Scientific Papers. Series D. Animal Science. Vol. LIX, 2016
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

IDENTIFICATION OF THE OPPORTUNITIES TO MAINTAIN THE REPRODUCTIVE HEALTH AS A RESULT OF THE SPERM LIPID SPECTRUM STUDY

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Abstract

Phospholipids as a component of the lipid spectrum represent the main part of structural integrity of sperm cell membranes. It is considered as natural fecundity factor which contribute to improving of spermatozoa cell membrane function by regenerative processes. Therefore, the purpose of the study was to examine the content of phospholipids, at various stages of a sperm cryopreservation. In results of the conducted researches is established that the content of phosphatidylcholine prevail above all fractions of phospholipids, which consistently decrease during cryopreservation. These changes are inherent to bull and boar spermatozoa. However, due to the fact that the content of phosphatidylcholine prevails over the other phospholipids, it is necessary to consider that this indicator can be the criterion of a fertility rate of spermatozoa which defines reproductive health of organisms relating to studied species.

Key words*: phospholipids, reproductive health, bull and boar spermatozoa, sanogenic status, cryopreservation.*

INTRODUCTION

By now has been accumulated a significant amount of information demonstrating that phosphatidylcholine and other phospholipids possess high biological activity relating to the maintenance of the integrity of the membrane systems of cells, the processes of differentiation, proliferation and regeneration of biological membranes. It is proved the participation of phospholipids in the regulation of metabolic processes through control of the membrane receptors and enzymes activities, both inside and outside the cell. Being a structural element of lipoproteins and also participating in the processes of blood coagulation, phospholipids determine hemodynamic and the cholesterol level in the blood (Quin P.J. et al., 1980).

A large variety of biological activities of phospholipids can be explained by feature of their chemical structure, which is characterized by: the presence of charged groups in the hydrophilic part of the molecule, the presence of biologically available phosphorus, polyunsaturated fatty acids and choline − the active component of phosphatidylcholine (Мэдди Э., 1979).

Thanks to its indispensability as a building material of biological membranes, cellular and subcellular structures, phosphatidylcholine takes an active part in a huge number of physiological and biochemical processes at all levels of biological objects organization. It has the most direct influence on the normal functioning of both somatic and reproductive cells. Formation of a sufficient number of reproductive cells contributes a significant, but not the major role to the maintenance of reproductive health. The significant influence has their qualitative indicators, such as: morphological features, mobility, life expectancy and the ability to fertilize. All of these properties are formed in a long process of the reproductive cells maturation. During this period occur certain sequences of changes of qualitative and quantitative composition of external and internal layers of the lipidic membrane of cells.

In many experimental researches is shown the positive impact of essential phospholipids on the toxic, allergic, metabolic and other damage of cell membranes. In this case takes place

inclusion of exogenous phospholipids in the damaged membrane, which is decisive in restoring of its morphology and functions (Quin P.J. et al., 1980). Regenerative effect of essential phospholipids refers to any types of cells. Phosphatidylcholine eliminates damage of cell membranes with the restoration of their functions and in the spermatozoa too. So, phosphatidylcholine showed the considerable activity in the protection and restoration of reproductive cells after their destruction under cold shock. In connection with this, phosphatidylcholine can be considered promising cryoprotector in the composition of cryopreservation medium (Шишкина Л.Н. et al., 2010).

Also, phosphatidylcholine is involved in the fertilization process. So, during movement to the ovule, the structure of the cellular membranes lipids of spermatozoa undergoes a series of successive changes−capacitation, and then the activation or acrosomal reaction.

In the inactive state, for spermatozoa it is characteristic the stable state of membrane supported by the asymmetry of the phospholipid layers composition. Asymmetry of the phospholipid composition is a typical phenomenon for all the cells. It is due to the activity of ATP and the sulfhydryl-dependent lipid pump. During the activation process the symmetry of local area of the spermatozoon membrane is restored (we are talking about acrosome), which contributes to the merger with the ovule. At this time in particular phosphatidylcholine has the triggering effect on the acrosomal reaction manifestation (Мэдди Э., 1979).

For the cells of the reproductive system phosphatidylcholine is a source of building blocks for the biological membranes, biological active substances as phosphorus, choline and essential fatty acids.

In connection with the above mentioned, the purpose of the conducted research was to study the cryogenic changes of phospholipids as an indicator that characterizing the sanogenic status of farm animals reproductive cells.

MATERIALS AND METHODS

In the experiments was used the sperm of the bulls of Holstein and boars of Large White breeds.

Extraction of lipids was carried out by the method which is based on a destruction of the protein-lipid connection by the polar solvents with the subsequent extraction of lipids by non-polar solvents which are united in one mixture, containing water in the ratio 5:10:4.

Another applied method was the colorimetric determination of phosphorus without burning. The colorimetry was performed at 820 nm using a spectrophotometer type SF-26. A calibration curve was built according to the standard solution of monopotassium phosphate. Translation of phosphorus in the phospholipids was made on the grounds that a molecule of phosphorus is 1/25 part of the phospholipid mass.

Research of spermatozoa phospholipid fractions were made using the method described by the M. Kates (Геннис Р., 1997) with the use of plates 9x12 cm, which was coated with silica gel «L» and «LSL», of the firm «Hematol» in the ratio of 5:3. Extract of lipids was received according to the above described method, then extract was dried in rotary evaporator and the pellet was dissolved in 0.5 ml of mixture of chloroform:methanol (1:1). The received mixture of lipids was used for chromatography, and their separation was carried out in the system of chloroform:methanol:water (65:25:4).

The phospholipids identification was performed by comparing them with the standard solution of lecithin, Rf and chromatograms of the chicken eggs (Кейтс М., 1975).

