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Short Communication

Study of adenosine-5'-triphosphate (ATP) in spermatozoa of boars

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The data obtained suggest that the dialysis method of diluting the sperm of boars contributes to more effective protection of sperm during their cryopreservation than the traditional dilution of ejaculate. In this case, the preservation of adenosine-5'-triphosphate (ATP) and mobility was higher by 12 and 1 to 13.5% compared with control.

Key words: Preservation, adenosine-5'-triphosphate (ATP), mobility, freezing, membrane, sperm, boars.

INTRODUCTION

Energetic basis of sperm motility were worked out by many scientists of Mann (1964), Engelhardt and Spring (1957) and Kurbatov et al. (1988). Researchers have shown that the main role in the energetic belongs to the adenosine three phosphates. The adenosine-5'-triphosphate (ATP) content is an important factor in regulating the movement of sperm. According to the AD Ugarova et al. (1991) the ATP content in sperm of bulls and rams is 8.7 and 8.6 mg per 100 ml, and of boars - 3.3 mg. The parallelism between content of ATP and mobility of sperm is shown. The numbers of ATP strictly consumed in the process of sperm movement are manifested at a constant level by means of two interconnected processes of glycolysis and respiration. As a result, research of Platov (1973), Prokoptseva et al. (1974), Moroz (1982) Korban (1988) and Rustenova (1996) showed that a breach of mobility and reduced survival time of sperm after a temperature shock and freeze-thaw cycles may be associated with a decrease in ATP synthesis and as a result, there is need for research to establish a quantitative change in the ATP after a temperature shock and freeze-thaw cycles, the relationship with the ATP fertilizing capacity of sperm and to ascertain the influence of different methods of cryopreservation on the level of ATP content in spermatozoa. The aim of our experiments was to study the ATP content in the native boar sperm as well as, being subjected to freezing in different ways.

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MATERIALS AND METHODS

Research methods

Experiments were conducted on semen of 8 boars and 98 head of sows of Large White breed. After evaluating the sperm quality, parameters were diluted with lactose-chelate-citrate-potassium-unitiol-urea medium (LHTSKUM). After receipt of the ejaculate, it was kept at a temperature of 34℃ for 1 h. Subsequent cooling: 15 to 16℃ for 1.5 to 2.0 h, 8℃ for 1 h and 1℃ for 1 h . Frozen semen ranking was done in the fluoroplastic plate beads in 1 ml of liquid nitrogen vapor, and then the plate was lowered into liquid nitrogen. From each boar, 325 to 2600 pellets were frozen. Thawing was performed at 42℃. Dialysis was carried out by placing the sperm in dialysis bags of ge nitrocellulose membrane. Fertilizing sperm was determined by the ratio of covered mares gestating sows and expressed as a percentage. Extraction of ATP from the sperm was carried out by the method as described by Ugarova et al. (1991) with organic solvents. The ATP content was determined using the method bioluminescent portable luminometer EMILITE-1003A.

RESULTS

The results of an experiment of the study on the ATP content in spermatozoa of boars are presented in Table 1. Table 1 show that in the process of freezing and thawing, there is a sharp decrease in the level of ATP in the sperm of boars. At the 5th min after thawing, ATP

Table 1. Effect of cryopreservation on the ATP content and fertilizing ability of boar sperm.

Time of experiment	Mobility of sperm (%)	Sperm with intact acrosome (%)	ATP content in sperm, nmol /10^8 kl	Fertilization capacity (%)
Prior to freezing	81 ± 5.3	93.1 ± 4.2	26.8 ± 0.6	89.4 ± 8.74
5 min. after thawing	37 ± 1.9*	56.3 ± 2.8**	16.7 ± 1.3	47.2 ± 5.63**
60 min after thawing	21 ± 1.3*	43.8 ± 2.9**	7.1 ± 0.3*	32.4 ± 2.82**
120 min after thawing	18 ± 0.4**	37.7 ± 1.9**	5.2 ± 0.2*	24.6 ± 1.56*

* P <0.05; ** P <0.01.

Table 2. Influence of dilution of sperm before freezing on the ATP content in spermatozoa.

