Animal Reproduction

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Metformin ameliorates bovine zygotes development

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In vitro embryo production experiences some obstacles to its utilization in livestock system, like unsatisfactory yield besides suboptimal quality of resultant structures, owning that to many factors such as the cellular damages mediated by elevated reactive oxygen species (ROS) generation under *in vitro* conditions. The present work aimed to improve blastocyst outcome and viability via the addition of metformin (MET), which inhibits oxidants release from the respiratory chain. Cumulus-oocyte complexes (COCs) punctured from antral follicles (3-6 mm in diameter) of abattoir ovaries, were matured for 24 h, and then co- incubated in fertilization medium (20 µg/ml heparin) with sperm (2 million *per* ml) previously separated by mini-Percoll gradients. After 18 h presumptive zygotes were partially denuded, placed in culture media (synthetic oviductal fluid containing 3% fetal calf serum) supplemented with MET as following: 0 (MET0, n = 106), 0.05 (MET1, n = 107), 0.1 (MET2, n = 111) and 0.2 mM (MET3, n = 118), and cultured under 5% CO_2, 38.5°C and saturated humidity. At 48 and 168 h post fertilization, the cleavage and blastulation were verified (six replicates), and 5-6 blastocysts/group from four replicates were submitted to terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay (DeadEnd™ Fluorometric TUNEL System, Promega, Madison, USA) in order to evaluate the apoptosis index and total cell number. Data of cleavage and blastocyst rates and apoptosis index were analyzed by binary logistic regression (Proc Logistic) and total cell number was analyzed by linear mixed model (Proc Mixed) using the SAS Analytics software (version 9.1), considering the difference significant when $P \leq 0.05$. Cleavage rates were similar ($P > 0.05$) among MET0 (90.9 ± 2%), MET1 (88.4 ± 1.4%), MET2 (85.3 ± 3%) and MET3 (87.9 ± 2.8%). The highest blastocyst rate ($P < 0.05$) was found in MET1 (60.3 ± 2.5%) when compared to others (MET0 = 43.6 ± 7.3, MET2 = 30.4 ± 3.7 and MET3 = 48.1 ± 6.1%) whereas blastocyst rate in MET2 was lower ($P < 0.05$) than in MET3. No difference ($P > 0.05$) was found between MET0 and MET3. In MET0, the blastocyst total cell number (114.4 ± 8.1) did not differ ($P > 0.05$) from MET1 (106.2 ± 6.8) and MET3 (101.5 ± 5.4), but it was higher ($P < 0.05$) than in MET2 (90.2 ± 4.6). No difference ($P > 0.05$) was found among MET1, MET2 and MET3. Apoptosis occurrence showed no significant difference ($P > 0.05$) between the treatments (MET1 = 26.4 ± 1.9%, MET2 = 27.9 ± 1.9% and MET3 = 22.9 ± 2%); however, the MT0 presented a lower ($P < 0.05$) apoptosis index (24.5 ± 2.5%) than MET1 and MET2. In conclusion, metformin contributes to blastocyst production in a dose dependent manner. Dose of 0.1 mM metformin can be proposed as additive to embryo culture medium to enhance embryo production, although it does not reduce the apoptosis incidence. Financial support: CAPES, FAPEMIG and CNPq.