Scientific Papers. Series D. Animal Science. Vol. LX, 2017
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CRYOGENIC CHANGES OF IONIC COMPOSITION AND STABILITY OF THE SPERM BIOCOMPLEXES OF AGRICULTURAL ANIMALS AT ITS CONSERVATION

Ion BALAN, Gheorghe BORONCIUC, Vladimir BUZAN, Nicolae ROŞCA, Iulia CAZACOV, Melania BUCARCIUC, Ion MEREUŢA, Nadejda ZAICENCO

Academy of Sciences of Moldova, Institute of Physiology and Sanocreatology,
1 Academiei Street, MD 2028, Chișinău, Republic of Moldova, Phone: +373.22.73.96.07,
Email: vladimirbuzan@yahoo.com

Corresponding author email: vladimirbuzan@yahoo.com

Abstract

In the article are presented the literal references devoted to theoretical investigations of phase changes in biological membranes at the cryopreservation process, also the development and new experimental methods application in studying different physico-chemical manifestations that are reflected by such type of devices. Lowering the temperature causes the phase of reorganization of biological membranes. Dehydration of cells in the process of phase transition is accompanied by the deformation of biological membranes. The result of phase transitions in biological membranes there is a migration, aggregation, translocation, and other types of movement protein components. Low temperatures lead to destabilization of membrane structures, causing activation of peroxidation and changes in lipid composition, and this affects the functional state of cells. Own protective functions of the gametes aimed at the stabilization of lipids and lipid processes in the low-temperatures zone can be sufficiently manifested in conditions of purposeful regulation of them from the outside.

Key words: *lipids, phase transitions, biological membranes.*

INTRODUCTION

At the influence of low temperatures on biological membranes is a series of structural rearrangements in their organization, among which of particular importance are phase transitions of lipids and their lateral separation in bilayer with the formation of clusters and aggregation of protein and lipid components. The role of the inducing element in the damage of biological objects at low temperature is to phase transitions of the lipids from liquid-crystal state into a gel (Белоус et al., 1982). The process of phase transition of lipids leads to the formation of a rigid membrane structure, the plasticity of which is markedly different from the state of the membrane at physiological temperatures, when the fatty acid chains of the lipids are in liquid crystalline state. At temperatures below the phase transition, in the gel phase the mobility of fatty acid chains is limited and the movement of them anisotropic. The phase transition temperature of lipids increases with their intensity. It depends on the length of the chemical bond of fatty acid chains and decreases with increasing of polar radical of lipids, the nature of the interaction of lipids with proteins, and to a large extent determined by the molar ratio of cholesterol:phospholipid in biological and model structures. The latter plays a dominant role in the regulation of structurally-dependent functions of cells related with state of lipid membranes. According to existing data, cholesterol has a specific effect on the packing of lipid membranes and in the certain concentration shifts the phase transition to the area of lower temperatures or completely eliminates it. Therefore, in the biological membrane of erythrocytes that contain more than 33% of cholesterol, the liquid-crystalline state of lipids is maintained until a temperature of -20°C. On the basis of the detected linear decrease of temperature of the apparent phase transition in cholesterol-phospholipid suspensions with increasing content of cholesterol, it was concluded that the phase transition disappears when the molar ratio of cholesterol-phospholipids is equal to 1:1.

Temperature-dependent phase transition from liquid-crystal state to a gel is accompanied by the formation of a very rigid membrane structures that differ from the state of membranes at physiological conditions. At temperatures below the phase transition in the gel phase the mobility of fatty acid chains is very limited and their movement is anisotropic. Lowering the temperature leads to the transition of lipids in the new phase, and also to conformational changes in their structures. The phase transition temperature of the lipids depends on the length, degree of saturation of fatty acid chains and the chemical nature of the polar radicals of lipids (Бондаренко et al., 2002).

In this regard, the objective of the research was to investigate the cryogenic changes in the ion composition of spermatozoa and stability of protein-cholesterol complexes of sperm of farm animals in the cryopreservation process.

MATERIALS AND METHODS

The main experimental work was carried out in the laboratory of Cryosanocreatology "V. Nauc". The object of investigations was the sperm of roosters of the Rhode Island breed. The experimental material was frozen in the form of granules with a volume 0,1-0,2 ml on the fluoroplastic plate surface at a temperature of -110 – -120°C. In the study of mass transfer through the plasma membrane was determined the content of Na^+, K^+, Li^+ and Ca^{+2} ions by flame photometry using the device ПФ-2. The allocation of plasma membranes using two-phase polymer system was performed according to the method of N. Ivanov, I. Porfirov in our modification (Наук В.А. et al., 1993). The stability of protein-cholesterol complexes were determined by the amount of loosely bound cholesterol (Кейтс, 1975).

