Animal Reproduction

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Bovine oocyte and cumulus cells can exhibit distinct response to heat shock during in vitro maturation

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Heat shock (HS) increases the generation of reactive oxygen species (ROS) in bovine oocytes and reduces developmental competence (Ascari et al., Dom. Anim. Endocrinol. 60:50-60, 2017). However, its effect on the surrounding cumulus cells has not been fully elucidated although those cells play an important role on oocyte competence. Zinc oxide nanoparticles (ZnO-NP) can modulate the generation of ROS in mammalian cells (Saptarshi et al., Nanomedicine, 10:2075-2092, 2015). This study evaluated the effect of ZnO-NP and HS during in vitro maturation of cumulus cells-oocyte complexes (COCs) on the generation of ROS and mitochondrial activity in oocytes and their surrounding cumulus cells. Immature COCs were randomly distributed in four in vitro maturation (IVM) groups: Control (IVM under 38.5°C for 24h), ZnO-NP (IVM at 38.5°C + 1 µg/mL ZnO-NP for 24h), HS (IVM under 41.5°C for 24h) and HS+ZnO-NP (IVM under 41.5°C + 1 µg/mL ZnO-NP for 24h). In the first trial (seven replicates), in vitro matured oocytes were in vitro fertilized, and the presumptive zygotes were cultured for eight days. Blastocyst data was analyzed by the Logistic procedure of SAS 9.0. In the second trial (two replicates), cumulus cells were removed from oocytes after IVM. The denuded oocytes and respective cumulus cells were stained with CellROX green, MitoTracker Red CMXRos and Hoechst 33342 reagents (Thermo Fischer). Images were captured using epifluorescence microscope and fluorescence intensity calculated by ImageJ software. Data was analyzed by the Mixed procedure of SAS. Values are shown as mean±SEM. The lowest ($P<0.05$) blastocyst rates were found in HS (22.4±8.7%) and HS+ZnO-NP (18.4±3.2%) groups and the highest ($P<0.05$) was found in ZnO-NP (52.2±7.3%) group when compared to control (42.1±9.2%). Oocytes of ZnO-NP (22.7±1.1 arbitrary units [a.u.]), HS (23.4±1.3 a.u.) and HS+ZnO (22.6±0.9 a.u.) groups displayed more ROS ($P<0.05$) than oocytes of control group (18.3±0.9 a.u.). Mitochondrial activity increased ($P<0.05$) only in the HS+ZnO-NP group (35.4±2.6 a.u.) and no differences ($P>0.05$) were found between control (21.7±2.5 a.u.), ZnO-NP (27.9±2.8 a.u.) and HS (28.0±2.9 a.u) groups. In contrast to heat-shocked oocytes, heat-shocked cumulus cells (HS group) displayed lower ($P<0.05$) generation of ROS (6.9±0.2 a.u.) and mitochondrial activity (3.7±0.1 a.u.) than cumulus cells of control (10.2±1.1 and 6.8±0.8 a.u., respectively) and ZnO-NP (10.3±0.7 and 6.3 ±0.4 a.u., respectively) groups. The generation of ROS (8.9±1.4 a.u.) and mitochondrial activity (6.7±1.1 a.u.) in cumulus cells of HS+ZnO-NP group were similar ($P>0.05$) to control group. In conclusion, bovine oocytes and the surrounding cumulus cells can exhibit a distinct response to HS during IVM. The lower ROS generation and mitochondrial activity induced by HS on cumulus cells can be modulated by ZnO-NP supplementation in the IVM medium.

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