Statistical processing of the research was done using the Student's t-test.

RESULTS AND DISCUSSIONS

Phosphatidylcholine is the main and the most versatile phospholipid, as presented in the various cell membranes. Therefore, in the studies were examined the changes of phospholipids under the influence of cryopreservation factors.

As a result of conducted experiments is established, that in the gametes of all types of studied animals in the greatest numbers are represented by a fraction of phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PEA) and cholesterol (C) (Table 1).

Table 1. Content of phospholipids in the technological processing of the animal sperm, mg/100g of spermatozoa

Lipids	Elements of technological processing		
	dilution	refrigeration	defrosting
	M ± м	M ± м	M ± м
Boar, n = 8			
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*	518.0 ± 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*
The ratio of PC/SM	4.13	4.37	4.46
Bull, n = 7			
Phosphatidylserine	221.9 ± 9.9	189.4 ± 8.3	156.9 ± 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 ± 16.2	140.7 ± 18.0*
Cholesterol	415.6 ± 10.9	379.0 ± 10.6	342.0 ± 10.6*
The ratio of PC/SM	6.93	6.63	6.94

Note: * P<0.05 the difference is statistically authentic in compared with diluted sperm.

In the quantitative content of the individual phospholipids and cholesterol are observed species peculiarities. So, in gametes of the boar less contained phosphatidylserine (PS) and phosphatidylcholine while the sphingomyelin (SM), phosphatidylethanolamine and cholesterol are in large quantities. Minor components of the boar gametes are presented by the phosphatidylserine and of the bull gametes – by the phosphatidylserine and cardiolipin (CL).

In addition, as a result of the conducted research it was shown that in the total content of phospholipids of the fresh dilute gametes of bulls and boars were not revealed significant specific differences.

Cooling and retention of the diluted bull seed at the temperature of 4˚C lead to a significant decrease of the total quantity of sperm phospholipid fractions.

These changes take place due to the fractions such as phosphatidylcholine and phosphatidylethanolamine. These, in our opinion, testify to the high vulnerability of these fractions, in the process of preservation-depreservation, compared with other fractions of phospholipids.

The results presented in the table 1 also show that the ratio of PC/SM is more than 67% in the bull sperm comparing with boar sperm. This follows that the phospholipids of their membranes have higher bilayer mobility.

In further technological processed can be traced decrease of the rest phospholipids factions.

Therefore, at freezing-defrosting sperm of bull and of boar the changes of content of phospholipids fractions, unlike protein fractions, have unidirectional character – their content is decreased. In technological processing of the semen of agricultural animals the content of different phospholipids fractions in gametes, regardless of the species of animal, go down which proves the non-specific nature of changes in their content at cryopreservation.

The output of membrane-bound phospholipids as a result of the influence of low temperatures is due to a breach of hydrophobic, ionic and lipid-protein interactions. A much larger decline was observed in the content of total lipids in bull spermatozoa after the freezing of sperm in comparison with boar spermatozoa, apparently fits mainly on the above type of loss, because observes more uniform change in phospholipid composition on all fractions.

Low-temperature processing of sperm stimulates the development of lipid peroxidation (LPO) (Абрамченко В.В., 2001) and apparently activates phospholipases. LPO, in turn, at biological freezing is an important element of pathology, contributing to the reduction of mobility and respiratory activity of spermatozoa. At the present it is known that the easily oxidable phospholipids are the CL and PEA, but PC on the contrary, inhibits oxidation processes. The last is present in large quantities in bull sperm, and sensitive to the LPO "PEA and CL" – in boar sperm, which indicates

higher stability of bull spermatozoa to redox influence.

The obtained data in the light of modern understanding of the role and functions of lipids in maintaining and strengthening reproductive health, with adequate certainty shows that the lipid composition of the sperm of different types of farm animals, possessing specific characteristics, determines their cryoresistance. Phospholipids are interrelated in membrane structure and correlate with sanogenic condition of cells. Protective effect of exogenous lipids, as well as specific changes in their composition in the area of low temperatures, indicates a significant role of these compounds in maintaining of the homeostasis in extreme environmental conditions.

The fact that, when cooled significantly reduced amount of PC and PEA suggests that this factor affects biological membranes on both sides at the same time as the first phospholipid is located mainly on the outer, and the second – on the inner side (Борончук Г.В. et al., 2008).

Significant role in preserving bilayer structure of biological membranes belongs to the PC and SM. Phosphatidylcholine is the main structural component of the membranes. It has a high interchange activity, represents specific activator of a many membrane-bound enzymes, serve as a metabolic precursor to SM as well as a source of lipid messengers and bioactive compounds: lysophosphatidylcholine, diglycerides and arachidonic acid; it has a great importance for the membrane permeability.

Unlike the PC, SM performs regulatory role, his presence in the membrane betrays special rigidity. Also, SM promotes the increase of microviscosity of the lipid phase of the membrane (Скатков С.А., 2003). On this basis it should be assumed that the PC may serve as a marker for characterizing of the functional spermatozoa state.

CONCLUSIONS

Generalization of the results of the conducted researches allows making the following conclusions:

1. In the process of sperm cryopreservation of farm animals, there is a reduction of the content of phospholipids at all technological stages.
2. From all spermatozoa phospholipids, quantitatively most representative is phosphatidylcholine.
3. The predominance of PC in bull spermatozoa and SM in boar spermatozoa indicates more rigid membranes structure of the latter species of animals.
4. The ratio PC/SM can be used as a criterion for the functional state of thawed spermatozoa of farm animals that predetermine their reproductive health.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology of the Academy of Sciences of Moldova and was financed from the Project 15.817.04.01A “Nutrition in accordance with constitution types. The impact of nutrition on the sanogenity of male gametes”.

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