Statistical processing of digital material was done by the method E. Merkurieva using the Student's t-test.

RESULTS AND DISCUSSIONS

Cryogenic changes in biological membranes can be reduced to the realization of a number of physico-chemical mechanisms. Among them it should be noted: the phase transition of lipids, changes in the structure of water, segregation and aggregation of proteins and lipids, biochemical changes in the structure of membrane components, lipid peroxidation, the violation of the barrier properties of the membranes. The listed processes are risky in the maintenance of the functional state of defrosted material.

As a result of our research was established the change of the ions concentration of Na^+, K^+, Li^+ and Ca^{+2} in the sperm of roosters in the cryopreservation process (Table 1).

Table 1. Cryogenic changes in ionic composition of the rooster sperm

Concentration of ions	Investigated material	
	Spermatozoa	Plasma
Native material		
Na^+	147.9 ± 5.48	407.0 ± 22.80
K^+	156.8 ± 27.90	35.7 ± 2.52
Li^+	0.73 ± 0.03	0.58 ± 0.01
Ca^{++}	7.53 ± 0.16	12.0 ± 0.49
Defrosted material		
Na^+	187.6 ± 5.32*	280.6 ± 19.0*
K^+	43.3 ± 4.58*	47.8 ± 2.97*
Li^+	0.17 ± 0.04*	0.30 ± 0.07*
Ca^{++}	13.16 ± 0.36*	7.66 ± 0.74*

Note: * Cryogenic changes are statistically authentic.

From table 1 it follows that the state of membrane lipids has a significant effect on the cell functions. It is at the stage of the phase transition of lipids occurs a sharp change in the membrane permeability for ions and metabolic products. In this regard, the nature of phase transitions of lipids in membranes is important not only to determine their cryoresistance, but

also for predicting the results of cryopreservation.
Accounting of the phase transition of lipids acquires special significance, if we take into account the fact that namely in the given case increases the level of membrane cryodamage.
Considering the nature of the phase transition of the lipids and the influence of temperature on these processes, it should be noted that the membrane lipids are amphipatic compounds, i.e. they contain both a polar hydrophilic end and a long hydrophobic part formed by fatty acid chains.
The general interest are the data about lipid mixtures wich containing cholesterol, in connection with the ability of the latter to regulate the liquid crystal compound in lipid bilayer (Балан et al., 2005).
According to the Belous (Белоус et al., 1982), lowering the temperature and reducing the level of cell hydration is accompanied by a deformation of the plasma membrane of cells and activation of phosphatidylinositol cycle. In the author's opinion, the increase of ionized Ca^{++} greatly changes the polymeric and conformational state of the proteins cytosol and the cytoskeleton. Polymerization of cytoskeleton proteins is accompanied by the appearance on the plasma membrane of spinules. Via deforming the membrane, they change the function of ion pumps. The specified structural changes of proteins contribute to the processes of phosphorylation, which areactivated when the temperature drops.
The formation of myosin hydration shells and its conformational state at the temperature changes - are closely related processes. The leading in the conformational rearrangements of the protein upon changing the temperature are the factors associated with the existence of thermolability of the physico-chemical parameters of water and end up with thermolability of the interactions of the protein macromolecule with the aqueous medium (Жилякова et al., 1991).
Cryogenic changes of cellular or other biological structures conditioned by phase transitions of proteins or lipids, are in direct dependence on the degree of membranes hydration. Therefore, the study of its role in maintaining the structure of membranes is one of the approaches to clarify the mechanisms of cryodamage and cryoprotection of biological objects.
Under the influence of the negative temperatures there is a change in the structure of water. The structure of vicinal water is close to the structure of liquid crystals. The vicinal water is characterized by temperature anomalies, which can be considered as phase transitions.
The existence of four regions of temperature anomalies (from +14 to +16°C, from +29 to +32°C, from +44 to +46°C and from +59 to +62°C) suggests, at least, phase transitions between the five structural types of water. It turns out that not only vicinal water influences the functioning of the membranes, but also the membrane structures determine the state of the vicinal water. According to Drost-Hansen (Drost-Hansen, 1973), the specific structure of water adjacent to the surface depends on the surface properties. The processes taking place in membranes during freezing of various fractions of water are of great importance for the successful implementation of the cryopreservation problem.
The extreme conditions of cryopreservation have a significant impact on the dynamics of components of the cells plasma membranes (Болдырев et al., 2006). With decreasing of temperature in the membranes take place the phase transitions.
Regulation of aggregation and segregation of proteins in the membrane is carried out by means of ionic bridges between charged groups of phospholipids and proteins, as well as the sulfhydryl-disulfide bonds, which condition the state of the protein complex. Through the method of fluorescent probes Belous with co-researchers (Белоус et al., 1982) found that in the case of changes in temperature and osmotic conditions of the medium, the nature of the protein-lipid interactions is modified. The authors suggest that the primary processes that induce sensibilization of cells to cooling, developing at the level of the protein cytoskeleton associated as a part of membrane lipids and with certain integral proteins. This is evidenced by the slow reversibility of structural changes of proteins, for which are characteristic more prolonged relaxation and reparation processes.
For biological membranes is characterized the

