are usually the preferred category for vitrification, metformin can be useful to generate embryos with the aim of cryopreservation. Nevertheless, the role of metformin on apoptosis in blastocyst requires further investigation. Financial support: CNPq and Fapemig.

Keywords: antioxidants; respiratory chain; expanded blastocyst.