149 EFFECT OF RESVERATROL ANALOGUE ON DEVELOPMENT OF *IN VITRO*-FERTILIZED BOVINE EMBRYOS

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Oxidative stress is one of the main effects of *in vitro* culture. Generation of reactive oxygen species (ROS) by embryos can be enhanced by the sub-optimal *in vitro* culture conditions and are associated with a delay in embryonic development. However, supplementation of culture medium with antioxidant agents can minimize the effects of ROS (Guérin *et al.* 2001 Hum. Reprod. Update **7**, 175–189). Resveratrol is an example of a potent antioxidant, and modifications in its structure can improve its biological activity. This study evaluated the effect of AR33 (formula with patent pending), an analogue of resveratrol with high antioxidant activity, on embryo development. Bovine cumulus-oocyte complexes recovered from ovaries collected at the slaughterhouse were *in vitro* matured for 24 h and oocytes were *in vitro* fertilized for 20 h, both at 38.8°C under 5% CO_2 in air and high humidity. Partially denuded presumptive zygotes were randomly distributed in 4 treatments (with 6 replicates): 0 μM (control, $n = 347$), 0.1 μM ($n = 337$), 0.5 μM ($n = 277$), and 2.5 μM ($n = 343$) of AR33. The base medium was SOFaa supplemented with 2.5% FCS and incubation conditions were 38.8°C under 5% CO_2 in air and high humidity. Half of culture medium was renewed (feeding) at Day 3 and 5 post-fertilization. Cleavage was evaluated at Day 3 and blastocyst rates at Day 7 and 8 post-fertilization. Data were analysed by logistic regression considering the significance level of $P < 0.05$. Values are shown as mean ± SEM. Cleavage rate was higher ($P < 0.05$) for 2.5 μM (69.0 ± 4.4%) than for 0, 0.1, and 0.5 μM AR33 (62.1 ± 2.0%, 60.7 ± 5.9%, and 56.7 ± 5.8%, respectively). At Day 7, the blastocyst rate was similar ($P > 0.05$) among 0.1, 0.5, and 2.5 μM (18.1 ± 5.4%, 17.5 ± 2.9%, and 19.4 ± 3.3%, respectively) and all of them were higher ($P < 0.05$) than 0 μM AR33 (12.4 ± 2.5%). At Day 8, there was again no difference ($P > 0.05$) among 0.1, 0.5, and 2.5 μM AR33 (21.0 ± 5.0%, 18.4 ± 2.1%, and 24.6 ± 3.3%, respectively) but only 0.1 and 2.5 μM showed higher ($P < 0.05$) blastocyst rate than 0 μM AR33 (15.2 ± 2.5%). In conclusion, the synthetic analogue of resveratrol tested in this study can improve bovine embryo development in culture medium supplemented with 2.5% FCS under 5% CO_2 in air. A concentration of 2.5 μM AR33 can be a choice for further studies.

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150 PROTEOME OF BOVINE CUMULUS CELLS AS RELATED TO OOCYTE MORPHOLOGY AND *IN VITRO* EMBRYO PRODUCTION

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The present study was conducted to study the effect of cumulus-oocyte complex (COC) morphology on subsequent *in vitro* embryo development and to assess the proteome of their corresponding cumulus cells (CC). Cow ovaries were obtained at an abattoir and COC aspirated from 3–8 mm follicles. The COC were defined as type I (TI): homogeneous ooplasma and ≥4 layers of compact CC; type II (TII): granular ooplasma and ≥4 layers of slight expanded CC. Fifty COC had ~500,000 CC. Cumulus cells were frozen in ammonium bicarbonate and immediately lyophilized for proteome analysis. Other selected COC were matured *in vitro* in TCM-199-supplemented media for 24 h. After maturation, CC were collected (T24) and processed as described above. The remaining COC were fertilized with sperm from a fertile bull and zygotes, cultured *in vitro* until Day 7. Ten blastocysts per group were stained (Hoechst 33342) and blastomeres, counted for assessment of embryo quality. The CC proteins were obtained from the following groups: immature type I (TIT0) and type II (TIIT0), and *in vitro* matured type I (TIT24) and type II (TIIT24). For protein extraction, we used sonication (30 min, 4°C), freezing and unfreezing in liquid nitrogen, and maceration. The CC proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and identified by ESI-MS/MS. Differences in cleavage, embryo rates, and blastomere numbers were analysed by *t*-test. Protein expression difference was set at 2.5-fold ($P < 0.05$). *In silico* protein interactions were investigated using STRING v. 10.0. There were no differences in cleavage (88 ± 4 *v.* 89 ± 8%) and embryo rates (36 ± 7 *v.* 33 ± 8%) between COC of TI ($n = 220$) and TII ($n = 161$), respectively. Blastomeres were also similar in TI (101) and TII (104) groups. Major proteins expressed in all CC were α-enolase, β-actin, oestradiol 17-β-dehydrogenase 1, glutathione S-transferase, glyceraldehyde 3-phosphate dehydrogenase, heat shock protein β-1, histone H2B type 1-N, histone H4, mitochondrial malate dehydrogenase 2, protein disulfide isomarase A6, triosephosphate isomerase, tubulin α-1C chain, and vimentin. Glyceraldehyde 3-phosphate dehydrogenase appeared to be more expressed in TIT0, whereas tubulin α-1C chain and vimentin had greater expression in TIIT24. As evidenced by *in silico* analysis, most CC proteins interact among themselves, participating in complex networks involving intracellular signalling and other events. In conclusion, there are no difference in embryo development when using compact and early-expanded COC, indicating that both types can be selected for IVP. Protein profile of cumulus cell may serve as a marker for *in vitro* embryo competence.

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