difference in the lipid composition on the both sides of the bilayer. The asymmetric distribution of phospholipids in the membrane is provided by the following three mechanisms. The first mechanism is related to the thermodynamic probability of distribution of phospholipids in accordance with the stereoconfiguration of their molecules. This is evidenced by the fact that the bilayer in the preparation of liposomes from a mixture of phospholipids is characterized by asymmetrical distribution of components: the outer part of the lipid bilayer during the formation of liposomes, by the predominance of phosphatidylcholine, and the internal - of phosphatidylethanolamine. This distribution of phospholipids contributes to the formation of bends and the formation of a gradient of flexibility.

The second mechanism is realized by differences in the composition of the medium, the surrounding bilayer in natural and experimental conditions. From the extracellular side of the membrane, the medium is characterized by a high content of Mg^{++} and Ca^{++}. From the cytoplasmic side, the membrane is exposed to contact with Mg^{++} and K^{+} ions. Differences in the ionic composition of the extra- and intracellular medium also contribute to the mobility of the bilayer.

The third mechanism is due to the enzymatic factors (Юрченко et al., 2002). At the same time the asymmetry of bilayer is provided by enzymes of lipid metabolism and lipid-carrying proteins. The latter are a group of proteins of different specificity - from highly specific, providing the exchange of membrane components, to the relatively low-specific, binding and transferring to membranes, or vice versa lipids of different classes. The transfer of lipid molecules is realized in the form of complexes with these proteins-carriers. While protein-lipid complexes acquire a hydrophilic nature.

Elucidation of the mechanisms of cryogenic changes in biological systems at different levels of organization is one of the fundamental problems of modern Cryobiology. Numerous data of special literature indicate that one of the most cryolabile structures of the cell are biological membranes (Наук, 1991). Herewith the change of their barrier properties leads to a variety of cellular modifications (Holban et al., 2000). The study of barrier properties of membranes is due to the theoretical and practical significance of this issue.

A necessary condition of cellular homeostasis is to maintain the functional activity of biological membranes. Experimental studies performed in our laboratory demonstrate that at all stages of cryopreservation occur the membrane modification, the nature and intensity of which is determined by the composition of synthetic mediums and modes of technological processing of biological material (Table 2).

Table 2. The stability of protein-cholesterol complexes in the sperm of bulls depending on the conditions of cryopreservation

The experimental variants	Content of loosely bound cholesterol, μg/10^9 spermatozoa
Fresh diluted semen	274 ± 21
After cooling and keeping the sperm at 2-4°C for four hours under conditions	
- anaerobic	230 ± 22
- aerobic	207 ± 13
- ordinary	223 ± 24
After freezing and thawing of semen, cooled in conditions	
- anaerobic	207 ± 22*,**
- aerobic	171 ± 17*
- ordinary	159 ± 17*

Note: * Cryogenic changes are statistically authentic.
** Statistically authentic changes in comparison with the ordinary conditions.

From Table 2 it follows that the change of cryopreservation conditions contributes to the preservation of protein-cholesterol complexes and thereby the functional state of the membranes.

