

ANALYSIS OF THE IMPACT OF NANOMATERIALS ON THE VIABILITY OF SPERM

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Abstract

Ultrafine silica (UFS) is widely used in the manufacture of drugs as an auxiliary substance, since it is physiologically harmless and compatible with the biological system within certain concentrations, and has a developed surface covered with hydroxyl groups that exhibit high adsorption properties for many substances. Replacing hydroxyls with synthetic or natural compounds enables to create on its basis immobilized biologically active nanomaterials of prolonged and adsorption action. Attached on the surface of the UFS of some carbohydrates or protein, it was possible to obtain nanomaterials which, when added to the standard cryomedium, contributed to an increase in the survival rate of the thawed sperm of bulls. The results of experimental studies on the interaction of ejaculated and cryopreserved sperm of bulls and boars with nanoparticles of ultrafine silica are a continuation of these studies related to technology which represent not only cryopreservation of gametes, but also thawing.

Nanomaterial of the brand A-300 with $S_{sp} = 285 \text{ m}^2/\text{g}$ (Kalush, Ukraine) was used in the research, which was pre-heated for 2 hours at 200°C and then added to the thawed spermatozoa of the bulls and boars at a final concentration of 0.001%. The analysis of the results showed a different effect of the nanomaterial used by us as an additive to the environment with thawed sperm. Thus, nanomaterial in 0.001% concentration slowed down the activity of thawed ejaculated sperm, which manifested itself in the elongation of the total survival time of gametes of bulls and boars to 6.0 hours.

The article reflects the prospect of further biotechnological research using nanomaterials of various origins in the system of preservation and rational use of genetic resources of farm animals.

Keywords: ejaculating of spermatozoa, bulls, boars, nanomaterials, ultrafine silica, cryopreservation

Introduction

Cryopreservation of sperm, oocytes and embryos is widely used in modern reproductive biotechnology of humans and animals and is especially relevant for solving the issue of preserving the gene pool of farm animals (Bagirov, 2009; Mikitenko, 2011; Sviridov, 2009). The decisive reception during cryopreservation of semen of the males, which is intended for further artificial insemination of females, is its dilution. Improvement of the sperm motility environment remains an urgent task today, since after the freezing / thawing process, the sperms lose their activity by 30-40% of the initial value, which affects their fertility.

Stallions' and boar's semen have a large number of secretions of the adrenal glands and are prone to oxidative reactions that are undesirable during long-term preservation. Constantly

searching for substances that would eliminate the negative effects of the freezing/thawing process (Eskin, 2007; Vierula, 1983).

It is known that ultrafine silica (UFS), which at certain concentrations is physiologically harmless and compatible with the biological system, is used as an auxiliary substance for the manufacture of a number of drugs (Chuiko, 2003). It has a developed surface-coated hydroxyl group that exhibits high adsorption properties for many substances. The replacement of hydroxyls with synthetic or natural compounds enables the production of immobilized biologically active nanomaterials of prolonged and adsorption action on its basis. The sputum on the surface of the UFS of some carbohydrates or a protein made it possible to obtain nanomaterials which, when added to the standard cryo-media, contributed to the increase in the survival rate of the thawed spermatozoa of the bulls (Galagan, 2006; Klymenko, 2008; Nedava, 1990). To preserve the biological integrity of the sperm after the cryopreservation, the defining conditions of their thawing are important. The method and rate of thawing of the sperm are decisive importance for maintaining its viability. It is shown that during thawing of cryopreserved semen, gametes are destroyed as a result of the process in what occurs during recrystallization, dehydration and hypertonic salts (Eskin, 2007). Therefore, now it became necessary to develop a method for the use of nanomaterials during the thawing of cryopreserved sperm males. Our research was aimed at studying the interaction of thawed ejaculated sperm of bulls and boars with nanoparticles of highly dispersed silica.

Materials and methods

The research was carried out at the Laboratory of Biotechnology at the Institute of Animal Breeding and Genetics named after M.V.Zubets National Academy of Agrarian Science of Ukraine. The semen of the bulls of the Holstein breed (Strohl 379536/678, Tom 379545/345 and Tripple 244) and the boars of the Mirgorod breed (Dnipro 641, Komish 853, Kokhanyy 289) were used. It is stored in the bank of genetic resources of animals of the Institute of Animal Breeding and Genetics named after M.V.Zubets of NAAS.

In the experiments, the effect of UFS brand A-300 with $S_{sp} = 285 \text{ m}^2/\text{g}$ (Kalush, Ukraine) was studied on thawed semen. The nanomaterial was preheated for 2 hours at 200°C ., and then added to the sperm cultivation medium at a final concentration of 0.001%. The effect of UFS on sperm boar and bovine sperm (individually and on average) was analyzed for their activity in percentages and the vitality index in hours.

