P. A. Trotskiy. Cryopreservation of oocyte-cumulus complexes of cows with different bioactive substances

Implementation of biotechnological process in livestock should be considered not only in terms of selection process intensification (obtain of embryos in vitro, their transplantation) and to a greater extent as development of effective methods of freezing and long storage of mammalian cells, including ova and embryos. Application of biotechnology in livestock breeding increases rate of genetic progress, preservation of gene pool of breeds via banks of sperm, embryos and cryobank of oocytes, obtaining and regulation of progeny of the desired sex, providing genetic evaluation of gametes and embryos, and it will enable to use genetic potential of animals after culling by age, replicate and create new genotypes with desired properties repeatedly.

Solution to this problem is to improve medium and conditions of gametes and embryos freezing. Although the overall development of cryopreservation method is through simplification of the equilibration and vitrification solutions which would be able to ensure the full development frozen-thawed gametes. Addition of biologically active substances to the solution for cryopreservation contributes to protection of gametes during freezing and thawing, and determination of the consistent patterns of these substances will improve procedures of frozen-thawed oocyte cultivation outside the body. So it is necessary to deepen the fundamental research on the mechanisms of formation of a mature ovum of cows obtained from frozen-thawed oocytes to obtain embryos.

The aim of the research is to conduct comparative analysis of different biologically active substances in equilibration and vitrification solutions at cryopreservation of oocyte-cumulus complexes of cows.

Material and methods of the research. The objects of experimental studies were oocyte-cumulus complexes of black-and-white cows. The oocytes with homogeneous fine-grained ooplasm, undamaged pellucid zone, thick or partially loosened cumulus were used for freezing.

The gametes of cows were treated by equilibration solution before freezing and then were transferred into vitrification solution. All the equilibration (10% glycerol + 20% propanediol) and vitrification (25% glycerol + 25% propanediol) solutions for cryopreservation of cows' oocyte-cumulus complexes were prepared in Dulbecco phosphatebuffered saline with addition of 20% fetal serum of cows, $1x10^{-4}$ M unithiol, $1x10^{-6}$ M acetylcholine and without addition of bioactive substances.

The research on adding some biologically active substances (fetal serum of cows – version A, unithiol – version B, acetylcholine – version C, without addition of biologically active substances – version D and not frozen cells of the control group (K)) in equilibration and vitrification solutions at freezing the oocyte-cumulus complexes of cows was carried out.

It was found by the results of experimental studies that introduction of the above-mentioned components into the equilibration solution for freezing cows' oocyte-cumulus complexes increased their cryoresistance, as evidenced by increasing indicator of maturation of frozen-thawed gametes outside the body to metaphase-2 of

meiosis after 27-hour cultivation by 5,4-23,0% and decreasing indicator of number of oocytes with chromosomal abnormalities by 2,9-15,3%.

The introduction of biologically active substances into the vitrification solution and subsequent cultivation during 27 hours after freezing and thawing cows' oocyte-cumulus complexes showed that the indicator of maturation of frozen-thawed gametes outside the body to metaphase-2 of meiosis increased by 3,9-16,4% and the indicator of number of oocytes with chromosomal abnormalities decreased by 2,9-8,4%.

Comparative analysis of the results of *in vitro* fertilization of cows' frozen-thawed ova which were frozen using fetal serum of cows (version A) and without it (version - B) has shown a positive effect of adding it to equilibration and vitrification medium at freezing gametes of cows; it led to increase of obtaining bovine embryos *in vitro* by 11,5%.

Thus, the analysis of experimental results showed different efficiency of using fetal serum of cows, unithiol, acetylcholine in the equilibration solution for freezing cows' oocyte-cumulus complexes. The advantage of using these biologically active substances in the vitrification solution for cryopreservation of cow's gametes by the indicator of maturation of frozen-thawed oocyte-cumulus complexes outside the body to metaphase-2 of meiosis wasn't established.

Conclusions.

1. Introduction of fetal serum into cryopreservation solution increases cryoresistance of cow's oocytes to cooling leading to increasing the indicator of the matured frozen-thawed gametes outside the body to metaphase-2 of meiosis by 23,0% and the obtained bovine embryos *in vitro* by 11,5%.

Keywords: **cryopreservation, oocyte-cumulus complexes, cryoprotector, vitrification solution, *in vitro* maturation, embryos**