S. I. Kovtun, N. P. Galagan, O. V. Shcherbak. Evaluation of biological activity of nanobionaterials

The main role in modern technology of long-term preservation of livestock gene pool is not only in conditions of low temperature conservation of reproductive cells and embryos, but also in composition of biomedia which are able to preserve their maximum integrity during this process. That is why cryomedia have been permanently improved in order to provide maximum vitality of cells after deconservation. Previously it was found that admixture of slight amount of high disperse (nanosized) silica (UFS) to the standard LGY-cryomedium for bull sperm freezing result in the increase of gametes survival after deconservation. As for UFS, it is widely used in preparation of drugs as a supporting substance, because in certain concentration limits it is physiologically non-harmful and compatible with biological systems. Such SiO_2 has the developed surface, covered by hydroxyl groups, which demonstrates high adsorption activity with respect to a lot of substances. Replacement of hydroxyls by synthetic or natural compounds makes it possible to synthesize on this base immobilized biologically active preparations with prolonged and adsorption action. Thus, immobilization of some carbohydrates on UFS surface allowed us to obtain nanobiomaterials (NBM) which, being admixed to some cryomedia, provided higher survival of gametes after their defrosting in comparison with initial SiO_2.

The aim of present work was obtaining NBM, based on UFS, bovine serum albumin (BSA) and N-acelylneuraminic acid (N-ANA) and also examination of its biological activity using ejaculated bovine gametes of Holstein bulls (Strolh 379536/678, Tom 379545/345 and Tryplle 244), which are kept more than 29 years in the Bank of Animal Genetic Resources of Institute of Animal Breeding and Genetics nd. a. M.V.Zubets of NAAS.

NBM UFC/N-ANA was obtained by impregnation of UFS, surface of which was preliminary heated during 2 hours at 200^{o} C. NBM UFS/BSA and UFS/BSA/N-AHK were obtained by non-covalent adsorption of biomolecules. They were added to bovine gametes on the stage of their deconservation in concentration 0,001 %. Effect of NBM on spermatozoa was estimated in percents using the index of vitality according to activity of their movement.

It was found out that after defrosting of bovine spermatozoa they demonstrated average activity of about 50,0 ± 5,77%. The same index of gametes activity in the control (without NBM admixture) lowered during 30 minutes only 3,3%, and reached 46,7 ± 6,01%. In experimental groups after 30 minutes the most active ones were gametes, which were in contact with UFS/BSA/N-ANA (56,7 ± 8,82 %). Gametes mixed with UFS demonstrated the lowest activity. In comparison with the control it decreased by 10 % and by 20 %, in comparison with UFS/BSA/N-ANA. Thus, admixture of UFS in concentration 0,001 % to deconservated bovine spermatozoa, stored in frozen state for considerable time, is inappropriate.

In presence of NBM UFS/BSA, unlike to UFS/BSA/N-ANA, the mobility of gametes decreased only by 1,7 %. At the same time, in presence of NBM without

protein – UFS/N-ANA, the decrease of mobility by 11,7 % was observed. It testifies in favour of possible stabilization of mobile cells number in presence of protein in NBM. But at low concentrations of nanoparticles in the media, containing cells, the probability of their contact with cell surface is insignificant. So, it may be assumed that this effect is observed due to interaction of NBM with components of semen plasma and cryomedium and this may result in redistribution of forms of water.

After 60 minutes of experiment, the most active were gametes in compositions with UFS/N-ANA (48,3 ± 4,41 %) and UFS/BSA/N-ANA (51,7 ± 8,82 %). In the control during this period the lower mobility was observed (41,7 ± 7,26 %) in comparison with upper mentioned samples and higher mobility by 13,4 % and 1,7 % in comparison with BSA and UFS/BSA. After 1,5 hours of the experiment both in control and experimental samples the gradual decrease of mobility was observed.

Summarizing the estimation of biological activity of NBM, the most promising was UFS/BSA and UFS/BSA/N-ANA. The first NBM provided for initial increase of spermatozoa mobility up to level 55,0 ± 5,77 %, whereas UFS/BSA/N-ANA, as it was shown previously, – up to 56,7 ± 8,82 %. Difference between them was not practically observed, but special role of protein was noted as a surface active substance. But mechanisms of activity of each NBM seem to be different. As for N-ANA in NBM, according to its functional properties it is able to provide for increase of chemical affinity of nanomaterials to certain components of semen or corresponding cell receptors, in contrast to protein.

Thus, we have proved the possibility to increase the level of mobility of deconservated bovine spermatozoa, previously stored for a long period in liquid nitrogen, caused by addition of NBM based on UFS and upper mentioned biomolecules, which result is particularly important further, on the initial stages of egg fertilization.

Keywords: **bulls, nanobiomaterials, ultrafine silica, ejaculated spermatozoa, preservation of the gene pool, cryopreservation**