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VIABILITY OF SPERM CELLS OF BOARS AT THE ADDITION OF ULTRA FINE SILICA TO CRYOPRESERVATION AND DEFROSTING MEDIA

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Aim. In modern conditions the priority task of preserving biological diversity is increasing the role of agriculture in its maintenance. Within the system of long-term preservation of genetic resources of farm livestock, urgent improvement is needed in the field of media for dilution, cryopreservation and storage of genetic material of animals; technological elements of cryopreservation of genetic material of animals and biotechnological means of obtaining embryos from cryopreserved genetic material outside of the organism. **Methods.** It is known that the medium for boar sperm cryopreservation is added the following components: hydrophilic extract of oaken silkworm pupa, aqueous extract of propolis, bovine serum albumin, water-soluble components of yolk and lipoproteins, extract of crude sunflower oil, proline, trimethylglycine (betaine) and nanomaterials. **Results.** The results of experimental studies on the interaction between cryopreserved ejaculated sperm cells of boars with nanoparticles of ultra fine silica (UFS) and saccharose were presented. It is noteworthy that the studies are related both to the technology of gamete cryopreservation and its de-preservation. Nanomaterial A-300 with S_{spec}= 285 sq.m./g (Kalush, Ukraine) was used in the studies after previous heating for 2 h at 200°C and surface modification with the carbohydrate – saccharose. UFS/saccharose was added to the cultivation medium for defrosted sperm cells (2.9 % sodium citrate solution) as well as prior to freezing into lactose-yolk-glycerin cryomedium in the concentrations of 0.1; 0.01 and 0.001 %. The analysis of obtained results demonstrated different impact of nanomaterial, used by us as an additive to media. It was established that after defrosting, boar sperm cells with UFS/saccharose in 0.001 % concentration demonstrated the activity at the level of 14.2 % and the total time of their survival was 4.0 h. UFS/saccharose concentration of 0.001 %, applied during the cryopreservation of ejaculated boar sperm, ensured 25.0 % activity after defrosting with the total survival time of 5.5 h. **Conclusions.** The article reflects the promising nature of conducting further biotechnological studies with the application of nanomaterials of different origin in the system of preservation and rational use of genetic resources of farm livestock.

Keywords: ejaculated sperm cells, boar, nanomaterial, ultra fine silica, cryopreservation, gene fund preservation.

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INTRODUCTION

In modern conditions the priority task of preserving biological diversity is increasing the role of agriculture in its maintenance. Within the system of long-term preservation of genetic resources of farm livestock, urgent improvement is needed in the field of media for dilution, cryopreservation and storage of genetic material of animals; technological elements of cryopreservation of genetic material of animals and biotechnological means of obtaining embryos from cryopreserved genetic material outside of the organism [1].

The fertilizing capacity of cryopreserved sperm cells depends on numerous factors, in particular, the composition of diluents and technological processes of preparing ejaculates to freezing and defrosting of sperm doses. Diluent components mitigate the negative effect of cold shock and ensure the integrity of acrosome and plasmatic membranes of sperm cells. It is known that

the medium for bull sperm cryopreservation is added the following components: glutathione, soy lecithin instead of chicken egg yolk, complex of biologically active substances (oestrophanum, eosin, unitiol, glutathione, l-cysteine) [2]. The medium for boar sperm cryopreservation is added the following components: hydrophilic extract of oaken silkworm pupa [3, 4], aqueous extract of propolis [5], bovine serum albumin, water-soluble components of yolk and lipoproteins, extract of crude sunflower oil, proline, trimethylglycine (betaine) and nanomaterials [6].

According to approximate estimations, at present there are over 800 different products, made via nanotechnologies. In 2007 the global sale of nanomaterials was estimated at 147 billion US dollars, and in 2015 this index increased up to 3.1 trillion US dollars. Nanoproducts have already been used in energetics, chemical and construction industry, cosmetics production. The application of nanotechnologies and nanomaterials in food industry and environment protection is also a promising approach. Positive results have already been obtained from the application of nanotechnological preparations in modern agriculture and veterinary practice. The specialists of the US state program "National Nanotechnology Initiative", established in 2000, determine nanotechnology as "science, engineering and technology conducted at the nanoscale which is about 1 to 100 nanometers with the purpose of obtaining fundamental knowledge about the nature of phenomena and properties of different materials in the nanometer scale and of creating and using structures, devices and systems, which acquire new properties due to their nanosizes" [7].