Time of ATP experiment	Common diluted semen		Dialysis treatment	
	Mobility of sperm (%)	ATP content nmol /10^8 kl	Mobility of sperm (%)	ATP content nmol /10^8 kl
Prior to freezing	82.3±5.9*	26.3±2.1	82.3±5.9	32.8±2.2**
5 min after thawing	31.6±2.7	15.8±1.*	33.8±2.3	19.2±0.9**
60 min after thawing	19.4±1.1	5.2±0.6*	22.3±1.6	7.0±0.3*
120 min after thawing	14.6±1.6	4.6±0.2*	19.8±0.9	6.2±0.5*

*, P<0.05; **, P<0.01.

level decreased by 37.7% compared with native samples. At the same time by 44% mobility and 36.8% of the number of sperm with damaged acrosome also decreased. Further incubation of the samples thawed at 37°C promoted a significant decrease in mobility and ATP content in spermatozoa. 2 h after thawing, the ATP content in sperm decreased by 5 times compared with the initial level after thawing (P<0.01). Consequently, in the process of freezing and thawing in boar, spermatozoa occur with significant destructive changes of membrane structures resulting in rapid ATP hydrolysis by enzymes which are contained in the cells and seminal plasma. It is established that glycolysis is unable to compensate the loss of ATP if breathing is impaired. Spermatozoa of boars quickly lose mobility in anaerobic conditions. Breathing in boar sperm is the main energy process ensuring the synthesis of ATP and as a result of complete combustion of one molecule of glucose to carbon dioxide and water during respiration, 38 ATP molecules are formed, while in the process of glycolysis of only two molecules. Accumulation of the energy of oxidation in the phosphate bond is subjected to conjugation of respiration and phosphorylation. However, perhaps the oxidation is not associated with the synthesis of ATP - the "free oxidation" in which the energy released in the breath of boar sperm is not transferred to the phosphorylating system and is released as heat. These two ways of oxidizing are synchronous and space is limited in the cell; phosphorylating oxidation is localized on the inner mitochondrial membrane and free on their surface. Correlation depends on its huge number of factors; the functional state of sperm, the degree of organization and order of the mitochondrial membrane and external influences. Swelling and disintegration of the structure of the sperm of boars during the cryopreservation leads to an increase in the proportion of free oxidation.

Inherent aerobic metabolism of boar sperm reduces resistance to low temperatures. The sharp decline in fertility-bath cryopreserve boar sperm from 89.4 to 47.2% can be explained by the predominance of aerobic processes in their power. The effect on the level of ATP in the sperm of different ways of diluting boar sperm before freezing was studied (Table 2). Analysis of Table 2 showed that the method of diluting the sperm before freezing have some influence on the motility and ATP content in cryopreserved boar sperm. In the conventional dilution of samples, there was much lower sperm motility (2.2 to 5.2) than in samples with dialysis treatment. Similar results were observed for the ATP content and after 5 min of dilution, ATP content in the samples treated by the dialysis was higher by 3.4 nmol/10^8 kl (or 12.1%) than in control. The superiority of the ATP content (1.6 to 1.8 nmol/10^8 kl) in gametes with dialysis treatment remained within two hours of storage. Received data indicated that the dialysis method of diluting boar semen promotes more effective protection of sperm cryopreservation processes.

This is probably due to better conditions for the stabilization of membrane structures of sperm in the process of dialysis treatment. At the same time, it may be that the low molecular weight components are disposed from the ejaculate, thus, having an impact on biodegradable membranes of boars spermatozoa.

Conclusion

In the process of freezing and thawing of boar, spermatozoa are significant destructive changes of membrane structures resulting in rapid ATP hydrolysis by enzymes, which are contained in the cells and seminal plasma. The sharp decline in fertilizing capacity of cryopreserved boar semen is due to the predominance of aerobic processes in their power. These findings are consistent with the findings of other researchers. According to the study of Moroz (1988) in boar, sperm glycolysis is unable to compensate the loss of ATP. According to them, by complete combustion of one molecule of glucose to CO_2 and H_2O, 38 ATP molecules is formed during respiration while in the process of glycolysis - 2 molecule. In the works of Moroz (1982) and Korban (1988) studied the processes of ATP synthesis in boar semen by direct measurement of the oxidative phosphorylation (P / O) on the entire sperm. To measure the amount of oxygen, the authors used the method of Paleography. It was found that the uncoupling of electron transport system is a major rapid cause of loss of motility and fertilizing capacity of sperm in boar. The superiority of the ATP content of spermatozoa from the dialysis treatment remained within two hours of storage. Studies of Rustenova (1996) established that the use of dialysis is to avoid osmotic shock sperm in the process of dilution and injection of glycerol and increase their resistance to cooling and freezing. Studies also suggest that the dialysis method of diluting boar semen before freezing provides the best cryoprotective effect on the sex gametes boars and promotes the preservation of ATP in sperm as compared with the conventional method of dilution of ejaculates.

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