Animal Reproduction

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OPU-FIV

Assessing nuclear and cytoplasmatic configuration in prepubertal bovine oocytes after *in vitro* ou *in vivo* maturation

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Genetic improvement programs extensively investigate the reduction of generation intervals. Oocytes from prepubertal female animals are prioritized for multiplying high-quality animals before sexual maturity. This study aims to evaluate nuclear maturation, quantify lipid droplets, and assess mitochondrial activity in oocyte from Nelore females. Moreover, comparing prepubertal and pubertal stages undergoing *in vitro* (IVM) or *in vivo* (IFOT) maturation. A total of 33 calves (aged 6-8 months) and 19 multiparous cows (aged >36 months) underwent OPU sessions. Viable COCs followed either for IVM or IFOT. In summary, groups of 25 COC were loaded into microtubes with 400 µL of IVM medium, consisted of TCM-199 with Earl's salts (Gibco® BRL, ON, Canada) supplemented with 5% FBS, 0.01IU/mL FSH, 0.1 mg/mL L-glutamine, Cysteamine (0.1mM) and antibiotic (amikacin, 0.075 mg/mL) and covered with 300µL of silicone oil, incubated for 18 hours at 38.5°C and 5% CO2 in a portable incubator (Minitub®). For *in vivo* maturation, groups of 25 immature COCs from both donor categories were injected into the dominant follicle of pubertal synchronized heifer. On day 9, recipients presenting one dominant follicle ≥10 mm, 30h of P4 withdrawal, were selected. The IFOT was then performed, along with the application of 50µg of Lecirelin. After 18h of maturation, COCs were removed from IVM or retrieved by OPU from the recipients. Following, the COCs were denuded, fixed, and stained with Lacmoid (BEZ-IVM, n = 71, COW-IVM, n = 72, BEZ-IFOT, n = 88, and COW-IFOT, n = 83) to the assessment of the meiotic stages in a phase-contrast microscope (Nikon E200). Another subset (BEZ-IVM, n = 12, COW-IVM, n = 10, BEZ-IFOT, n = 21 and COW-IFOT, n = 11) was incubated, subsequently fixed and evaluated for lipid droplet area using microscopy fluorescence (New Orleans, USA) (Bodipy), with images quantified by ImageJ® (NIH, USA) and mitochondrial quantification. Mitochondrial evaluation used 400 nM of MitoTracker Deep Red fluorescent dye, with data presented as mean pixel values. The Chi-square test was used for the meiotic progression evaluation and ANOVA followed by Tukey's test for relative lipid area (%) and mitochondrial activity (number of pixels), respectively, with a significance level of 5%. The percentage of oocytes completing nuclear maturation was similar ($p>0.05$) between BEZ-IVM and COW-IVM groups (67.6% and 63.9%, respectively). However, BEZ-IFOT (47.7%) and COW-IFOT (47.0%) groups had lower rates ($p\leq0.05$) than *in vitro* groups. BEZ-IVM had a larger relative area occupied by lipid droplets ($p\leq0.05$) compared to other groups, which did not differ ($p>0.05$). Greater mitochondrial activity was observed in BEZ-IVM and COW-IVM oocytes compared to those matured *in vivo* ($p\leq0.05$), with no difference between *in vivo* groups ($p>0.05$). In Summary, using IFOT as an *in vivo* maturation system was effective, however, probably the maintenance time of COCs inside the follicle needs to be adjusted to reach similar patterns of MII stage oocytes.