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SPECIFICITY OF MEMBRANE-BOUND ENZYMES ACTIVITY OVER THE CRYOPRESERVATION OF FARM ANIMALS SPERM

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Abstract

The intensity of the vital processes in spermatozoa in addition to the transmembrane defects is determined through activity of membrane-bound enzymes. The purpose of our research was to study the activity of membrane-dependent enzymes in the sperm of different animals species at cryopreservation. In experimental studies were used physiological, cryobiological and statistical methods that were held on the plasmatic membranes of sperm of the boar of Large White breed and the roosters of Rhode Island Red breed. The obtained results in the study of the activity of Mg^{+2} (Na^{+} + K^{+}) - ATPase in the membranes of spermatozoa of the diluted sperm, and after freezing and thawing of sperm of rooster and boar attest substantial destruction of the plasmatic membranes of these breeds. Activity of 5'-nucleotidase in isolated rich fractions of plasmatic membranes of native spermatozoa of rooster and boar not suffer essential changes at cryopreservation of sperm these species. Our research revealed minor changes in the activity of alkaline phosphatase in the membranes of native and frozen-thawed rooster and boar spermatozoa. The study of the above enzymes of plasmatic membranes of spermatozoa of rooster and boar are certain theoretical and practical interest. They allow obtaining new data on the chemical composition of the membranes, reveal the specific relationship between the content and ratio of the structural components of membranes and the resistance of rooster and boar spermatozoa to low temperatures.

Keywords: *membrane, spermatozoa, freezing, enzymes, farm animals*.

INTRODUCTION

Increase of productive and reproductive indices of farm animals can be achieved by improving methods of artificial reproduction. But this requires the availability of high-quality sperm, the indicators of which are reduced in the process of cryopreservation. Stabilization of functional parameters of reproductive cells is possible on the basis of fundamental research at the subcellular level. The intensity of the vital processes in spermatozoa besides the transmembrane defects (Белоус et al., 1982) is determined also on the activity of membrane-bound enzymes.

Previously completed studies on enzymes characteristic for plasmatic membranes of gametes males have showed that their activity depends on the type of animals. For example, in the membranes of spermatozoa from native sperm of bull the activity of ATPase is almost 10 times higher than in the membranes of spermatozoa of ram, the activity of which in the technological processing of sperm decreases in plasmatic membranes of both species of animals. Thus observed the same tendency of activity of ATPase in the membranes of sperm cells of the bull, which is almost 10 times higher than in the membranes of spermatozoa of the ram after thawing of sperm. Investigation of alkaline phosphatase in the native membranes of spermatozoa showed that the activity of this enzyme also varies depending on the species. At the cryopreservation of sperm the activity of this enzyme increases in plasmatic membranes of spermatozoa of bulls, whereas in the membranes of gametes of ram is a slight decrease (Борончук et al., 2008).

Based on the above, the purpose of the paper was to study the activity of membrane-dependent enzymes in the sperm of different species of animals during cryopreservation.

MATERIALS AND METHODS

The experimental investigations were carried out using the plasmatic membranes of spermatozoa of the boar of Large White breed and the roosters of Rhode Island Red breed.
Native sperm was divided into two parts. The first part (without dilution) was subjected to analysis served as control. The second part was diluted with synthetic mediums of corresponding type of animal. Both parts were cooled and freezed as granules on the surface of fluoroplastic plates at a temperature of minus 100-110°C. Defrost of sperm was carried out at 40°C, using a specially designed aerodynamic devices that can reduce the contact between the solid and liquid phase formed when heated. The selection of plasmatic membranes was performed using a polymer system, consisting of dextran with a molecular mass 500000 D and polyethylene glycol - 6000 D (Ivanov et al., 1981).

Determination of activity of membrane enzymes Mg^{+2} (Na^+ + K^+) - ATPase (CE 3.6.1.3), 5`-nucleotidase (CE 3.1.3.5) and alkaline phosphatase (CE 3.1.3.1) was performed in accordance with recommendations by the same authors. All the processing of the membranes was carried out at 4°C.
The obtained data were processed using the Student's t-test.

RESULTS AND DISCUSSIONS

Based on the above were continued research to elucidate the activity of Mg^{+2} (Na^+ + K^+) - ATPase, 5`-nucleotidase and alkaline phosphatase of the plasmatic membranes. They showed that the cooling, freezing and thawing of rooster and boar sperm have different effect on them.
However, in isolated plasmatic membranes of thawed spermatozoa of both species was observed a significant reduction the activity of these enzymes (Table 1).

Table 1. The activity of Mg^{+2} (Na^+ + K^+) - ATPase, 5`-nucleotidase and alkaline phosphatase of isolated plasmatic membranes of spermatozoa at cryopreservation of rooster and boar sperm (µmol/h/mg protein)

Stage of technological processing	Mg^{+2} (Na^+ + K^+) - ATPase	5`-nucleotidase	Alkaline phosphatase
Rooster			
Dilution (control)	16.23±2.20	8.09±0.86	1.19±0.08
Freeze-thawing	9.57±0.56*	7.39±0.59	1.17±0.06
Boar			
Dilution (control)	28.03±1.30**	4.49±0.98**	3.02±0.36**
Freeze-thawing	18.85±1.43*. **	3.17±0.66**	2.48±0.37**

*The difference is statistically authentic
** Statistically authentic differences breed

