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CHANGES IN CUMULUS CELL GENE EXPRESSION UNDER THE CONDITION OF EXPERIMENTAL IMMUNE-MEDIATED INFLAMMATION

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During folliculogenesis bi-directional communication between the human oocyte and cumulus cells (CCs) is essential for oocyte development. It is suggested that functions of CCs can indirectly reflect oocyte's competence [1, 2, 3]. It has been identified that expression levels of some genes in CCs can characterize the oocyte ability to undergo meiotic maturation, successful fertilization and early embryonic development [3, 4]. Among them are cyclooxygenase 2 (COX2), gremlin 1 (GREM1) and hyaluronan synthase 2 (HAS2), which play an important roles during oocyte development, ovulation and fertilization [5, 6, 7]. *It is known that presence of an inflammatory process in the body can cause ovarian dysfunction [8].* In our work we used two models of inflammatory process in mice to assess the changes in mRNA expression of COX2, GREM1 and HAS2 in ovarian CCs and oocyte meiotic maturation: 1) immune complex- mediated pathology induced by bovine serum albumin (BSA); 2) endotoxemia induced by endotoxin of gram-negative bacteria – lipopolysaccharide (LPS). In addition, we studied effect of pharmacological inhibition of nuclear enzyme poly (ADP-ribose) polymerase-1 (PARP-1) with 4-hydroxyquinazoline (4-HQN) on gene expression and oocyte developmental competence, as it was showed the role of PARP1 in the pathogenesis of various immune-mediated diseases [9].

Materials and methods. Female mice (18-20 g, outbred albino mice) were randomly divided into 5 groups: 1/ mice immunized with BSA 6 times every 7 days according to the scheme: 1) 150; 2) 175; 3) 200; 4) 225; 5) 250 and 6) 275 mg of BSA per kg of body weight; 2/ mice immunized with BSA and receiving 4-HQN (100 mg/kg) twice each week; 3/ mice injected with LPS (E. coli 0111 :B4) at dose of 3 mg/kg; 4/ mice treated with 4-HQN, one hour before LPS injection; 5/control animals received an equal volume of normal saline solution. 24 h after the treatments, the mice were euthanized under ether anesthesia and their ovaries were sampled. Cumulus-oocyte cellular complexes were extracted mechanically and were cultured at 37°C. The number of metaphase I and metaphase II oocytes was counted after 4 and 20 hours of culture, respectively. Reverse transcriptase polymerase chain reaction analysis was performed to detect the expression levels of interest genes in CCs.

Results. BSA immunization caused the predominant activation of the humoral link of the immune response to antigen administration; LPS treatment induced predominant activation of cells of non-specific resistance and proinflammatory cytokine release. Both experimental approaches resulted in similar ovary impairment characterized by reduced expression of COX2 ($p<0.01$), GREM1 ($p<0.01$) and HAS2 ($p<0.05$) genes in CCs, and failed oocyte meiotic maturation at metaphase I and II stages compared to that of control mice ($p<0.01$). The administration of PARP1 inhibitor 4-HQN to BCA- or LPS-treated mice significantly improved ovarian function. The mRNA expression of COX2, GREM1 and HAS2 in CCs was enhanced ($p<0.05$). The changes in gene expression were accompanied by improved oocyte meiotic maturation ($p<0.01$).

Prospects for further research. Our data suggest that PARP1 is involved in the pathogenesis of ovarian dysfunction caused by both BCA immunization and LPS *treatment*. We suppose that this enzyme can be an attractive target for the therapy of inflammatory disorders in ovary. However, the detailed mechanisms of beneficial action of PARP inhibitors in both immune complex- and endotoxin- mediated ovarian pathology require further investigation.

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