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PHOSPHOLIPID SPECTRUM AT CRYOTECHNOLOGICAL STAGES OF GAMETES PROCESSING OF FARM ANIMALS

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Abstract

Due to the fact that the bulk of the lipid bilayer of biological membranes is phospholipids, the purpose of the researches, the results of which are presented in this paper, was to study the phospholipid composition and its cryogenic changes during preservation of bulls, rams and boars sperm. Ram gametes are characterized by a high content of phosphatidylcholine and phosphatidylethanolamine. The minor components of boar gametes are phosphatidylserine; ram - sphingomyelin and bull - phosphatidylserine and cardiolipin. By thin-layer chromatography it was found that phospholipids of gametes are represented by the following fractions: 1. Phosphatidylserine; 2. Phosphatidylcholine; 3. Sphingomyelin; 4. Phosphatidylethanolamine; 5. Cardiolipin. Of these, the largest amount is contained in the fractions of phosphatidylcholine and phosphatidylethanolamine, the content of the remaining fractions is slightly lower, namely phosphatidylserine, sphingomyelin and cardiolipin. With the passage of the technological stages from dilution to thawing of biological material, the phospholipids content gradually decreases.

Key words*: phospholipids, cryopreservation, gametes.*

INTRODUCTION

Phospholipids belong to the class of highly specific lipids, are components of cell membranes and organelles contribute to the metabolic process. Currently, new properties of phospholipids have been discovered, one of which contributes to an increase in the intensity of reproduction of animals involved in activation and acrosomal reactions, as if they were specially created by nature to stimulate the reproductive system of humans and animals (Skatkov, 2002). Diet fortified with phospholipids along with other biotechnological methods (Narijnii et al., 2001) such as hydrotherapy, the introduction of placental preparations, herbal preparations based on *Rhodiola rosea*, *Silybum marianum* and others (Vodeannikov et al., 1999; Djamaldinov, 2006), improves the activity of the reproductive system. For example, in the studies of (Narijnii et al., 2014) as an addition to the ration of boars were used the drug Moslecitin which contains essential phospholipids, which acted very effectively on sperm indicators and sows fertility when fed for 45 days and 90 days.

MATERIALS AND METHODS

The main experimental studies were carried out in the laboratory "Physiology and Reproductive Health" of the Institute of Physiology and Sanocreatology.

The object of the study was the semen material of bulls of the Black-Motley; rams of the Karakul, Tsigay and East Friesian; boars of Large White breeds. The experimental animals were kept under conditions corresponding to zoo-veterinary requirements.

The optimal composition and concentration of the components of cryoprotective mediums during freezing of the animal genome was determined by mixing isotonic solutions of the studied substances in an arithmetic or geometric series (Milovanov, 1962).

Seed was frozen in the form of granules with a volume of 0.1-0.2 ml on a fluoroplastic plate at a temperature of –110…–120°C. The material was thawed and carried out in a water bath or

using a constructed aerodynamic device using a dry and wet method.

As the base medium for cryopreservation of the bull, ram and boar sperm, respectively, was used the medium of (Kononov et al., 1975; Nagase et al., 1964; Watanabe et al., 1976; Kopeika, 1986). In the study of biochemical parameters from the composition of the medium the yolk is excluded.

Quantitative determination of phospholipids by phosphorus is based on the ability of ammonium molybdenum acid to form a phosphorus-molybdenum complex in an acidic medium with inorganic phosphate, which, after reduction, gives products colored blue in proportion to the phosphorus content.

Samples were evaporated on a rotary evaporator. The precipitate was washed with acetone and washed with chloroform. A mixture of lipids was applied to chromatographic plates. The separation of phospholipids was carried out in a system of chloroform: methanol: acetic acid: distilled water (65: 43: 1: 4). Staining was performed on crystalline iodine. Chromatograms were photographed and processed using an NF-4 densitometer (GDR).

RESULTS AND DISCUSSIONS

As we mentioned above, in the conditions of the laboratory of "Physiology and Reproductive Health", using the method of thin layer chromatography, the amount of cholesterol and five phospholipid fractions were identified and determined, of which: the phosphatidylcholine fraction. Phosphatidylcholine (PC) is one of the two most massive phospholipids of the brain membranes. The nitrogen-containing base, which carries a positive charge - choline - is connected by an ether bond with the radical of phosphoric acid, which ensures the balance of electric charges. In most cases, saturated fatty acids predominate in the 1st position of glycerol, and in the 2nd unsaturated. The hydrophobic apolar tails of phosphatidylcholine are the same as those of phosphatidic acid.