The results obtained in the study of activity of Mg^{+2} (Na^+ + K^+) - ATPase in the spermatozoa membranes from the diluted sperm, and after freezing and thawing of rooster and boar sperm attest substantial destruction of the plasmatic membranes of these breeds. For example, in the process of cryopreservation of sperm the activity of this enzyme is reduced from 16.23±2.20 to 9.57±0.56 µmol/h/mg protein at rooster and from 28.03±1.30 to 18.85±1.43 µmol/h/mg protein at boar. And since this enzymatic system performs transport function and the role of the transformer of energy, accumulated in ATP for active transport of Na^+ and K^+ on the membrane, the revealed changes of active transport of Mg^{+2} (Na^+ + K^+) - ATPase shows the large deenergization of the plasmatic membranes of rooster and boar spermatozoa. Further analysis of the data shows that in the membranes of boar spermatozoa in the process of freeze-thawing of sperm occurs most significant decrease of activity of Mg^{+2} (Na^+ + K^+) - ATPase, which indicates more labile relation of this enzyme with membrane of boar spermatozoa ($P<0.01$).
In addition, numerous studies show that treatment of ATPase with various detergents, phospholipases, and solvents leads to their

inactivation (Bagatolli et al., 2010; Болдырев, 1990). This highlights need for participation of lipids to the manifestation of mentioned enzymes activity. It should be noted that from all factions of phospholipids the greatest increased activity of ATPase causes negatively charged phospholipids - phosphatidylserine and phosphatidic acid (Болдырев, 1990; Ипатова, 2005). It follows that the one of possible causes of the substantial inactivation of Mg^{+2} (Na^{+} + K^{+}) - ATPase membranes boar spermatozoa after thawing in comparison with such indicators of membranes sperm of rooster, may be most lower content of phosphatidylserine at all stages of technological processing of the material (Наук, 1991).

Activity of 5`-nucleotidase in isolated rich fractions of plasmatic membranes of native rooster spermatozoa was higher than in the boar spermatozoa, which is 8.09±0.86 against 4.49±0.98 μmol/h/mg protein, respectively. In the process of cryopreservation of rooster and boar spermatozoa occurs a slight decrease of activity of this enzyme, namely 7.39±0.59 at rooster and up to 3.17±0.66 μmol/h/mg protein at boar. It follows that this enzyme is not suffer essential changes in the cryopreservation of sperm of these animals.

The representative of associated enzymes with the plasmatic membrane, is alkaline phosphatase, which participates in the mechanism of realization of the physiological action of cyclic AMP by releasing of orthophosphates and phosphorylated proteins substrates (Gotoh et al., 2007; Ипатова, 2005). Our research revealed insignificant changes in the activity of this enzyme in the membranes of native and freeze-thawed rooster and boar spermatozoa. So, alkaline phosphatase activity is respectively 1.19±0.08 and 1.17±0.06 μmol/h/mg protein in the membranes of native and thawed spermatozoa of rooster. At boar the activity of this enzyme is reduced from 3.02±0.36 in the plasmatic membranes of native spermatozoa to 2.48±0.37 μmol/h/mg protein in the membranes of thawed spermatozoa. The obtained data are consistent with research results of the functional indices of spermatozoa after freezing and thawing of bull and ram sperm (Борончук et al., 2008). The analysis of the received results allow to note that changing the activity of Mg^{+2} (Na^{+} + K^{+}) - ATPase, a 5`-nucleotidase and alkaline phosphatase during cryopreservation are the result of changes in the molecular organization of plasmatic membranes, the restructuring of which is carried out in the process of dilution, cooling, freezing and thawing of sperm of these animals breeds.

The comparative analysis of breeds features of the membrane-bound enzymes activity of spermatozoa of these species showed that the activity of Mg^{+2} (Na^{+} + K^{+}) - ATPase and alkaline phosphatase is higher in plasmatic membranes of boar spermatozoa. The activity of these enzymes constitute respectively in membranes of native spermatozoa 16.23±2.20 and 1.19±0.08 at rooster, 28.03±1.30 and 3.02±0.36 μmol/h/mg protein at boar. In turn, the activity of 5`-nucleotidase is higher in the membranes of rooster spermatozoa, which at the cryopreservation is reduced from 4.49±0.98 to 3.17±0.66 at boar and from 8.09±0.86 to 7.39±0.59 μmol/h/mg protein at rooster. However, the obtained data indicate that the membrane-bound enzymes of plasmatic membranes of rooster and boar spermatozoa have the species specificity, as their activity differs between them on a statistically significant difference. At the same time, for boar spermatozoa membranes are the most species-specific Mg^{+2} (Na^{+} + K^{+}) - ATPase and alkaline phosphatase, whereas for rooster – 5`-nucleotidase.

Thus, the study of the above enzymes of plasmatic membranes of rooster and boar spermatozoa are certain theoretical and practical interest. They allow obtaining new data on the chemical composition of the membranes, reveal the specific relationship between the content and ratio of the structural components of membranes and the resistance of rooster and boar spermatozoa to low temperatures.

This offers the possibility to limit the scope of searches conditions of cryoprotection and improve the quality of sperm after long-term preservation outside the body.

CONCLUSIONS

The researches allow making the following conclusions:

1. As a marker for the determination of the functional state of boar and rooster sperm may be used the activity of Mg^{+2} (Na^{+} + K^{+}) - ATPase, which is more labile.
2. The process of cryopreservation of boar and rooster sperm initiates a reduction of the activity of membrane-bound Mg^{+2} (Na^{+} + K^{+}) - ATPase, whereas the activity of the 5`-nucleotidase and alkaline phosphatase remains stable.

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