

PHOSPHOLIPIDS, PHOSPHOLIPID FRACTIONS AND POSSIBILITY OF THEIR STABILIZATION DURING HYPOTHERMIC STORAGE OF BOAR SEMEN

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Abstract

Lipids are integral and essential components of the membranes that perform multiple functions in the life of germ cells. They provide the energy reserve, the solubility of various vitamins, the physical thermoregulation of activity of membrane-bound enzymes and other. In accordance with modern data about the molecular organization of biological membranes, the lipids are represented mainly by amphiphile connections: phospholipids, glycolipids and cholesterol. At the same time phospholipids make up more than 60% of the total number of membrane lipids. In combination with cholesterol they provide fluidity and plastic properties of biological membranes. Based on the above, the purpose of the conducted research was to study the content of phospholipids, their fractions and to determine the possibility of their regulation during hypothermic storage of boar semen at 16-18°C. Using the method of thin-layer chromatography allowed allocating and identifying 6 phospholipid fractions in the reproductive cells of the boar. Among them prevail phosphatidylcholine and phosphatidylethanolamine, whereas phosphatidylserine and cardiolipin presents minor fractions. In the process of dilution of sperm is observed multidirectional change in their content. It is established that targeted stabilization of the content of phospholipid fractions at the level of native spermatozoa is possible by improving of synthetic mediums. The obtained experimental data can be used for solving the problems of reproduction of pigs.

Key words: lipids, biological membranes, phospholipid fractions, spermatozoa, boar semen.

INTRODUCTION

Reproduction is one of the significant problems in the complex of measures aimed at further increasing the livestock of pigs which with the development of the industry is becoming increasingly topical.

Currently, the main method allowing in the shortest terms to implement a massive improvement of the breed qualities and increase their productivity, is artificial insemination, enabling intensive use of high-value boars-improvers. The efficiency of breeders is determined not only by their breeding advantages, but also by the quality of the semen, which varies among different breeders and varies depending on feeding, housing, use of animals, from ambient temperature and other factors (Яковлев, 2007). Therefore there is a need for a careful checking of the material used. Among the qualitative indicators should include the content of phospholipids, which have high biological

activity relative to maintaining the integrity of membrane systems of cells, the processes of differentiation, proliferation and regeneration of biological membranes. It is proven the involvement of phospholipids in the regulation of the membrane receptors, enzymes and metabolic processes both inside and outside the cell (Скатков, 2002). Being structural elements of lipoproteins, as well as participating in the process of reproduction of animals, phospholipids determine the resistance of germ cells to adverse environmental conditions (Hayk, 1991). A great variety of biological activity of phospholipids can be explained by the peculiarity of their chemical structure, which is characterized by amphipathicity (the ability of the phospholipids to hydrophobic and hydrophilic interactions), the presence of charged groups in the hydrophilic part of the molecule, the presence of biologically available phosphorus, of polyunsaturated fatty acids and choline - the active component of phosphatidylcholine (Борончук et al., 2008).

The main role in preserving of the fertilizing capacity of ejaculate is assigned to the homeostasis of the components of seminal plasma, to the maintenance of the viability of spermatozoa, the stability of their membranes, protection against the acidic environment of the genital tract of females (Oresti et al., 2011). The lack of phospholipids leads to a reduction of reproductive function of spermatozoa (Антонов et al., 2012).

In connection with the stated above, the purpose of the conducted research was to study the phospholipid spectrum of boar spermatozoa, as carriers of genetic information and to explore the possibility of its stabilization during the hypothermal phenomenon.

MATERIALS AND METHODS

In our experiments we used the sperm of boars breeders of Landrace breed which contained in the conditions of relevant veterinary requirements of the breeding enterprise "Moldsuin hibrid". The sperm was received by fractional method. After receiving it was divided into three parts. The first variant served as a control (native sperm). In the second variant, the sperm was diluted 1:1 with medium containing optimal amounts of L-carnitine, in the third variant, the semen was diluted with medium containing L-carnitine 1:1 and one hour after reaching the temperature of 16-18 °C - with medium 2:1 containing ferric sulfate. As a basis it was used the medium consisting of glucose, EDTA and sodium citrate. Spermatozoa were separated from plasma by centrifugation with subsequent laundering of their with isotonic solution of sodium chloride. Thin-layer chromatography of lipids was performed on glass plates coated with a mixture of silica gels types "L" and "LSL" of company "CHEMAPOL" (Keйrc, 1975). Fractionation of phospholipids was carried out in the system chloroform-methanol-distilled water in the ratio 28:12:2. Staining of phospholipid fractions was carried out in the iodine vapor. The densitometry of chromatographic bands was performed using a computational technique using a special program "Sorbfil TLC Videodensitometer". The identification of chromatographic bands performed against standard solutions of lecithin and cholesterol,

by special staining reactions, on Rf value and the comparison results of original photos and lipids in our studies (Hayk, 1991).

Digital material was processed using the Student's t-test.

RESULTS AND DISCUSSIONS

One of the main technological parameters at the preservation of semen used for artificial insemination of sows is its dilution. Improving of the mediums for dilution of sperm, still, remains an actual problem in the field of reproduction of farm animals. The boar sperm in native form to a large extent diluted by the secrets of the accessory glands and exposed to the action of internal and external environmental factors. Among them we should mention, lipid peroxidation and accumulation of toxic products of this process (Alvarez et al., 1995; Goni et al., 2008). However, redox reactions play an essential role in maintaining the functional activity of the reproductive cells. In this connection great interest represents not only inhibition but also the activation of these reactions. The results of conducted researches are presented in figure 1.