After adopting the Law of Ukraine "On Ratification of the Convention on Biological Diversity", ratifying the Interlaken Convention in 2007 and the Global Plan of Action for Animal Genetic Resources, signing the Nagoya Protocol on Access to Genetic Resources, Ukraine started the work in optimizing and applying nanomaterials in the technology of preservation and rational use of genetic resources of domestic breeds of swine to improve the intensity of applying the reproductive potential of breeders using cryopreservation of ejaculated sperm cells.

To substantiate the most efficient directions in applying nanomaterials in animal breeding, it is reasonable to improve the methods of obtaining embryos *in vitro*, cryopreserving gametes and embryos with the use of nanomaterials. Due to this fact, the aspects of ultra fine silica (UFS) – one of modern and promising nanomaterials – is considered. It was demonstrated that the addition of UFS in some concentrations to the standard cryomedium may stimulate the viability of bull sperm cells. UFS is pyrogenous; its surface layer consists of a large number of hydroxylic groups and has a high sorption capability regarding different molecules. In biological media, silica nanoparticles demonstrate their ability to bind the cells via intermolecular interactions; in this case a cell is still alive, but it may lose its activity, and, as a result, have deceleration of metabolic processes.

UFS is widely used as a supplementary treatment means in medical practice. The modification of its surface with some biomolecules, for instance, mono- and oligosaccharides, which promote better motility and survival of sperm cells of bulls, rams and humans, when added to the cryomedium, allowed creating promising nanomaterials on their basis [8–11].

It was demonstrated that in case of adding 0.001 % concentration of UFS/saccharose to lactose-yolk-glycerin cryomedium, the de-preserved epididymal sperm cells of boars had 10.0 % higher activity compared to the control (cryopreservation without the addition of nanomaterials, the activity of sperm cells after defrosting of 10.0 %) with the preservation of this level for 2 h. It should be noted that prior to freezing the activity of these gametes was at the level of 50 % [12].

Thus, our studies were aimed at estimating the biological activity of nanomaterials (on the basis of UFS, whose surface had previously been modified with carbohydrate – saccharose) under conditions of adding it to boar sperm cells after defrosting.

MATERIALS AND METHODS

The study was conducted in the Laboratory for Reproduction Biotechnology of the Institute of Animal Breeding and Genetics n.a. M. V. Zubets, NAAS.

The experiments studied the impact of nanomaterial UFS/saccharose on the viability of boar sperm cells of Myrhorod breed (Dnipro 641, Komysh 853, Kokhany 289), preserved in the bank of genetic resources of the animals at the Institute of Animal Breeding and Gene-tics n.a. M.V. Zubets, NAAS. Nanomaterial UFS/saccharose is a ultra fine silica (UFS), chemical formula SiO_2 of brand A-300 with S_{spec} = 285 sq.m./g (Kalush, Ukraine), whose surface was modified with saccharose at the O.O. Chuiko Institute of Surface Chemistry, NAAS of Ukraine. UFS/saccharose was added to the cultivation medium for defrosted sperm cells (2.9 % sodium citrate solution) as well as prior to

freezing into lactose-yolk-glycerin cryomedium in the concentrations of 0.1; 0.01 and 0.001 %. The impact of UFS/saccharose on the viability of boar sperm cells (each one separately and using the average indices) was analyzed in terms of their activity in per cent and the survival index in hours of cultivation in the thermostate at +37 °C.

Six ejaculates were used to conduct the studies and transported at 7 °C to the laboratory, where the main qualitative indices of sperm were determined. The duration of transporting ejaculates to the laboratory did not exceed six hours.

The estimation of the study results for quantitative and qualitative indices was conducted via the analysis of tables and charts. The obtained results of studies were processed using descriptive statistics method based on the estimated arithmetic mean (M), deviation from the indices of arithmetic mean error (m) (software package ×7, version 2.0.0.9).

