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CRYOGENIC CHANGES OF LIPID DURING PRESERVATION OF SPERM ANIMAL FARM

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Abstract

The solution of the problems of cryopreservation of sperm of farm animals is determined largely by the intermolecular interactions of cellular components of reproductive cells. Therefore, the aim of the research was the study of the contribution of the lipid components in the manifestation of adaptive-compensatory changes of gametes of animals in hypothermic conditions. Experimental investigations were carried out using sperm of the boars of Large White breed, the roosters of Rhode Island Red breed and the bulls of Black and White breed. Isolation of plasmatic membranes of spermatozoa was conducted according to the method developed by Ivanov and Porfiry (1981) in our modification (Наук et. al, 1993). The obtained results proved that the phospholipids in the process of cryopreservation of sperm of farm animals are exposed to the greatest changes, while the cholesterol content is more stable. Also, the molar ratio phospholipids:cholesterol was changed in the direction of value "1" after cooling and freezing of sperm. Decrease of the ratio phospholipids:cholesterol is one of the mechanisms in the system of adaptive-compensatory reactions of spermatozoa at the influence of low temperatures. The study of the adaptation mechanisms at the cellular and molecular levels attracted the attention of an increasing number of researchers, because in this direction is possible prospects of solving the problems of cryogenic changes during preservation of sperm.

Keywords: *lipids, cryopreservation, sperm, cholesterol, plasmatic membranes.*

INTRODUCTION

The stabilization of intermolecular interactions of cellular components of reproductive cells may be the solution of the problems of cryopreservation of sperm of farm animals. An important role in the vital functions of gametes belongs to the plasmatic membrane. Due to the strictly coordinated work of membrane mechanisms is supported cellular homeostasis, is carried out the fine regulation of functional activity in response to the impact of environmental factors and changes within the cells (Наук, 1991).

In the functional gametes must be supported the specific liquid-crystalline status of lipid systems (Болдырев, 1990). It is important for the cell that the status of lipid phase is determined by strictly specific chemical composition of lipids and could easily be broken in the changing conditions of environment. Such damage causes a number of adaptive-compensatory changes in the structure of biological membranes. These changes are very important for maintaining the structural and functional activity of gametes.

The foregoing was the motive of the research the contribution of the lipid components in the manifestation of adaptive-compensatory changes of gametes of animals in hypothermic conditions.

MATERIALS AND METHODS

The experimental investigations were carried out using sperm of the boars of Large White breed, the roosters of Rhode Island Red breed and the bulls of Black and White breed. The animals were housed in vivarium conditions with observance of veterinary requirements. The isolation of plasmatic membranes of spermatozoa was conducted according to the method developed by Ivanov and Porfiry (1981) in our modification (Наук et. al, 1993). Cholesterol was determined by the method of Ilka (Покровский, 1969), at wavelength 665 nm using a spectrophotometer SF-46. Principle of the method is based on the degree of degradability of biocomplexes and extraction

from them of loosely bound cholesterol with organic solvents.

The study of phospholipids of spermatozoa were conducted by the method of Keith (Кейтс, 1975) with the use of plates 9x12 cm, which is applied the mixture of silicogel L and LSL firm Chemapol in the ratio 1:0,6. The extract of lipids was received according to the technique described above, then dried at a rotary evaporator and the sediment was dissolved in 0,5 ml of a mixture of chloroform:methanol (1:1). The received mixture of lipids used for thin-layer chromatography, and their separation was carried out in the system chloroform:methanol:water (65:25:4).

Statistical processing of data was performed using a Student's t-test.

RESULTS AND DISCUSSIONS

In stabilization of biological structures at the stages of low temperature preservation of great importance have the protein-lipid interactions. In this connection it is of interest to study the biochemical structure of plasmatic membrane to clarify the reasons of different stability of gametes at the effect of low temperatures (Table 1).

Table 1. Cryogenic changes of the ratio protein:lipid in plasmatic membranes of gametes of farm animals

Animal species	Ratio of protein:lipid of membranes		
	Native gametes	Cooled gametes	Thawed gametes
	$x \pm s_x$	$x \pm s_x$	$x \pm s_x$
Bull	0.43 ± 0.014	0.3 ± 0.11	0.25 ± 0.019*
Boar	0.17 ± 0.009	-	0.20 ± 0.004*
Rooster	0.40 ± 0.042	0.4 ± 0.08	0.52 ± 0.041*

*Statistically reliable cryogenic changes

As the table shows, the highest ratio of protein:lipid in plasmatic membranes noted in the gametes of a bull, which is well endure freezing of cells (Наук, 1991). In the membranes of the gametes of the boar, which are characterized by high sensitivity to cooling and freezing, it was discovered lowest ratio of these components. The value of this indicator in the membranes of gametes of the rooster occupies an intermediate position.

