

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Supplementation of Oleic Acid to Bovine Oocyte Maturation Medium Improves Embryo Quality Parameters

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In vitro embryo production (IVP) is a key biotechnology for improving genetic progress and reproductive outcomes in cattle. The quality of embryos produced *in vitro* is largely affected by the maturation medium (IVM) composition, especially the presence of fatty acids that influence oocyte developmental competence. Stearoyl-CoA desaturase 1 (SCD1) converts stearic acid (SA), a saturated fatty acid, into oleic acid (OA), a monounsaturated fatty acid known to enhance oocyte viability and embryo development. This study evaluated the effects of SA and OA supplementation during IVM on bovine embryo quality, assessed by total cell number, neutral lipid content, and apoptotic index. Oocytes and cumulus-oocyte complexes were collected from slaughterhouse-derived ovaries. A total of 544 oocytes were distributed across six experimental groups: BSA control (n=92), Ethanol control (n=95), 25 μ M SA (n=110), 50 μ M SA (n=76), 100 μ M OA (n=89), and 200 μ M OA (n=82). Oocytes were matured for 24 h at 38.5 °C in a 5% CO₂ atmosphere, fertilized *in vitro* for up to 24 h, and cultured for 7 days until the blastocyst stage. Embryos were fixed in 4% paraformaldehyde (Electron Microscopy Sciences, USA) and stained with Hoechst 33342 (Thermo Fisher Scientific, Waltham, USA), Nile Red (Molecular Probes, Inc., Eugene, OR, USA) and TUNEL (apoptosis, DeadEnd Fluorometric TUNEL System, Promega, Madison, USA). Embryos were analyzed by fluorescence microscopy (EVOS M5000) and images were evaluated using ImageJ software (NIH). Data were analyzed by one-way ANOVA followed by Tukey's test (GraphPad Prism 10; P<0.05). Cleavage rates ranged from 73.7% (50 μ M SA) to 82.9% (200 μ M OA) and the blastocyst rate was significantly higher in the 200 μ M OA group (51.2%) compared to the BSA control (32.6%; P<0.05). The total cell number per blastocyst was significantly higher in the 200 μ M AO group (152.5 $\pm$ 39.2) than in the BSA control (142.6 $\pm$ 52.6; P<0.05) while the average number of apoptotic cells was lower (0.05 $\pm$ 0.04 vs. 0.2 $\pm$ 0.2; respectively P<0.05). Lipid content was reduced in the 200 μ M OA group (0.0001 $\pm$ 0.00007) compared to the BSA control (0.0002 $\pm$ 0.0002; P<0.05). Supplementation with 200 μ M AO during IVM significantly improved bovine embryo quality by optimizing cellular dynamics, reducing apoptosis and lipid accumulation, reinforcing its potential in IVP protocols. These findings confirm previous results (Melo et al., Animal Reproduction, vol. 21, no. 3, p.111, 2024), regarding the positive contribution of AO to the competence for early development.

Financial Support: Fapemig and CNPq.