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THE INFLUENCE OF LIPID FACTOR ON THE MANIFESTATION OF ADAPTATION-COMPENSATORY REACTIONS OF GAMETE PLASMA MEMBRANES OF FARM ANIMALS

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Abstract

By the methods of spectrophotometric, chromatographic and morphological studies established that as a result of influence of the temperature factor takes place adaptation-compensatory reactions in the gamete plasma membranes of farm animals. As a result of the adaptive-compensatory reactions in the plasma membranes of bull and boar gametes to the influence of temperature factor occur a shift of the phase transition of lipids to a lower temperature. In plasma membranes the amount of phospholipids predominates over cholesterol. During the stages of cryopreservation of cock semen there is a decrease in the amount of cerebrosides of 1, 5, 6, and 8 fraction, which compensates by increasing of quantity of cerebrosides third faction. In a similar direction changes the phospholipid-cholesterol ratio, which tends to unity, that is, in the direction of eliminating the phase transitions of lipids. The observed processes may be a consequence of a change in the activity of the phospholipase system, which is a gamete protective reaction in response to the action of extreme cryopreservation factors. The obtained results of the studies of fatty acid composition of plasma membranes testify to its change under the action of cryogenic factors. The accumulated material allows us to note the manifestation of specific reactions characteristic of individual fatty acids during freezing and thawing of biological objects. The change in the content of phospholipids and the saturation of fatty acids of plasma membranes testifies a complex adaptive-compensatory reactions aimed at preserving the viability of gametes in the new conditions of their stay.

Key words*: gametes, temperature factors, adaptation reactions.*

INTRODUCTION

Anthropogenic impact on the environment has become so significant that it has become impossible to cease it, it remains to mitigate this action or adapt to new conditions. In the formation of regulation of compensation mechanisms, in response to the action of extreme factors of cryopreservation on spermatozoa, lipids are assigned one of the leading roles. In this regard, the great interests are adaptive-compensatory reactions of plasma membranes under the action of temperature factor on them. Due to the strictly coordinated work of membrane mechanisms, cellular homeostasis is maintained, fine regulation of functional activity is performed in response to the influence of environmental factors and changes within the cell (Vie et al., 2000).

All cell membranes, including plasma membranes, are mobile, fluid formations that have common structural features: they are ensembles of lipid and protein molecules held together by weak non-covalent intermolecular interactions. Hydrophobic interactions of long nonpolar chains of fatty acids with the remains of hydrophobic amino acids lying on a surface of these proteins play a key role in stabilization of the immersed position of membrane proteins (Белоус et al.,1982). When changing individual parameters of the medium, and, above all, temperature, there may be a transition of system in a solid crystalline state or in a liquid state, devoid of a certain structure. This much-needed state of the lipid phase membranes for the cell due to a strictly defined chemical composition of lipids and can easily be disturbed at changing the environment conditions. Such disturbance causes a number of adaptive-compensatory changes in the composition of biological membranes. These changes are very important for preserving their structural and functional activity of gametes.

In this regard, the purpose of the research was to study the contribution of lipid component membranes in the implementation of adaptive-compensatory reactions.

MATERIALS AND METHODS

The experimental studies were carried out using the semen material of Black-Motley bulls, Large White boars and Rhode Island breeds cocks, which were kept in conditions meeting modern veterinary requirements. Determination of the content of phospholipids, cholesterol and cerebrosides was carried out by the described methods (Кейтс, 1975). The isolation of plasma membranes was carried out according to the method that was improved by us (Иванов et al., 1983). Statistical processing of digital material was carried out using the Student's t-test.

RESULTS AND DISCUSSIONS

Fundamental researches in the field of cryobiology have quite definitely proved that lipids play an important and sometimes decisive role in a number of processes taking place in the cell in normal and in pathology conditions contributing to the stabilization of its functional homeostasis. In this regard were investigated cryogenic changes of phosphorlipids and cholesterol of the bull and boar gametes (Table 1).