Apparently, the decrease of protein:lipid ratio during defrosting is more beneficial for maintaining the usefulness than its increase, since the sperm of the bull in the membranes which reduced this ratio, it is better kept at cryopreservation than the sperm of other species of animal (Борончук et al., 2008).

In connection with the original structure of cholesterol and its ability to regulate viscosity of membranes in the following studies were studied cryogenic changes during the process of preservation of sperm of the bull and a boar. The results of these studies are presented in Table 2.

Table 2. The content of loosely bound cholesterol in the process of cryopreservation of gametes of farm animals

Animal species	The amount of cholesterol (mg/1mlrd) in gametes after:		
	Dilution	Cooling	Thawing
	$x \pm s_x$	$x \pm s_x$	$x \pm s_x$
Bull	415.6 ± 10.9	379.9 ± 10.6*	342.0 ± 10.6*
Boar	482.0 ± 4.0	456.4 ± 10.4*	424.2 ± 11.4*

*Statistically reliable cryogenic changes

From data of Table 2 it follows that the number of loosely bound cholesterol is greatest in the gametes of the boars and least - for the bulls. Therefore, the biological membranes of gametes of the boar are distinguished by a reduced elasticity, which is consistent with studies of Nauc (1991). The cooling process significantly affects at the concentration of loosely bound cholesterol in gametes of investigated species of animals that should be considered when developing of new synthetic mediums and techniques.

By the fundamental research in the field of cryobiology quite definitely was proved that

lipids play an important and sometimes decisive role in a number of processes flowing in the cell in norm and in pathology contributing to the stabilization of its functional homeostasis (Schäfer, 2011). In this regard, were investigated the cryogenic changes of phospholipids and cholesterol in gametes of the bull and of the boar (Table 3).

Table 3. Phospholipids, cholesterol, and their ratio in the process of cryopreservation of sperm of the bull and a boar

The studied parameters in gametes of:			
Bull		Boar	
After dilution	After thawing	After dilution	After thawing
$x \pm s_x$	$x \pm s_x$	$x \pm s_x$	$x \pm s_x$
Phospholipids (mol %)			
3.8 ± 0.06	2.3 ± 0.10*	3.6 ± 0.09	2.8 ± 0.04*
Cholesterol (mol %)			
1.1 ± 0.04	0.9 ± 0.04*	1.3 ± 0.05	1.1 ± 0.03*
The molar ratio of phospholipids:cholesterol			
3.54	2.62	2.86	2.56

*Statistically reliable cryogenic changes

The presented data show that after cryopreservation the amount of phospholipids is 60.5%, and cholesterol 81.8% of native bull sperm, while in the sperm of the boar the studied characteristics, respectively amounted to 77.7 and 84.6%.

The table also shows that the number of the examined lipid in diluted material is almost on the same level. The cryopreservation process leads to decrease of the specified indicator to statistically significant value which may be due to: 1) involvement of lipids in energy metabolism, 2) increasing the activity of phospholipases, 3) increased of free radical process of lipid peroxidation.

Reducing the amount of cholesterol in the spermatozoa of the bull and of the boar after cryopreservation, apparently, can occur as a result of his decompaction in the lipid bilayer membranes or by loosening of lipid micelles in the structure of membranes (Богач et al., 1979).

At similar change is exposed and phospholipid-cholesterol ratio. The results obtained clearly show that the molar ratio of phospholipids:cholesterol in spermatozoa is changed in the direction of value "1" after cooling and freezing of sperm, namely in the direction of ratio which eliminates the phase transitions of lipids, or at least moving it to the area of lower temperature. It should be noted that the tempo of approximation to value "1" is the most high in the case of experimenting with the sperm of the bull. This is another proof of predominance of its cryoresistance in comparison with the sperm of the boar.

