Evaluation of biological activity of ultrafine silica based nanomaterials in the system of biotechnology research

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The main role in modern technology of long term preservation of livestock gene pool is played not only by conditions of low temperature conservation of reproductive cells and embryos, but also by composition of biomedia which are able to preserve their maximum integrity during this process. That is why cryomedia are permanently improved in order to provide maximum vitality of cells after deconservation. Previously [1] it was found out that admixture of slight amount of high disperse (nanosized) silica (UFS) to the standard LGY-cryomedia 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 [2]. Such SiO_2 has developed surface, covered by hydroxyl groups, which demonstrates high adsorption activity with respect to number of substances. Displacement of hydroxyls by synthetic or natural compounds makes it possible to synthesize on this base immobilized biologically active preparations with prolonged and adsorption action [3]. Thus, immobilization of some carbohydrates on UFS surface allowed us to obtain nanobiocomposites (NBC) which, being admixed to some cryomedia, provided for higher survival of gametes in comparison with initial SiO_2 after their defrosting [4, 5]. The aim of present work was obtaining NBC, based on UFS, protein 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 gene resources of Institute of Animal Breeding and Genetics nd. a. M.V.Zubets of NAAS.

NBC UFC/N-ANA was obtained by impregnation of UFS, surface of which was preliminary heated during 2 hours at 200° C. NBC 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 NBC 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 50,05,77 %. The same index of gamete activity in the control (without NBC admixture) lowered during 30 min only 3,3 %, and reached 46,76,01 %. In experimental groups after 30 min. the most active were gametes, which were in contact with UFS/BSA/N-ANA (56,78,82 %). The lowest activity demonstrated gametes mixed with UFS. 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 NBC UFS/BSA, unlike to UFS/BSA/N-ANA, the mobility of gametes decreased only by 1,7 %. At the same time, in presence of NBC without protein, UFS/N-ANA the decrease of mobility by 11,7 % was observed. It testifies in favor of possible stabilization of mobile cells number in presence of protein in NBC. 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 NBC with components of semen plasma and cryomedium and this may result in redistribution forms of water [6].

After 60 minutes of experiment, the most active were gametes in compositions with UFS/N-ANA (48,34,41 %) and UFS/BSA/N-ANA (51,78,82 %). In the control during this period the lower mobility was observed (41,77,26 %) in comparison with upper samples and higher mobility by 13,4 % and 1,7 % in comparison with BSA and UFS/BSA. After 1,5 hours of experiment both in control and experimental samples the gradual decrease of mobility was observed.

Summarizing the estimation of biological activity of NBC, the most promising was UFS/BSA and UFS/BSA/N-ANA. The first NBC provided for initial increase of spermatozoa mobility up to level 55,05,77 %, whereas UFS/BSA/N-ANA, as it was shown previously, - up to 56,78,82 %. 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 NBC seem to be different. As for N-ANA in NBC, 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 spermatozoids, previously stored for a long period in liquid nitrogen, caused by addition of NBC based on UFS and upper mentioned biomolecules, which result is particularly important further, on the initial stages of egg fertilization.

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