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THE INFLUENCE OF HIGH TEMPERATURE ON THE PROTEIN FRACTIONS OF BULL SEMEN

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Abstract

Proteins are the most important organic substances which are part of the composition of living cells. Since the specific capabilities of spermatozoa depend on the protein, occurs the necessity of studying the characteristics of changes of the spectrum of the protein fractions of sperm depending on the ambient temperature. In this experiment was studied the effect of temperature of 45°C on the spectrum of protein fractions of the bull semen. From the data of the conducted research it is observed that when sperm of bull was exposed to a temperature of 45°C for 1 minute, has been a trend of increasing the quantity of albumin up to 4.2% and at 10 minutes - up to 3.3% of the total volume. When exposed to a temperature of 45°C for 1 minute the total amount of globulins, typically does not significantly change. The content of α-globulins tends to decrease, while the β- and γ-globulins do not support the changes. After 10 minutes of stress the amount of albumins is also increasing in comparison with the control sample and the quantity of total globulins decreased, especially α - and γ-globulins, while the content of β-globulin is not changed. Thus, the hyperthermic stress of bull semen for 1 minute is reflected only on the quantity of albumin, which has a tendency to increase. The influence of hyperthermic stress for 10 min is manifested by the tendency of growth of the percentage content of albumin and insignificant decrease in the concentration of total globulins, including α-globulin.

Key words*: protein fractions, spermatozoa, high temperature, hyperthermic stress, bull semen.*

INTRODUCTION

Intensification of reproduction of farm animals is impossible without fundamental studies of biochemical changes occurring in seed material in the process of manipulation with it. On the basis of experimental data it is possible to develop the technological methods of stabilizing the functional activity of the reproductive cells and use them for artificial insemination of animals.

Sperm of cattle is a complex system, the basic structural elements of which make up the spermatozoa. Their chemical composition is dominated by organic substances, which determine the viability and functionality.

Among of organic substances included in the composition of living cells, from the viewpoint of structural and biological roles, the most important are the proteins. They form the basis of protoplasm. The specificity of biological processes mainly depends on the composition of proteins with different structure and function. It is known that the reproduction and development of organisms is determined by the properties of conjugated protein - nucleoproteins of spermatozoa and egg cells (Mereuţă, 2010; Исаева et al., 2007).

From a chemical point of view the proteins belong to physiologically very active substances. Protein fractions that may exist in many states are markedly different from each other. Proteins, characterized by a high cooperative subjected to transformation with a relatively small change and limiting manifestation of the maximal cooperatively is the transition between two states. It is known that the chemical activity of proteins determines the physiological properties of cells (Борончук et al., 2003; Фурдуй et al., 2003; Юрченко, 1988).

The purpose of the research, the results of which are presented in this paper, was to study the characteristics of changes of the spectrum of protein fractions of sperm in depending on the ambient temperature, because the specific

opportunities and tolerance of cells, including sperm inevitably depend on proteins.

MATERIALS AND METHODS

The determination of the content of protein fractions was performed through method developed by Oll and Makford with changes of Carpiuc S.A. by the description of Holban D. M. et al. (Голбан et al., 1988).

The principle of the method is based on the properties of phosphate solutions of various concentrations to precipitate proteins. The value of optical density of solutions of different protein fractions was determined using spectrophotometric methods. We used the spectrophotometer SF-26. The color intensity of the solution was determined at a wavelength of 720 nm. The content of the separated fractions are calculated in percent. As the subject of research was used sperm from 5 bulls of Black and White breed at the age of 3 years. In research were used 68 of ejaculate. For stressing of bull semen. as a stress factor was chosen hyperthermia (45°C). Hyperthermic stressing of sperm was performed in a water bath where the temperature was maintained at 45°C. We studied the effects of short-term (1 minute) and long-term stress (10 minutes) at changes in protein fractions of sperm of the bull. To each experimental sample matched control sample (35°C).

Digital materials are statistically processed using the Student's t-test.

RESULTS AND DISCUSSIONS

Proteins which have chemical individuality. responds differently to different stress factors. This experiment consist of the study of influence of hyperthermic stress on the spectrum of protein fractions of bull semen. Obtained results are shown in table 1.

The data of table show that at the effects on spermatozoa of cattle for 1 minute. compared with control. there has been a shift towards increasing of amount of albumin from 1.9 to 4.2%. After 10 minutes of stressing of albumin increased in comparison with the control variant on 13.3% of the total content of protein fractions.

Table 1

The spectrum of the protein fractions of bull semen during of hyperthermal stress (45 °C)

Name of proteins	Content of protein fractions (%)		
	Control	The experimental variant	
		1 min	10 min
Albumins	1.9±0.43%	4.2±1.03%	13.3±1.01%*.**
Globulins	98.1±2.89%	95.8±1.19%	86.7±4.34%
α-globulins	6.6±1.17%	4.1±1.45%	3.3±0.84%*
β-globulins	32.7±4.38%	31.5±0.99%	30.6±4.8%
γ-globulins	58.8±3.13%	60.2±1.15%	52.8±3.38%

* P<0.05 compared with indicators from control sample

** P<0.05 the difference is statistically authentic between the variants of experience.

The amount of globulin at stressing for 1 minute did not undergo any significant changes. The content of α-globulin decreases from 6.6 to 4.1%, while β- and γ-globulin showed no obvious changes. When exposed for 10 minutes the concentration of globulin was decreased from 98.1 to 86.7% compared with the control. Also decreased the level of α-globulins from 6.6 to 3.3%, and γ-globulins from 58.8 to 52.8%, while the concentration of β-globulins has not changed significantly. Statistically authentic changes are observed not only at modification of content of albumin but and α-globulin in particular. Process of its variability , apparently, is aimed at increasing the adaptability of the whole cellular system of gametes to the action of unfavorable factors of hypothermia and, obviously, can be regarded as a manifestation of cell adaptation to the stress effect of cold, and therefore can provide a higher level of survival of gametes.

