Animal Reproduction

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Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

The influence of ovarian follicular niche on oocyte development

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Resumo

The follicular environment is essential for the development of a gamete. Extracellular vesicles (EVs) present in the follicular fluid (FF) act in intercellular communication, carrying bioactive contents, such as miRNAs and mRNA, contributing to the oocyte maturation. The first aim of this work was to evaluate through RNAseq the RNA contents of small EVs from the FF and its relation with the oocyte competence to reach the blastocyst stage. Therefore, the small EVs from the FF were collected from 3 to 6 mm diameter follicles using a retrospective model previously described. To track its competence, FF and oocyte were individually recovered from follicle dissection of slaughterhouses ovaries collected without selection. Then, were matured, parthenogenetically activated and cultured individually for 7 days. Oocytes and their respective follicular fluids were classified and separated in three groups, accordingly to developmental competence: IM Group (Incompetent Mature): Oocytes matured in vitro but did not cleave after parthenogenetic activation; IC Group (Incompetent Cleaved): Parthenotes cleaved after parthenogenetic activation, but blocked the development at 3rd or 4th cell cycle; BL Group (Blastocysts): Oocytes which developed to the blastocyst stage. Differential gene expression, exclusive and HUB genes analyses of the transcriptomic data were performed, and transcriptional alterations among the more competent (BL) and less competent (IC and IM) groups ($q<0.1$) were observed. Interestingly, several gene transcripts found significantly altered among these groups were related to signaling pathways associated with cell proliferation and meiosis modulation such as WNT, PI3K-Akt and Hippo signaling pathways. Due to its key function in all pathways, GSK3 (Glycogen Synthase Kinase 3) was selected as a candidate gene potentially regulating these signaling pathways and the oocyte competence. To test this, we matured the oocytes for 8 hours in maturation medium depleted of FSH/LH under 3 conditions: 1) CTL (control); 2) DKK1 treatment that indirectly stimulates GSK3B through DKK-1 (Dickkopf related protein 1) inhibition of the WNT signaling pathway; 3) CHIR treatment that directly inhibits GSK3B through the chemical probe CHIR99021. After treatment, oocytes were washed and matured for 16 hours in regular maturation medium. Maturation rates were observed, then mature oocytes were parthenogenetically activated and in vitro cultured. Thus far, 6 replicates were performed, and the blastocysts rates, CTL (41±4.1%; N=264), DKK1 (47.9±4.2%; N=249) and CHIR (45.2±4.1%; N=260) were similar among the groups ($p<0.05$). The retrospective model identified crucial genes and pathways associate with oocyte competence. Initial attempts to modulate these pathways failed in enhance the oocyte competence. Currently we are testing different periods of treatment and molecularly investigating the alterations caused in the oocyte following GSK3 modulation.

Acknowledgments

CAPES.