Table 1. Phospholipids, cholesterol and their ratio in the process of cryopreservation of bull and boar semen

Investigated indicators in gametes			
Bull		Boar	
After dilution	After thawing	After dilution	After thawing
M±m	M±m	M±m	M±m
Phospholipids (Mole %)			
3.8 ± 0.06	2.3 ± 0.10*	3.6 ± 0.09	2.8 ± 0.04*
Cholesterol (Mole %)			
1.1 ± 0.04	0.9 ± 0.04*	1.3 ± 0.05	1.1 ± 0.03*
Molar ratio of phospholipids: cholesterol			
3.54	2.62	2.86	2.56

*Cryogenic changes are statistically authentic

From the table it follows that the amount of studied lipids in the diluted material is practically at the same level. The process of cryopreservation leads to a decrease in this indicator by a statistically significant amount, which may be due to: 1) the involvement of lipids in energy metabolism; 2) increased activity of phospholipases; 3) the enhancement of the free radical process of lipid peroxidation.

The decrease in the amount of cholesterol in gametes of bull and boar after their cryopreservation can apparently occur as a result of its "decompaction" in lipid bilayer of membranes or due to destruction of lipid micelles in the membrane structure (Белоус et al., 1982).

The phospholipide-cholesterol ratio undergoes similar changes. The obtained results convincingly show that the molar ratio of phospholipids: cholesterol in gametes changes in the direction of one after cooling and freezing of the semen, that is, in the direction of the ratio eliminating the phase transitions of lipids or, at least, moving it to the zone of lower temperatures. It should be noted that the rate of approach to one is the highest in the case of experimentation with the bull semen. This is further evidence of the prevalence of his cryoresistance compared to boar seed.

Considering the fact that the maximum activity of phospholipases occurs in the temperature range of the lipid phase transition (Katkov, 2002), as well as the fact that it is the initiating link in the basic biochemical rearrangements, it can be assumed that a decrease in the ratio of phospholipids: cholesterol is one of the mechanisms in the system of adaptation-compensatory reactions of gametes to the action of low temperatures. At the same time, it is necessary to assumed that along with the value of the ratio of phospholipids: cholesterol or the content of these components, their dynamics in the process of cryopreservation of the seed, as an important mechanism for adapting gametes to low temperatures, also becomes important. Nevertheless, the change in this ratio cannot be a positive phenomenon if it is associated only with the loss of phospholipids - the most important functional and structural components of biological membranes, since the loss of phospholipids leads to a significant deterioration in the physiological, morphological and biochemical state of gametes (Наук et al., 1991). Consequently, the positive effect of this mechanism can be manifested only in the presence of exogenous lipids, for example,

seminal plasma lipids, egg yolk, etc., and can also be caused by the processes of synthesis or resynthesis of endogenous substrates. Thus, the inclusion of a system of phospholipases, aimed at changing the ratio of phospholipids: cholesterol can be considered as an intrinsic protective function of the cell.

Phospholipids and cholesterol are able to realize lipid-lipid interactions.

Many researchers have attempted to explain the formation of the cholesterol-phospholipid complex by hydrogen bonds between the hydroxyl group of the sterol and the oxygen atoms of the phospholipid phosphate. However, this type of bond is unlikely, because, firstly, the ester groups of phospholipids should be hydrated; secondly, the inclusion of cholesterol in various phosphatidylcholine liposomes did not change the NMR spectrum (Богачет al., 1979).

The cyclopentaneperhydrophenanthrene structure of the sterol molecule is unique, since its cyclic part and side chain have great opportunities for the manifestation of intermolecular interactions, various reactions and transformations, which include the ability of hydrogen atoms to be replaced by different radicals, the presence of double bonds, several hydroxyl groups, carbonyl carboxyl groups in various combinations, spatial configurations (authors). Therefore, it is possible to form a very large number of individual compounds with different specific properties, including intermolecular interactions.

Due to the fact that the bulk of lipids of biomembranes are phospholipids, which can be fractionated, in the next series of experiments have been studied the cryogenic changes of individual phospholipids in plasma membranes of the cock gametes (Table 2).