Given the fact that the maximal activity of phospholipases refers for the temperature range of the phase transition of lipids (Белоус et al., 1982), and also that he is the initiator of the main biochemical changes, can be assumed that the decrease of the ratio phospholipids:cholesterol is one of the mechanisms in the system of adaptive-compensatory reactions of spermatozoa at the influence of low temperatures. However, it is necessary to admit that along with the magnitude of ratio of phospholipids:cholesterol or content of these components separately, an important role is played their dynamics in the process of cryopreservation of sperm, as an important mechanism of adaptation of spermatozoa to low temperatures (Polyansky, 2010). The positive effect of this mechanism can occur only in the presence of exogenous lipids, such as lipids of seminal plasma, yolk of chicken eggs etc. and can also be caused by the processes of synthesis or resynthesis of endogenous substrates. Thus, the inclusion of the system of phospholipases aimed at changing of the ratio of phospholipids:cholesterol can be considered as own protective function of cell.

Many researchers tried to explain the complex formation of cholesterol-phospholipids by hydrogen bonds between the hydroxyl group of sterol and the oxygen atoms of phospholipids. However, the relationship of this type is not

probable, since, firstly, the ether groups of phospholipids should be hydrated, secondly, the inclusion of cholesterol in different phosphatidylcholine liposomes not change the NMR spectrums (Богач et al., 1979). The structure of the molecule of sterol is unique because of its cyclic part and side chain has great opportunities for the manifestation of intermolecular interactions, different reactions and transformations. These include the ability of hydrogen atoms being replaced by various radicals. Additionally, the presence of double bonds, few of hydroxyl groups, carbonyl and carboxyl groups in various combinations define a spatial configuration of cholesterol. Therefore is possible the formation of a very large number of individual compounds with various specific properties, including to enter into intermolecular interaction (Борончук et al., 2008). Consequently, cholesterol can be used in composition of cryoprotective mediums.

Thus, at the study of the influence of cooling on the status of various cellular components, the important point is the account of cryogenic changes of proteins and lipids, as these changes result in complex adaptive-compensatory processes in cryobiological systems and the possibility of full or partial rehabilitation of the object after cryopreservation.

CONCLUSIONS

The researches allow making the following conclusions:

1. In the process of cryopreservation of sperm of farm animals to the greatest changes are exposed phospholipids, while the cholesterol content is more stable.
2. Cryogenic changes of the content of phospholipids and cholesterol are most pronounced in the sperm of the bull, while in the sperm of the boar studied indicators are less pronounced.
3. The content of phospholipids in the native spermatozoa of the bull and of the boar is practically at the same level, while the amount of cholesterol is higher in the spermatozoa of the boar ($P<0.05$).
4. High amount of cholesterol in the spermatozoa of the boar plumping biological membranes, making it more fragile, which can reduce their cryoresistance compared with those of bull's spermatozoa.
5. Enhancing the effectiveness of cryopreservation of sperm of farm animals is possible by the introduction in the synthetic mediums of exogenous lipids.
6. Stabilization of adaptive-compensatory reactions can be implemented by changing of the structure of lipid components of cryoprotective mediums.

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REFERENCES

Polyansky A.A., Volynsky P.E., Arseniev A.S., Efremov R.G., 2010. Adaptation of a membrane-active peptide to heterogeneous environment. II. The role of mosaic nature of the membrane surface. J. Phys. Chem. Lett., 1, p. 3108-3111.

Schäfer L.V., de Jong D.H., Holt A., Rzepiela A.J., de Vries A.H., Poolman B., Kilian J.A., Marrink S.J., 2011. Lipid packing drives the segregation of transmembrane helices into disordered lipid domains in model membranes. Proc. Natl. Acad. Sci. U.S.A., 108, p. 1343-1348.

Белоус А.М., Бондаренко В.А., 1982. Структурные изменения биологическ ихмембранп риохлаждении. Киев: науковадумка, 256 р.

Богач П.Г., Финагин Л.К., Мирошниченко З.Н., 1979. Холестерин в плазматической мембране. Биохимия животных и человека. Киев: Наукова Думка, Вып. 3, р. 39-47.

Болдырев А.А., 1990. Введение в мембранологию. Москва: МГУ, 209 р.

Борончук Г.В., Балан И.В., 2008.Структурно-функциональные и биохимическиеизменения в биологически хсистемахприкриокон сервации. Chişinău: Tipografia AŞM, 633 p.

Кейтс М., 1975. Техникалипидологии. М.: Мир, 270 р.

Наук В.А., 1991. Структура и функцияспер миевсе льскохозяйственны хживотныхприкриокон сервации. Кишинев: Штиинца, 200 р.

Наук В.А., Борончук Г.В., 1993. Методика выделения плазматических мембран гамет сельскохозяйственных животных. Кишинев: Известия АН МССР, серия биол. и хим. наук., N 1., р. 50-53.

Покровский А.А., 1969. Биохимические методы исследования в клинике. М.: Медицина, р. 159-160.