RESULTS AND DISCUSSION

It was established that after defrosting sperm cells demonstrated the average activity at the level of 16.7 ± ± 3.33 %. This index in the control decreased only by 1.7 % within 30 min (15.0 ± 2.89 %). After sperm cells stayed in the medium, containing UFS/saccharose in the concentrations of 0.1; 0.01 and 0.001 %, for 30 min, there was a decrease in the activity of gametes by 7.5; 5.0 and 2.5 % compared to the initial activity (Fig. 1). It is noteworthy that after 1.5 h since the start of the study the activity of sperm cells in the control was 9.2 %, and similar activity was noted in the experimental groups within this time period, namely, 10.0 % for 0.001 % concentration of UFS/saccharose; 9.1 and 6.7 % for 0.01 and 0.1 % concentration of UFS/saccharose. The total survival time of sperm cells in the control was five hours, and in the experimental group with 0.001 % concentration of UFS/saccharose this index was 30 min higher.

It was demonstrated that the addition of UFS/saccharose in the concentration of 0.001 % to the medium (2.9 % sodium citrate solution) had positive impact on the viability of defrosted sperm cells, which is manifested with slow decrease in the activity and prolonged total survival period of gametes. Taking this fact into consideration, we estimated the biological activity of UFS/saccharose during cryopreservation of boar sperm cells. UFS/saccharose was added to lactose-yolk-glycerine cryomedium in three concentrations directly prior to freezing sperm cells.

It was established that sperm cells, frozen with UFS/saccharose in the concentration of 0.001 %, demonstrated the highest activity on average after defrosting, which was 25.0 ± 1.44 % (Dnipro 641 – 25.0 %; Komysh 853 – 27.5 %; Kokhany 289 – 22.5 %, respectively) which was 5.0 % higher compared to 0.01 % concentration and 7.5 % higher compared to 0.1 % concentration of UFS/saccharose (Fig. 2).

After 30 min since the start of the experiment, there was a decrease in the activity of gametes in all the groups, for instance, this index (without adding UFS/saccharose) decreased only by 1.7 % in the control (15.0 ± 2.89 %; Dnipro 641 – 20.0 %; Komysh 853 – 15.0 %; Kokhany 289 – 10.0 %, respectively) in 0.001 % concentration – by 2.5 % and by 5.8 and 5.0 % in the concentrations of 0.01 and 0.1 %. Within the following 30 min (one hour since the start of the experiment), the activity in some groups was almost at the same level, as this index in the control was 10.8 ± ± 3.82 % (Dnipro 641 – 15.0 %; Komysh 853 – 10.0 %; Kokhany 289 – 7.5 %, respectively), in the concentration 0.01 % 10.8 ± 2.21 % (Dnipro 641 – 12.5 %; Komysh 853 – 10.0 %; Kokhany 289 – 10.0 %, respectively), in 0.1 % concentration 10.0 % (Dnipro 641 – 12.5 %; Komysh 853 – 10.0 %; Kokhany 289 – 7.5 %, respectively). In case of freezing sperm cells with UFS/saccharose in 0.001 % concentration, the activity within this time period was 19.2 % (Dnipro 641 – 17.5 %; Komysh 853 – 22.5 %; Kokhany 289 – 17.5 %, respectively).

The total period of survival for sperm cells in the control and in case of freezing sperm cells with 0.1 % concentration of UFS/saccharose was 3.2 h (Dnipro 641 – 4.0; Komysh 853 – 3.0; Kokhany 289 – 2.5 h, respectively,), and in experimental groups where sperm cells were frozen with 0.001 % concentration, this index did not exceed 4.8 h (Dnipro 641 – 5.5; Komysh 853 – 4.5; Kokhany 289 – 4.5 h, respectively), with 0.01 % concentration the survival was 3.7 h (Dnipro 641 – 4.5; Komysh 853 – 3.5; Kokhany 289 – 3.0 h, respectively).

