

T. I. Kuzmina, S. I. Kovtun, E. S. Usenbekov, O. A. Epishko, V. N. Stefanova. Analysis of indicators of fertility of porcine oocytes that have finished growth phase *in vivo* aspirated from the follicles of different diameters

The selection of competent oocytes to completion of meiosis *in vitro*, fertilization or reconstructing (cloning, transgenesis) is the initial stage of cell reproductive technologies in animal husbandry. The development of effective methods of early prediction prospective potencies for extracorporeal maturation and fertilization of oocyte is the actual problem of rapidly developing embryo technologies. Numerous factors determined developmental competence of the oocytes. Brilliant cresyl blue (BCB) staining has been used for selection of oocytes from several mammalian species, including pigs (Ericsson S. et al, Theriogenology, 39(1): p.214, 1993). BCB determines the intracellular activity of glucose-6-phosphate dehydrogenase, which plays an important role in cell growth, as a key enzyme in the pentose phosphate cycle. The enzyme activity in the growing oocyte increases, opposite in the oocytes that have finished growth phase it decreases (Alm et al., 2005). BCB - diagnostics of the initial population of oocytes based on staining with vital dye brilliant cresyl blue have proposed as an effective indicator of completion of oocyte growth phase.

The aim of the present study was to evaluate the developmental competence of porcine oocytes that have finished growth phase (BCB⁺) *in vivo* depending on diameter (d) of follicles (d <3 mm, 3 –5 mm, <6 mm).

Before *in vitro* maturation compact cumulus oocyte complexes were incubated in BCB solution (13 µM) for 90 minutes. Treated oocytes were divided into BCB⁻ (colourless cytoplasm) and BCB⁺ (coloured cytoplasm). We have found that different diameter follicles contain both growing oocytes and oocytes that have finished growth phase *in vivo* (follicles d <3 mm – 71%; follicles d 3 - 5 mm – 86%; follicles d 6 – 8mm – 86%). Only BCB⁺ oocytes were used in the experiments. The medium used for oocyte maturation was NCSU 23 supplemented with 10% follicular fluid, 0.1 mg/ml cysteine, 10 IU/ml eCG and 10 IU/ml hCG. Follicular fluid was collected from follicles with 3 - 6 mm in diameter. Oocyte cumulus complexes were cultured in maturation medium with pieces of wall (600 – 900 µm length) from non atretic healthy follicles (d 3 – 6mm). After 20 – 22 h of culture, oocyte cumulus complexes and pieces of wall were washed and transferred into the same maturation medium but without hormonal supplements for another 20-22 h of culture. After *in vitro* maturation, oocytes were fertilized *in vitro* and embryos were cultured by standard protocols (Kuzmina et al., 2008). We have estimated oocyte maturation, quality of early embryos including status of chromatin (Tarkowsky, 1966). All chemicals used in this study were purchased from Sigma-Aldrich. Data were analyzed by Chi² – test.

Oocytes that have finished their growth phase of examined species have shown high potency to maturation in all groups of experiment (follicles d <3 mm – 78%; follicles d 3 –5mm – 79%; follicles d 6 – 8 mm– 85%). Level of oocyte with degenerative chromatin had not significant differences in all groups of experiments. We did not find significant differences between the level of cleavage and blastocyst

in all groups of experiments. Percentages of cleavage and blastocyst in the groups were: follicles d <3 mm– 43% (27/63) and 29% (18/63); follicles d 3 – 5 mm– 46% (45/98) and 35% (34/98); follicles d < 6 – 8 mm–48% (28/58) and 28% (16/58) (χ^2 test). Analysis of morphology and chromatin abnormalities in embryos has not shown significant differences between the groups of experiment.

Developmental competence of *Sus Scrofa Domesticus* oocytes that have finished growth phase *in vivo*, isolated from the follicles of various diameters (<3 mm, 3 – 5mm and 6 – 8mm) was analyzed. There were no significant differences in the level of cleavage and embryos on the blastocyst stage and their morphological characteristics. The findings suggest the equal potency to the maturation and fertilization of oocytes that have finished growth phase *in vivo*, independently of diameter of follicles.

Keywords: oocyte, follicle, embryos, *Sus Scrofa Domesticus*, BCB-test, *in vitro*