It should be noted that these changes occur only at long-term hyperthermic exposure, but probably and at short-term. In this regard, in the next series of experiments it was determined the short-term impact of hyperthermic temperatures (45°C) on protein fractions of sperm of the bull. The results of researches are shown in the diagram (fig. 1).

The figure shows that at short-term hyperthermic influence are subjected to insignificant changes the protein fractions of albumins and α-globulins. At a temperature of 45°C the content of the albumin fraction is definitely growing, and the concentration of α-globulin decreases slightly. Other fractions of proteins in experimental conditions have only a tendency to decrease.

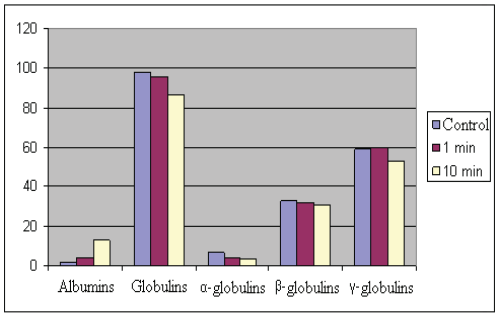

Figure 1. The protein fractions of semen of the bull at hyperthermal stress (45°C)

At short-term hyperthermic stress out of five revealed fractions only the content of albumin is increased but the magnitude of the concentrations of all other fractions is reduced. Because the total protein content of the seed of animals when exposed to high temperatures does not change, it can be explained by the denaturation of proteins and, in particular, these fractions.

It should be noted that the activity of proteins is caused by a sufficient (but not excessive) conformational flexibility. However, this condition is possible only in a limited temperature range, which lies within the optimum temperature of activity of gametes.

Significant temperature changes that occur in the process of conservation should disrupt the optimal ratio of lability and stiffness of cellular structures and thereby cause morphological and functional changes (Наук, 1991).

The reaction of protein macromolecules to the change of temperature are mainly determined by two temperature-dependent specific component included in the entropy of the system multiplied to the absolute temperature: conformational entropy and entropy is determined by hydrophobic interactions. In the field of physiological temperatures their influence is opposite on protein stability that has deep biological sense, since it smoothes the impact of temperature effects on the state of protein macromolecules (Александров et al., 1975).

It should be assumed that gametes must possess a variety of regulatory mechanisms that are, to a certain extent, can compensate for these changes. In the case where environmental factors on the strength and duration, prevail over the capabilities of these mechanisms, conformational mobility of protein molecules can be changed, and then in the cell at different levels of its organization, may occur destructive changes (Борончук et al., 2008).

The temperature change of any biological object can be considered as a powerful stressor effect, which causes a complex set of structural and functional changes (Фурдуй et al., 1992). In cellular systems during freezing don't have time to develop the adaptive change. However, the cooling of the gametes in the area of hypothermic temperatures during equilibration, although accompanied by a sharp decrease of metabolic processes, however, does not stop them completely. This is due to carrying out of adaptive-compensatory reactions.

Given that when the cells are gone from the body some time interval remain viable, can be considered possible to implement in them in this period of self-regulation processes due to the negative feedback aimed to restore the original level of living system as a whole (Юрченко, 1988). The study of adaptive-compensatory reactions of biological objects in the hypothermia is of great importance and is an integral part of the development of the theory of defense mechanisms in biology.

In the case of lack of effectiveness of adaptive-compensatory reactions in cells occur the temperature changes. It should be noted that the most labile cellular structures are membrane (Наук, 1991).

Thus, the hyperthermic stressing of semen of the bull for 1 minute reflects only the tendency towards increasing the percentage content of albumin and decrease of globulin. The outcome of exposure to hyperthermic stress for 10 minutes is manifested by changing upward of the percentage content of albumin, and is reflected downward at concentrations of α-globulin.

CONCLUSIONS

The researches allow making the following conclusions:

1. Changes of the protein spectrum of semen of the bull can be the result of the excess of intensity of the influence of the temperature factor over the adaptive-compensatory reactions of the spermatozoa.

2. Short-term stressing of spermatozoa of the bull does not cause significant changes in the quantitative composition of the investigated proteins.
3. The increased of duration of temperature exposure to ten times accompanied by an increase of content of albumin, while the total amount of globulins and their fractions are not subjected to significant changes.
4. Globular proteins of the sperm of bull are more resistant to the effects of temperature factor compared with their albumin fraction.
5. When creating temperature-protective mediums for sperm of the bull is necessary to consider the ability of their components to stabilize the adaptive-compensatory reactions and the preservation of proteins in particularly their albumin fraction.
6. The increase in the content of albumin fractions after thermal exposure of semen of the bull takes place by reducing the amount of globular proteins.

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