It should be noted that the cryocollection of ejaculated sperm cells of Myrhorod breed boars was first created in Ukraine (750 doses). The efficient application of the mentioned and new cryocollections will ensure the implementation of a complex of measures at the state level regarding the functioning of "virtual gene fund cryo-animal stocks" which is economically more profitable compared to keeping stocks of farm animals.

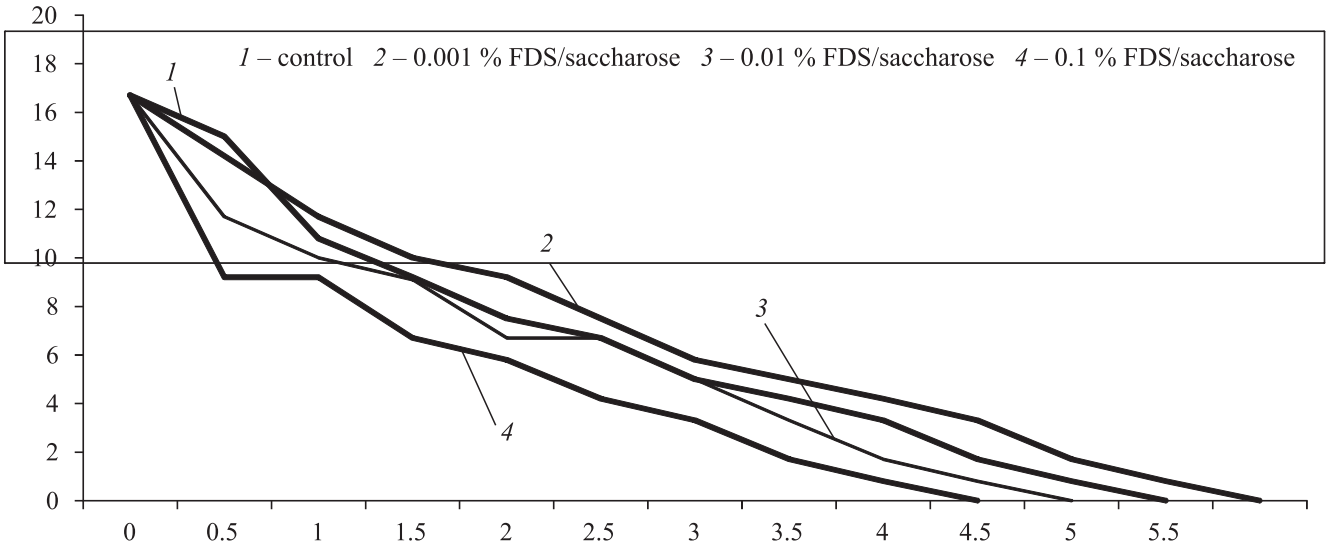

Fig. 1. The impact of UFS/saccharose on the viability of defrosted ejaculated sperm cells of boars

