Animal Reproduction

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IVM SYSTEM ALTERS KDM4C TRANSCRIPT LEVELS IN BOVINE OOCYTES

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Resumo

Maternal mRNAs play essential functions in early embryo development. During oocyte maturation, ZFP36L2 binds AU-rich transcripts, directing them to degradation and remodeling mRNA stores. Our previous data showed a difference in ZFP36L2 mRNA levels between oocytes matured in vivo vs in vitro. This study aimed to evaluate ZFP36L2 expression and activity in bovine cumulus-oocyte complexes (COCs) submitted to different in vitro maturation (IVM) protocols. Grade I and II COCs from 2-8 mm follicles were distributed into 3 IVM groups, containing 50 COCs per well and 400µL of the following IVM media: conventional IVM for 19 h [C-IVM; TCM199 Earle's salt and L-glutamine, 2.2g/L sodium bicarbonate, 50µg/mL gentamicin, 0,2mM sodium pyruvate (base medium) plus 0,5µg/mL FSH, 50mg/mL hCG e 10% fetal calf serum]; "physiological" IVM for 19 h (Ph-IVM; base medium plus 10ng/mL IGF-1, 100ng/mL AREG, 10-2 IU/mL rhFSH, 5µg/mL 17β-oestradiol e 150ng/mL progesterone); pre-IVM (PM-IVM; base medium plus 500ng/mL 17β-oestradiol, 50ng/mL progesterone, 50ng/mL androstenedione, 10-4 IU/mL rhFSH and 100nM NPPC) for 9 h followed by Ph-IVM for 19 h. Samples were collected at 0 h (germinal vesicle - GV), end of pre-IVM (PM-IVM 0 h), 9 h, and 19 h of IVM only oocytes in Metaphase II (MII) were collected. Oocytes and cumulus cells were separated, frozen and stored. Ten pools of 50 oocytes from 9 h IVM groups and 6 pools of MII oocytes were used for ZFP36L2 quantification by western blotting (WB). Twenty COCs/group were collected at 0 h and 9 h IVM, fixed and immunostained with ZFP36L2 antibody for protein detection, Hoechst 33342 for nuclei observation, and Alexa Fluor 647 Phalloidin for transzonal projections (TZP) visualization. Seven pools of 10 oocytes were analyzed for expression of ZFP36L2 and its target genes KDM4C, KDM5A, CCNE1, FBXO5 e FBXO43 by RT-qPCR. MII rates were analyzed by Chi-square test followed by Fischer's exact test. WB results were submitted to ANOVA followed by Tukey's test. Gene expression data were transformed by ΔΔCT and normalized by housekeeping genes ACTB e PPIA. Results considered 5% significance level. MII rates were different between PM-IVM (68.37b%) vs C-IVM (73.19a%) ($p=0.004$) and Ph-IVM (79,13a%) ($p<0.0001$). ZFP36L2 protein levels were similar among groups at 9 h IVM (C-IVM: 1048; Ph-IVM: 1059; PM-IVM: 1132; arbitrary units) and MII oocytes (C-IVM: 558; Ph-IVM: 674; PM-IVM: 677). PM-IVM was efficient to prevent GVBD and sustain TZP integrity for 9 h, visually similar to GV. No differences were observed for gene expression at 0 h (GV vs PM-IVM 0h). KDM4C was downregulated ($p=0.0078$) in oocytes from the PM-IVM compared to C-IVM (~43%) and Ph-IVM (~26%) at 9h IVM, and upregulated (1.5 fold) in MII oocytes from the Ph-IVM vs PM-IVM ($p=0.0137$). These data indicated that the IVM protocol did not alter ZFP36L2 mRNA and protein levels, but impacts KDM4C expression in oocytes.

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