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EXPRESSION OF SELECTED AQUAPORIN IN THE OVARIAN FOLLICLES OF PIG *(Sus scrofa f. domestica)*

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SUMMARY

Rapid and mass transport of water through the protein-lipid membrane is possible through the presence of specialized channels known as aquaporins (AQPs). These proteins create a system for controlling water flow especially in ovaries, fallopian tubes, uterus, placenta and fetal membranes to maintain normal reproductive function, embryo implantation, fetal growth and development. The comprehensive hormonal control over the reproduction process of the female is performed by the hypothalamic-pituitary-ovarian-uterine axis. Pituitary hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and growth hormone (GH) affect the ovarian function during the estrous cycle and pregnancy.

The above study aimed to define the role of FSH, LH, PRL and GH on expression of aquaporins 1, 5 and 9 in porcine ovaries during estrous cycle days: 2-4, 10-12, 14-16 and 18-20 and pregnancy days: 14-16 and 30-32. The *in vivo* studies have been applied to determine the expression of gene and protein of AQP1, 5 and 9, whereas *in vitro* studies have been performed to 1) analyze the effect of FSH, LH, PRL and GH on the expression of gene and protein of AQP1, 5 and 9 in granulose and theca cells derived from medium (10-12 days of the estrous cycle) and large (16-18 days of the estrous cycle) ovarian follicles; 2) the impact of FSH, LH, PRL and GH on subcellular distribution of AQP1, 5 and 9 proteins in granulose and theca cells derived from the medium and large ovarian follicles; 3) the influence of FSH, LH, PRL and GH on the volume of granulose and theca cells using aquaporins inhibitor PMB; 4) the effect of FSH, LH, PRL and GH on the expression on analyzed AQPs in granulose and theca cells cocultures during above listed days of the estrous cycle.

The significant increase in expression of AQP1 and 5 protein was demonstrated at day 18-20 of the cycle. Immunoloclization of the AQP1 protein was found in ovarian endothelium of blood

vessels, whereas AQP5 and 9 in granulose cells. No differences were found in basic expression of the *Aqp1, 5* and *9* genes in ovarian follicles in the studied days of the cycle and pregnancy. In turn significant differences in expression of analyzed genes were observed in granulose and theca cells. There was a significantly higher expression of *Aqp1* gene in granulose and theca cells during 14-16 days and in *Aqp5* during 10-12 and 18-20 days of the estrous cycle. In turn, the expression of the *Aqp9* gene was significantly higher in the granulose cells on days 10-12 and 18-20 of the estrous cycle. The highest expression of AQP1 and AQP5 proteins in granulose and theca cells of medium and large follicles was determined in the perinuclear region of cytoplasm and endosomes as well as in cytoplasmic membranes of both control and stimulated by FSH, LH, PRL and GH cells. The hypotonic environment caused the significant increase in volume of granulose and theca cells of medium and large follicles in both control and stimulated by FSH, LH, PRL and GH cells when compare to cellular volume at the beginning of the experiment. Application of PMB inhibitor blocked water transport to analyzed cells. The expression of *Aqp1* mRNA in co-cultures of granulosa cells derived from medium size follicles was significantly higher when compare to stimulating effect of FSH and GH on large follicles. Moreover the expression of *Aqp1* mRNA in theca cells derived from medium size ovarian follicles was regulated by LH and GH, while in large follicles only by LH. The abundance in expression of *Aqp5* gene in cocultured granulose cells from large follicles was observed after incubation with LH, PRL and GH. The expression of *Aqp5* mRNA in theca cells from medium and large follicles was stimulated by LH, and by PRL in these cells derived from medium size follicles. The significant decrease in expression of *Aqp9* gene in response to FSH, LH and PRL was demonstrated in coculture of granulose cells from large follicles. The inhibition of *Aqp9* gene expression was observed also in coculture of theca cells from medium size follicles exposed to LH and GH. However the significantly higher level of this transcript was detected in theca cells cocultures from large follicles stimulated by LH.