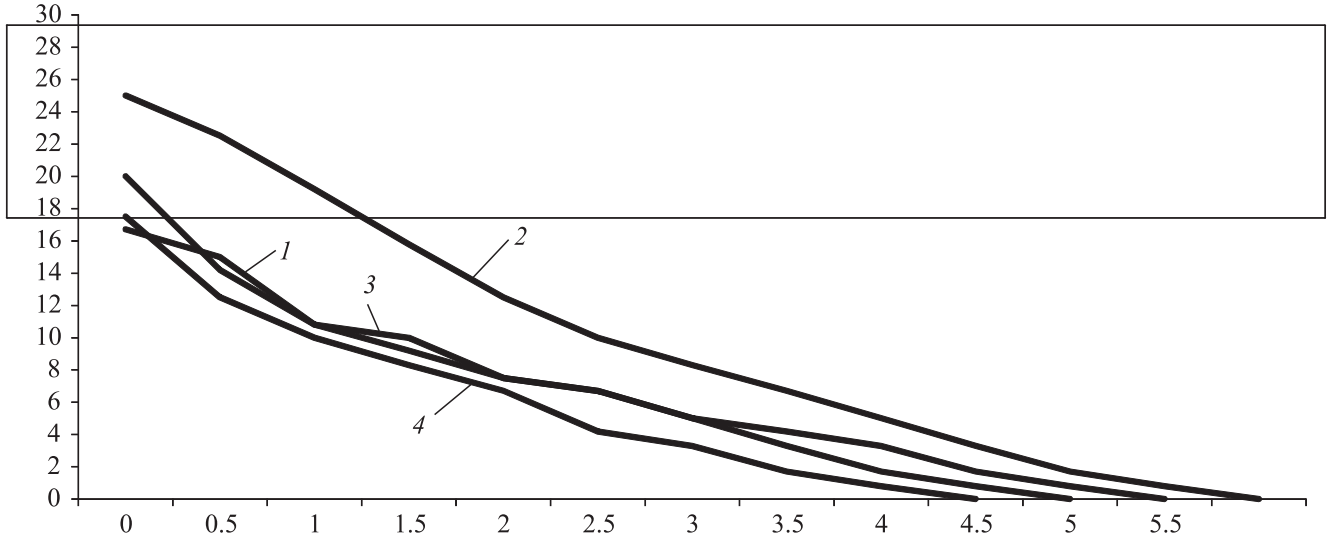

Fig. 2. The viability of cryopreserved ejaculated sperm cells of boars at the addition of UFS/saccharose

On condition of using cryopreserved ejaculated sperm cells for fertilization of porcine oocytes, which have matured *in vitro*, the level of embryo development will ensure the additional use of genetic potential of animals and improvement of complex biotechnological methods for implementation of tasks of preserving the gene fund of farm animals in Ukraine.

CONCLUSIONS

It was established that after defrosting, boar sperm cells with UFS/saccharose in 0.001 % concentration demonstrated the activity at the level of 14.2 % and the total time of their survival was 4.0 h.

It was demonstrated that lactose-glycerin-yolk-medium may be effectively used in case of cryopreservation of ejaculated sperm cells of boars. The application of such sperm cells may ensure a sufficient level of the effective formation of embryos both *in vitro* and *in vivo*.

UFS/saccharose concentration of 0.001 %, applied during the cryopreservation of ejaculated boar sperm, ensured 25.0 % activity after defrosting with the total survival time of 5.5 h.

Therefore, during the cryopreservation of biological objects the threshold concentrations of nanomaterials in cryomedia should be noted in its final definition with the consideration of the activity of specific types of cells. It will allow enhancing the efficiency of biomaterial cryopreservation.

The technology of cryopreserving genetic resources of farm animals, including "virtual gene fund cryo-animal stocks", will be implemented in close cooperation with the breeding farm, keeping domestic breeds of swine (State enterprise "Experimental farm named after Decemberists at the Institute of Swine Production and Agroindustrial Production, NAAS of Ukraine"). This will ensure efficient implementation of tasks in terms of preserving biodiversity and estimating agro-ecosystem balance, obtaining scientific research.

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Життєздатність сперматозоїдів кнурів за додавання високодисперсного кремнезему до складу середовищ для кріоконсервації та розморожування