The plasma membrane in hypertonic salt solutions and during freezing in the range of pre-eutectic temperatures are characterized by increased permeability only for cations while maintaining the barrier properties with respect to marker compounds of greater molecular weight such as sucrose. The authors showed that the increased permeability of membranes for cations is a reversible process, as evidenced by the absence of change in the permeability of plasma membranes for water molecules after freezing to different temperatures in the range (0) - (-16°C). Comparing the facts about the change of plasma membranes permeability for cations in the pre-eutectic temperature range, the authors concluded that hypertonic saline solutions in combination with a decrease of temperature are a risk factor leading to a change in the functional activity of plasma membranes at temperatures of the order of (0) - (-17°C).

Thus, it can be assumed that along with the ratio of cholesterol:phospholipids or cholesterol content their dynamics plays an important role in the process of cryopreservation as a possible mechanism of cells adaptation to low temperatures. However, the increase of this ratio can not be a positive phenomenon if it is associated only with the loss of phospholipids of the most important functional and structural components of biological membranes as the loss of phospholipids will lead to a significant deterioration of the physiological and morphological state of gametes (Борончук et al., 2003). Consequently, the positive effect of this mechanism can manifest only in the presence of exogenous lipids, for example lipids of seminal plasma, egg yolk and other components, and also may be due to the synthesis or resynthesis processes of endogenous substrates.

The presented material allows concluding that low temperatures lead to destabilization of membrane structures, causing activation of peroxidation and changes in lipid composition, and this affects the functional state of cells. Own protective functions of the gametes aimed at the stabilization of lipids and lipid processes in the low-temperatures zone can be sufficiently manifested in conditions of purposeful regulation of them from the outside.

CONCLUSIONS

The researches allow making the following conclusions:

1. The decrease of temperature causes the phase reorganization of biological membranes.
2. In the result of a phase transition of lipids there is a risk of ion permeability violation of plasma membranes.
3. Dehydration of cells during phase transitions is accompanied by the deformation of biological membranes.
4. As a result of active phase transitions in biological membranes take place migration, aggregation, translocation and other types of protein components movement.
5. Regulation of the resistance of spermatozoa to the action of low temperatures is possible by the introduction of cholesterol into the composition of synthetic mediums.
6. The maintenance of the bonds stability in the protein-cholesterol complexes of sperm is better achieved in the anaerobic conditions of cryopreservation.
7. The cryoprotective properties of synthetic mediums are manifested under condition of mandatory presence of exogenous lipids.

ACKNOWLEDGEMENT

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”.

REFERENCES

Drost-Hansen W., 1973. Phase transitions in biological systems: manifestations of cooperative processes in vaccinal water. Ann. N.Y. Acad. Sci., Vol. 204, l, 100-108.

Holban M., Balan I., Boronciuc Gh., 2000. Cercetări privind concentrația de ioni în spermatozoizii de cocoș în procesul crioconservării. The Bulletin of the European Postgraduate Centre of Acupuncture and Homeopathy. Chișinău, 4, 91

Балан И.В., Борончук Г.В., 2005. Белково-липидные и холестерин-фосфолипидные соотношения как показатель криорезистентности генома животных. Актуальные проблемы интенсификации производства продукции животноводства. Жодино, 43.

Белоус А.М., Бондаренко В.А., 1982. Структурные изменения биологических мембран при охлаждении. Киев: Наукова думка, 256.

Болдырев А.А., Кяйвяряйнен Е.И., Илюха В.А., 2006. Биомембранология: Учебное пособие. Петрозаводск: Изд-во Кар. НЦ РАН, 226.

Бондаренко В.А., Рамазанов В.В., 2002. Влияние гипертонических сред и барбитуратов на транспорт анионов в эритроцитах человека. Проблемы криобиологии, 1, 111-112.

Борончук Г.В., Балан И.В., 2003. Криомембранология. Кишинев: Типогр. АН Молдовы, 336.

Жилякова Т.А., Малеев В.Я., Николов О.Т., 1991. Состояние воды в липидных системах по данным СВЧ диэлектрометрии. Харьков, 39-45.

Кейтс М., 1975. Техника липидологии. Москва, Мир, 270.

Наук В.А., 1991. Структура и функция спермиев с/х животных при криоконсервации. Кишинев, Штиинца, 197.

Наук В.А., Борончук Г.В., 1993. Методика выделения плазматических мембран гамет сельскохозяйственных животных. Известия АН РМ, 1, 50-53.

Юрченко Т.Н., Белоножко А.П., Жуликов Е.П. et al., 2002. Динамика активности окислительно-восстановительных ферментов в тканях плаценты при ее гипотермическом хранении. Проблемы криобиологии, 1, 3-6.