As a result of the studies, the following phospholipid fractions were extracted from the rooster's gamete membranes: sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, cardiolipin and phosphatidic acid.

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Table 2. Cryogenic changes of phospholipids of plasma membranes of cock gametes

Phospholipids	Content of phospholipids (%) in	
	native membranes	thawed membranes
	M±m	M±m
Sphingomyelin	6.7 ± 0.15	7.3 ± 0.18
Phosphatidylcholine	13.4 ± 0.31	19.4 ± 0.05*
Phosphatidylserine	9.6 ± 1.30	7.7 ± 0.20
Phosphatidylethanolamine	10.2 ± 0.18	20.6 ± 0.15*
Cardiolipin	12.1 ± 0.32	0
Phosphatidic acid	48.0 ± 0.35	46.0 ± 0.29*

*Cryogenic changes are statistically authentic

In the largest amount are presented phosphatidic acid and phosphatidylcholine. These data indicate a massive saturation of the investigated lipids and a high biosynthetic potential in the process of spermatogenesis. The minor component of the membranes of gametes of the cock is sphingomyelin, which is based on glinozemnylinosport sphingosine (Крепс, 1981).

Cooling, freezing and thawing of semen significantly reduced the ratio of individual phospholipid fractions. Significant specific weight in lipid losses with decreasing temperature is occupied by phosphatidylserine, cardiolipin and phosphatidic acid. The analysis of the content of phosphatidylcholine and phosphatidylethanolamine indicates an increase in the percentage of these fractions, which is inconsistent with the literature data (Наук et al., 1991) and the results of the studies presented in Table 1.We believe that in this case we are not talking about increasing the number of these phospholipids, but a their less pronounced decrease in comparison with other lipids, about the changes in the chromatographic mobility of phospholipids and the complex structural and biochemical rearrangements in the process of cryopreservation of the cock semen.

The observed changes in the content of phospholipids of plasma membranes of rooster gametes during the cryopreservation testify to the development of complex adaptive-compensatory reactions, which are aimed at preserving the viability of gametes in the new storage conditions.

The adaptive role of lipids concerns not only those that make up the matrix of biological membranes, but also many lipids that are not included in membrane structures.

The gametes would not have coped with the problem of compensating the membrane processes necessary to maintain functional activity, if there was no universal biochemical mechanism based on the adaptive ability of lipids.

At organisms that are acclimatized to low temperatures notes a high ability to rebuild their lipid metabolism in such a way that the content of polyunsaturated fatty acids sharply increases in their cell membranes, which reduce the temperature of the phase-structural transitions of lipids (Огурцов,2012), and this creates favorable conditions for maintaining their liquid crystalline state, which is of important functional importance, since it ensures the functioning of lipid-dependent membrane-bound enzymes. The deepening of research in this direction necessitates the study of cryogenic changes and peculiarities of lipid components, such as fatty acids at preservation of the semen of various species of farm animals. In this aspect, studies have been conducted to study the fatty acid composition of plasma membranes of bull and boar gametes (Table 3).

Table 3. Fatty acid composition of plasma membranes in the process of cryopreservation of bull and boar gametes

Percentage of fatty acids in gamete membranes of:			
Bull		Boar	
After dilution	After thawing	After dilution	After thawing
M±m	M±m	M±m	M±m
Saturated fatty acids			
69.9 ± 1.98	67.5 ± 2.87	64.1 ± 0.93	65.1 ± 2.77
Unsaturated fatty acids			
A. Mono-unsaturated			
16.1 ± 0.91	23.0 ± 2.23*	23.3 ± 0.82**	21.8 ± 1.31
B. Di-unsaturated			
14.0 ± 1.34	9.5 ± 1.02	12.6 ± 0.45	12.5 ± 1.07
Total unsaturated fatty acids			
30.1 ± 1.61	32.5 ± 2.45	35.9 ± 0.93**	34.3 ± 1.93
Ratio of saturated: unsaturated fatty acids			
2.32	2.08	1.78	1.61

*Cryogenic changes are statistically authentic
** Breeds particularity are statistically authentic