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Ціль. В сучасних умовах пріоритетним завданням збереження біологічного різноманіття є підвищення ролі сільського господарства в підтримці біорізноманіття. В системі довготривалого збереження генетичних ресурсів сільськогосподарських тварин наразі потребують удосконалення середовища для розрідження, кріоконсервації та зберігання генетичного матеріалу тварин; технологічні елементи кріоконсервації генетичного матеріалу тварин та біотехнологічні прийоми отримання поза організмом ембріонів із кріоконсервованого генетичного матеріалу. **Методи.** Відомо, що до середовища для кріоконсервації сперми кнурів додають: гідрофільний екстракт лялечок дубового шовкопряда, водний екстракт прополісу, альбумін сироватки крові великої рогатої худоби, водорозчинні компоненти жовтка та ліпопротеїди, екстракт нерафінованої соняшникової олії, пролін, триметилгліцин (бетаїн), а також наноматеріали. Представлено результати експериментальних досліджень щодо взаємодії кріоконсервованих еякульованих сперматозоїдів кнурів з наночастинками високодисперсного кремнезему (ВДК) та сахарози. Слід зазначити, що дослідження стосуються не тільки технології кріоконсервації гамет, але й деконсервації. В дослідженнях використано наноматеріал марки А-300 із $S_{пит}$ = 285 м^2/г (м. Калуш, Україна), який попередньо прожарювали 2 год за температури 200 °С, поверхню якого модифікували вуглеводом – сахароза. ВДК/сахарозу додавали в середовище культивування розморожених сперматозоїдів (2,9%-вий розчин цитрату натрію), а також перед заморожуванням у лактозо-жовтково-гліцеринове кріосередовище у концентраціях 0,1; 0,01 та 0,001%. **Результати.** Аналіз отриманих результатів показав різний вплив використаного нами наноматеріалу в якості добавки до середовищ. Встановлено, що сперматозоїди кнурів з ВДК/сахароза у 0,001%-й концентрації після розморожування проявили активність на рівні 14,2 %, а загальний час їх виживаності становив 4,0 год. Застосована 0,001%-ова концентрація ВДК/сахарози під час кріоконсервації еякульованих сперматозоїдів кнурів забезпечила 25,0%-ову активність після розморожування із загальним часом виживаності 5,5 годин. **Висновки.** В статті відображена перспективність проведення подальших біотехнологічних досліджень з використанням наноматеріалів різного походження у системі збереження та раціонального використання генетичних ресурсів сільськогосподарських тварин.

Ключові слова: еякульовані сперматозоїди, кнур, наноматеріал, високодисперсний кремнезем, кріоконсервація, збереження генофонду.

Жизнеспособность сперматозоидов хряков при добавлении высокодисперсного кремнезема к составу сред для криоконсервации и размораживания

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Цель. В современных условиях приоритетной задачей сохранения биологического разнообразия является повышение роли сельского хозяйства в поддержке биоразнообразия. В системе длительного сохранения генетических ресурсов сельскохозяйственных животных необходимо усовершенствовать среды для разбавления, криоконсервации и хранения генетического материала животных; технологические элементы криоконсервации генетического материала животных и биотехнологические приемы получения вне организма эмбрионов из криоконсервированного генетического материала. **Методы.** Известно, что к среде для криоконсервации спермы хряков добавляют: гидрофильный экстракт куколок дубового шелкопряда, водный экстракт прополиса, альбумин сыворотки крови крупного рогатого скота, водорастворимые компоненты желтка и липопротеиды, экстракт нерафинированного подсолнечного масла, пролин, триметилглицин (бетаин), а также наноматериалы. В исследованиях использован наномате-

риал марки А-300 с Sудел = 285 м²/г (г. Калуш, Украина), который предварительно прожаривали два часа при температуре 200 °С, поверхность которого модифицировали углеводом – сахароза. ВДК/сахарозу добавляли в среду культивирования размороженных сперматозоидов (2,9%-ный раствор цитрата натрия), а также перед замораживанием в лактозо-желтково-глицериновую криосреду в концентрациях 0,1; 0,01 и 0,001%. **Результаты.** Представлены результаты экспериментальных исследований по взаимодействию криоконсервированных эякулированных сперматозоидов хряков с наночастицами высокодисперсного кремнезема (ВДК) и сахарозы. Следует отметить, что исследование касаются не только технологии криоконсервации гамет, но и деконсервации. Анализ полученных результатов показал различное влияние использованного нами наноматериала в качестве добавки к средам. Установлено, что сперматозоиды хряков с ВДК/сахароза в 0,001%-ной концентрации после размораживания проявили активность на уровне 14,2 %, а общее время их выживаемости составило 4,0 ч. Примененная 0,001%-ная концентрация ВДК/сахарозы при криоконсервации эякулированных сперматозоидов хряков обеспечила 25,0%-ную активность после размораживания с выживаемостью 5,5 ч. **Выводы.** В статье отображена перспективность проведения дальнейших биотехнологических исследований с использованием наноматериалов различного происхождения в системе сохранения и рационального использования генетических ресурсов сельскохозяйствен-ных животных.

Ключевые слова: эякулированные сперматозоиды, хряк, наноматериал, высокодисперсный кремнезем, криоконсервация, сохранения генофонда

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