Phosphatidylethanolamine (PE) - in this phospholipid, the remainder of phosphoric acid is etherified with amino alcohol ethanolamine. At pH 7, it carries a small negative charge. Phosphatidylethanolamine fatty acids are usually less saturated than phosphatidylcholine. Phosphatidylethphnolamine is also one of the most massive brain phospholipids.

By analogy with phosphatidylcholine, mammalian phosphatidylethanolamine contains relatively high amounts of arachidonic and docosahexaenoic acids. However, the PE has a smaller “polar head” than the PC, and, in contrast to it, is able to form additional hydrogen bonds using the terminal amino group.

Phosphatidylserine (PS) is a diacyl glycerophospholipid containing the amino acid serine in an ether bond, through the OH- group of serine, with phosphoric acid. Although PS is well distributed in nature in animals, plants, and microorganisms, it usually accounts for less than 10% of the total amount of phospholipids. The highest content of PS is found in brain tissues, but in some cases in the plasma membrane and endoplasmic reticulum (ER) of the cells its amount can reach 20% of all phospholipids. By its chemical structure, PS is an anionic phospholipid with three ionized groups: phosphate, amino, and carboxyl. Like other acidic lipids, it exists in nature in salt form. He is the third most abundant phospholipid in the brain. This phospholipid carries a negative charge and is among the phospholipids. Its plasmalogenous form is found in the brain, but in very small quantities.

Cardiolipin (CL) - diphosphatidylglycerol (sometimes poly) consists of a phosphatidylglycerol molecule in which the 3rd OH- group of the second glycerol residue is esterified with the phosphate group of the phosphatidic acid molecule. Thus, cardiolipin consists of three glycerol molecules connected by two diester bridges. The two OH- groups of both outer glycerin molecules are esterified with fatty acids. The inner mitochondrial membranes are rich in cardiolipin. It was first isolated from the muscles of the heart, where mitochondria are very numerous. The content of cardiolipin in the brain is small, significantly lower than in the heart, but it is constantly detected.

In the brain and in other animal tissues, sphingophospholipids are represented by sphingomyelin (SM), which contains a choline group and 4-sphingenin (sphingosine). Since the sphingosine base with a fatty acid is called

ceramide, sphingomyelin can be referred to as ceramide-1-phosphorylcholine. Sphingomyelin is contained in fairly significant amounts (5-10% of total phospholipids) in the white matter of the brain, especially in myelin, but also in gray matter and other tissues. This is a common saturated lipid free of polyenoic acids. About 2/3 of the sphingomyelin fatty acids from the gray matter of the brain are represented by stearic acid, while in sphingomyelin from the white matter of the brain, the main fatty acids are lignoceric and nervonic. Due to the phosphorylcholine group, sphingomyelin is a neutral lipid (Kreps, 1981; Ipatova, 2005).

In the conditions of our laboratory, the following phospholipid fractions were identified from the bull, boar and ram spermatozoa using standard solutions of lecithin and cholesterol, specific staining reactions and original photographs of chromatograms: phosphatidylserine (PS), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanoamine (PE), cardiolipin (CL), as well as cholesterol (C). The PC fraction included cholin plasmalogen (CP) and ethanolamine plasmolagen (EP), the PS - phosphatidylinositol (PI) fraction, and the CL fraction - phosphatidic acid (Nauk, 1991).

In accordance with modern ideas about the mechanisms of cryodamage of biological objects, an important place is given to changes in the state and content of lipids in them. Therefore, subsequent studies were devoted to the study of these particular chemical compounds.

As a result of the performed experiments, it was found that in the gametes of all species of animals under study contain the greatest amount of phospholipid fractions, such as phosphatidylcholine, phosphatidylethanolamine and cholesterol (Table 1).