The analysis of the data presented in the table shows the species specificity of the fatty acid composition of plasma membranes of freshly diluted gametes of the studied animal species. Thus, in the membranes of boar gametes, the total amount of unsaturated fatty acids is higher than that of the bull ($P<0.05$).The analysis of the data presented in the table shows the species specificity of the fatty acid composition of plasma membranes of freshly diluted gametes of the studied animal species. Thus, in the membranes of boar gametes, the total amount of unsaturated fatty acids is higher than that of the bull ($P<0.05$). Their increased content is due to a high number of mono-unsaturated ($P<0.01$) in particular oleic acid ($P<0.001$).This indicates a lower ordered laying of hydrocarbon chains, an increase of the liquid crystal phase in the membranes of boar gametes, which in turn should positively affect the physicochemical properties of lipids at low temperatures.

The smaller amount of unsaturated fatty acids in the plasma membranes of the bull gametes, with a greater cryoresistance compared to those in the boar, can be explained by the peculiarity of the phospholipid spectrum of the plasmatic membrane of gametes of the animal species under study. There are phospholipids in which the same molecule has in its structure not only

fatty acids capable of oxidation, but also reaction groups that can serve as traps for radicals and breaking off the chain of free radical lipid oxidation (Наук et al.,1991).

In the plasma membranes of the bull gametes, a tendency is observed for high content of saturated fatty acids with an even number of hydrocarbon atoms and a longer chain, which indicates their higher melting temperature of lipids than in the boar gametes.

The ratio of saturated: unsaturated fatty acids of plasma membrane is slightly higher in the gametes of the bull in comparison with those in gametes of the boar, which is not observed in a similar analysis of the results of experiments with gametes of freshwater and marine fish, with different possessing of cryostability (Drokin et al., 1996). The results of the conducted researches of fatty acid composition of plasma membranes testify to its change under the influence of cryogenic factors.

Similar data were obtained by Bulgarian researchers (Иванов et al., 1983) in the study of fatty acid composition of plasma membranes of ram gametes. The accumulated material allows us to note the manifestation of specific reactions characteristic of individual fatty acids during freezing and thawing of biological objects.

Changes in the composition of biological membranes appear to represent their adaptive response to changes in the environment. According to our data, the formation of such a response at the membrane level in bull gametes is mainly due to changes in the content of mono-unsaturated fatty acids. On adaptation, as a way to maintain the necessary degree of liquid and permeability of membranes with a decrease in ambient temperature, we meet in early reports (Selivonchick et al., 1977). Similar changes in the cryopreservation of semen of boar semen are not observed. Apparently this can be explained by its lower cryoresistance.

Thus, cold adaptation is accompanied by a greater unsaturation of fatty acids. This pattern is clearly manifested, despite the fact that the lipid composition of membranes is more specific, characteristic for gametes of various animal species. But always, depending on environmental conditions, saturated acids can be replaced by unsaturated (or vice versa), often resulting to an extension of the chain of fatty acids along with their desaturation. This leads to an increase in the concentration of long chain unsaturated acids in the case of adaptation to environmental conditions. Under other conditions, these changes may develop in the opposite direction.

CONCLUSIONS

The researches allow making the following conclusions:

1. As a result of the action of the temperature factor, take place adaptive-compensatory reactions in the plasma membranes of farm animals gametes.
2. At adaptation-compensatory reactions in the plasma membranes of the bull and boar gametes, in response to the action of the temperature factor, takes place the displacement of the phase transition of lipids to a lower temperature zone.
3. In plasma membranes, the amount of phospholipids predominates over the content of cholesterol.
4. At the stages of cryopreservation of the rooster semen, there is a decrease in the content of cerebrosides 1, 5, 6 and 8^{th} fraction, which is compensated by an increase in the number of cerebrosides of the third fraction.
5. Change of activity of phospholipase system is a protective reaction of gametes in response to the action of extreme factors of cryopreservation.
6. The change in the content of phospholipids and saturation of fatty acids of plasma membranes testifies to complex adaptive compensatory reactions aimed at maintaining the viability of gametes in the new conditions of their stay.

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