Analysis of the chromatograms shows that as a result of the fractionation of lipids were found 7 fractions: 1. sphingomyelin (SPH); 2. phosphatidylcholine (PC); 3. phosphatidylethanolamine (PE); 4. phosphatidylserine (PS); 5. cardiolipin (CL); 6. cholesterol (Chol) and 7. phosphatidic acid (PA). The most expressed content of such phospholipid fractions are SPH, PC which represent saturated phospholipids and PE. In addition, it is assumed that PC includes plasmeylcholines, and the fraction of PE – plasmeylethalamines (Hayk, 1991).

It turns out that the predominant fractions perform an essential role in the activity of biological membranes. So, the phosphatidylcholine is the basic, most versatile phospholipid, which is great represented in membranes and lipoproteins of different cells and tissues. This is the phospholipid, which is paramount structure-forming function. But along with this it also performs several other functions in particular – serves as a metabolic precursor for the other two major kinds of phospholipids – PE and SPH.

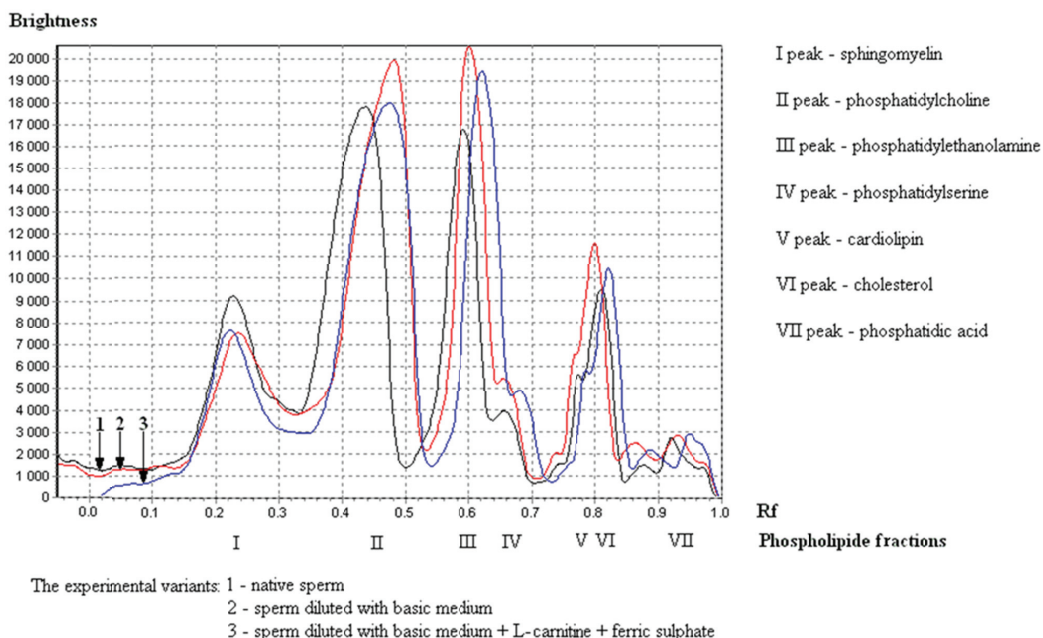


Figure 1. Phospholipid spectrum of boar spermatozoa during the hypothermal preservation and the influence upon it of the synthetic mediums of different composition

Important and indispensable role of PC, as a primary membrane phospholipid in the functioning, growth and development of cells is manifested in all tissues of animal origin, being significant the role of biological membranes in the activities of the cell and organism as a whole (Pomorschi et al., 2001). This is especially important for the sexual cells which are renewing relatively quickly – in a consequence of their high metabolic activity.

PE which represents an amine of phospholipids is involved in series of processes such as cell adhesion and endocytosis (Bazzi et al., 1992).

The SPH as a structural element of cell membranes is studied for many years, but only recently obtained proof of its regulatory role in important cellular processes. It is shown that, the presence in the membranes of SPH with its long-chain base and saturated fatty acid confers the stiffness to membrane.

It is established that the superficial changes of cell surface prevent the addition of SPH, on the basis of which it is assumed that the determining factor of morphological changes in the apoptotic cell is not the formation of ceramide, but the elimination from the surface of the SPH and its hydrolysis (Paris et al., 2001).

When the temperature is lowering to 16-18 °C the content of phospholipid fractions varies depending on the composition of mediums used for dilution and storage of semen of the boar. Thus, the number of SPH decreases slightly whereas the safety of PC and PE largely improved, which can be explained by the change of the chromatographic mobilities of the studied phospholipids (Hayk, 1991).

Change of phospholipid spectrum demonstrates corresponding reconstructions of biological membranes where the phospholipids represent 60–70% of the total amount of lipids, which have an affect the functional activity of spermatozoa.

CONCLUSIONS

The researches allow making the following conclusions:

1. Phospholipid spectrum of boar semen is mainly represented by sphingomyelin, phosphatidylethanolamine and phosphatidylcholine, whereas cardiolipin and phosphatidylserine are minor factions.
2. The content of phospholipid fractions is changed under the influence of hypothermal factor.

3. Stabilization of phospholipid is possible through targeted regulatory activity of redox processes at the dilution and storage of boar semen.
4. The ingredients of synthetic mediums can have a positive (or negative) impact on the biochemical status of boar spermatozoa by changing the chromatographic mobility of the phospholipid molecules.

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