For artificial insemination or for quality control sperm, it is thawed in isotonic 2.9% solution of sodium citrate. In our studies, an isotonic 2.9% solution of sodium citrate was supplemented with 0.001% UFS $t^\circ\text{C}200$.

Results

The analysis of the results showed different influence of nanomaterial used by us as an additive for optimization of the medium. Thus, nanomaterial UFS $t^\circ\text{C}200$ at a concentration of 0.001% slowed the decrease in the activity of the thawed ejaculated sperm of boars (Fig. 1, mean value). In one hour control, activity decreased by 5.9%, while spermatozoa with nanomaterial showed activity at 12.5%, which is 1.7% higher compared to control. A similar tendency was observed in the future. It should be noted that there was an extension of 1 hour of the total survival time of gametes boars that were from UFS $t^\circ\text{C}200$.

After adding UFS t°C200 at a concentration of 0.001% to the thawed sperm, the bulls observed somewhat different results. Thus, nanomaterial UFS t°C200 has sharply reduced the activity of the thawed ejaculated sperm counts (Fig. 1, mean value). In one hour control, activity decreased by 8.3%, while the spermatozoa that were with nanomaterial showed activity at the level of 28.3%, which is 13.4% lower than control. A similar trend was observed in subsequent time measurements. It should also be noted that the elongation of the total survival time of the gametes of the bulls, which were from UFS t°C200 for 1 hour, was noted.

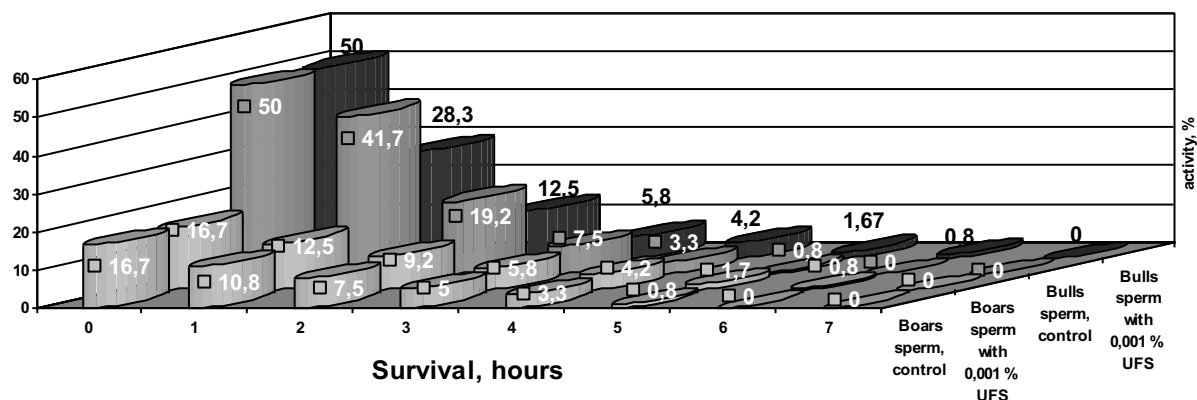


Figure 1. Effect of UFS t°C200 in the concentration of 0.001% on the viability of cryopreserved ejaculated sperm of bulls and boars

Summing up the obtained data, it was established that the addition of UFS t°C200 in the concentration of 0.001% to the thawed sperm of the bulls and boars did not contribute to the increase of gametes activity, at the same time, there was no negative effect on the total survival time, and, conversely, it's lengthening by 1 hour. It should be noted that the contribution of UFS t°C200 to the ejaculated sperm of the boars was somewhat lower than that of the bull's sperm.

Discussion

In our opinion, the biological activity of 0.001% of the concentration UFS t°C200 in relation to the thawed semen of the boars forms a series of "UFS-elements", which are formed as a result of recrystallization (residues of secretions of the sexual glands). Due to this kind of research, we observed no significant decrease in activity. In the case of biological activity in relation to the thawed sperm, the bulls do not rule out the special role of the silanol groups of the surface of the UFS in the manifestation of its bioactivity to the actual sperm. Thus, in the presence of UFS, the activity of spermatozoa decreased and the number of mobile cells diminished against the background of the absence of toxicity of this nanomaterial.

Conclusions

The analysis of the results showed different effects of the nanomaterial used by us as an additive to the environment with thawed sperm. Thus, nanomaterial in 0.001% concentration slowed the decrease in the activity of thawed ejaculated sperm, which was manifested in the elongation of the total survival time of the spermatozoa of bulls and boars up to 6.0 hours. We are presented with the prospect of further biotechnological research using nanomaterials of

various origins in the system of conservation and rational use of genetic resources of farm animals.

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