Table 1. The content of phospholipids at the technological processing of animals gametes, mg/100 g

Name of lipids	Elements of technological processing		
	Dilution	Refrigeration	Thawing
Bull			
Phosphatidylserine	221.9±9.9	189.4±8.3	159.9±5.4*
Sphingomyelin	238.1 ± 24.4	189.4 ± 20.2	140.7±17.9*
Phosphatidylcholine	1649.4± 40.6	1253.3± 64.0	979.4±78.5*
Phosphatidylethanolamine	607.1±11.3	541.1±16.7*	389.6±39.0*
Cardiolipin	216.5±17.9	173.2±16.6	140.7±18.0*
Cholesterol	415.6±10.9	379.9±10.6	342.0±10.6*
Ram			
Phosphatidylserine	199.7±23.5	151.5±13.8	119.9±11.6*
Sphingomyelin	132.6±12.9	107.3±11.6	88.4±8.0*
Phosphatidylcholine	1848.5±55.1	1685.6±27.6*	1602.3±24.2*
Phosphatidylethanolamine	630.1±22.8	559.3±11.6*	512.6±15.2*
Cardiolipin	246.2±16.2	202.1±12.7	157.8±15.2*
Cholesterol	426.0±8.0	409.1±12.8	364.9±12.9*
Boar			
Phosphatidylserine	94.7±13.6	66.3±9.5	52.1±6.9*
Sphingomyelin	356.1±16.3	307.8±11.2	274.6±9.5*
Phosphatidylcholine	1468.8±39.1	1344.8±24.4*	1226.3±24.1*
Phosphatidylethanolamine	639.2±14.5	562.5±11.9*	18.0±12.2*
Cardiolipin	217.8±13.9	151.5±10.1	113.6±10.1*
Cholesterol	482.0±4.0	456.4±10.4	424.2±11.4*

Note: *Statistically authentic differences compared with diluted semen.

In the quantitative content of individual phospholipids and cholesterol are observed specific features. Thus, boar gametes contain less phosphatidylserine and phosphatidylcholine with a higher content of sphingomyelin, phosphatidylethanolamine and cholesterol. Ram gametes are characterized by a high content of phosphatidylcholine and

phosphatidylethanolamine. The minor components of boar gametes are phosphatidylserine, bull - phosphatidylserine and cardiolipin, and ram - sphingomyelin.
In addition, as a result of the studies, it was shown that no significant species differences were found in the total phospholipid content of freshly diluted gametes of bulls, rams and boars.
Refrigeration and maintenance of diluted bull, ram and boar semen at 4°C leads to a significant decrease in the total number of gamete phospholipid fractions. These changes take place due to such fractions as phosphatidylcholine and phosphatidylethanolamine. This, in our opinion, indicates a greater vulnerability of these fractions, in the process of cryopreservation - deconservation, in comparison with other phospholipid fractions.
With further technological processing, a decrease is observed in the remaining fractions of phospholipids.
Therefore, during freezing and thawing of the bull, ram and boar sperm, changes in the content of phospholipid fractions, unlike protein fractions, are unidirectional character - their content decreases. At technological processing of farm animals sperm, the content of various fractions of phospholipids in gametes, regardless of the type of animal, decreases, which indicates the non-specific nature of the change in their content during cryopreservation.
Phospholipids are the main lipid component of cell membranes, they accompany fats in food and serve as a source of phosphoric acid necessary for human life. Phospholipids belong to the class of highly specific lipids and are components of cell membranes and organelles (mitochondria). They improve metabolic processes (Grishenko et al., 1994).
At the same time, new properties of phospholipids are currently discovered, one of which is that essential phospholipids contribute to the improvement and intensification of spermatogenesis (Grishenko et al., 1992). Of the essential phospholipids, the main one is phosphatidylcholine (lecithin). This phospholipid is involved in fertilization, namely in the activation of acrosomal reaction. In experiments with boars, the diet of which was enriched in phospholipids, it was demonstrated that phospholipids are a building material for cell membranes, cellular and subcellular structures. Phosphatidylcholine is involved in many biochemical and physiological processes, and has a direct effect on the functioning of cells, both somatic and reproductive (Gotsalka, 1969).
Skatkov (2002) found that poultry eggs and soybeans are the main sources of phospholipids. These sources of phospholipids, respectively, represent the components of the reproductive organs of animals and plants.

CONCLUSIONS

As a result of cryopreservation of farm animals sperm, the amount of phospholipid spectrum is gradually reduced, which obviously also occurs with other methods of preserving biological objects.
Fresh foods should contain more phospholipids since over time they can be subjected to peroxidation with the formation and accumulation of malondialdehyde, which has genotoxic, mutagenic, and carcinogenic effects.
The loss of phospholipids can be made up for by including them in the diet of humans and animals.
Animal feeding diets enriched with phospholipids affect the reproductive systems of both females and males.

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