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SPECIES FEATURES OF THE CONTENT AND CRYOGENIC CHANGES IN THE PROCESS OF PRESERVATION OF SPERM OF FARM ANIMALS

Vladimir BUZAN, Ion BALAN, Nicolae ROŞCA, Gheorghe BORONCIUC, Iulia CAZACOV, Melania BUCARCIUC, Ion MEREUȚA, Alexandru DUBALARI, Nicolai FIODOROV, Nadejda ZAICENCO

Institute of Physiology and Sanocreatology, 1 Academiei Street, MD 2028, Chișinău, Republic of Moldova

Corresponding author email: vladimirbuzan@yahoo.com

Abstract

Experimental studies by using the spectrophotometric method revealed that lipid peroxidation is species specific. At the same time, the number of diene conjugates in rooster sperm is more than twice than in the bull sperm. The content of this product is not subject to cryogenic changes. The amount of hydroperoxides in rooster sperm is an order of magnitude higher than in bull sperm. It should be noted that cryogenic changes of this indicator are observed only in the bull sperm. While the content of malondialdehyde increased in the sperm of both species in the process of cryopreservation. In the process of technological treatment of sperm of various species of animals, an increase in the content of lipid peroxidation products is caused mainly by malonic dialdehyde, and not to diene conjugates and hydroperoxides, and is also species-specific; the effect of the influence of certain antioxidants on the functional state of gametes depends on the degree of stabilization of intermediate and final products of lipid peroxidation. The conclusion is made about the cryolability of hydroperoxides and malonic dialdehyde, the content of diene conjugates is not affected by cryogenic changes.

Key words*: cryopreservation, gametes, lipid peroxidation.*

INTRODUCTION

The reproductive cells of animals basically perform the same functions, the main of which is the transfer of the genome to the future generation. However, they differ significantly in morphology, the number and structure of the constituent components, which determines their cryoresistance.

At the same time, lipids, and especially phospholipids, are the main components of biological membranes, they are involved in such central biological phenomena as biosynthesis, reception, intercellular interactions and signaling, biological transport, regulation of membrane-bound enzymes activity, formation of the immune response, bioenergetic transformations and other (Наук, 1991; Рогинский, 1990). The understandings of the character of lipid peroxidation in biological objects are based on a large experimental material. However, under the influence of various factors lipids may be subjected to peroxidation.

According to the research of (Владимиров et al., 1975), the main reactions at lipid peroxidation are proceeding in a certain sequence (Fig. 1).

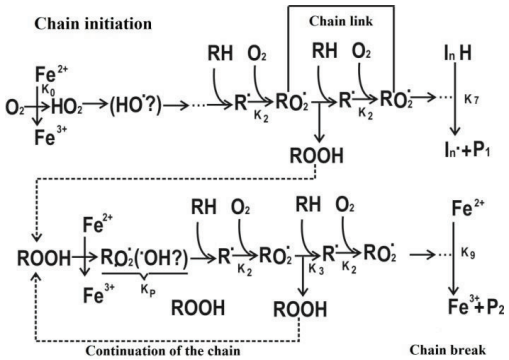

Figure 1. Scheme of reactions of lipid peroxidation (Владимиров, Ю.А. et al., 1975).

Where, RH - unsaturated residue in phospholipids; ROOH - corresponding hydroperoxide; R˙ - lipid radical; ROO˙ - lipid peroxide radical; HO˙$_2$ - superoxide radical; InH - radical reaction inhibitor; In - inhibitor radical; K_0, K_2 … - the individual reaction rate constants.

As can be seen from this scheme, lipid peroxidation is a chain of successive alternating reactions, the links of which are the stage of

initiation, branching, continuation and termination of the chains.

The only nonpolar cell regions are the middle hydrophobic zones of biomembranes, in which unsaturated fatty acids are located - the most susceptible to oxidative damage. Therefore, it becomes clear the value of lipid peroxidation in membrane destabilization and general cell pathology, since as a result of this process in the hydrophobic chains of fatty acids, hydrophilic areas are formed that significantly change the functioning of membrane structures (Владимиров et al., 1975).

Lipid peroxidation is a universal phenomenon of life, present everywhere, where there is molecular oxygen and where its active forms are formed under normal conditions: O_2, H_2O_2, OH, i.e. practically in every living cell and, first of all in biological membranes.

Lipid peroxidation is a normal process. Thus, free radical reactions, supported by special regulatory systems at a low stationary level, take part in normal metabolic processes and regulatory functions of the cell. By inhibiting or, conversely, accelerating lipid peroxidation can change the composition of cell membranes, their structural organization and functional activity of the cell. It was also established that the activity of membrane enzymes depends to a large extent on the lipid environment, therefore a change in the composition of biological membranes as a result of modification of lipid peroxidation processes causes inhibition of the activity of some and activation of other membrane-bound and membrane-dependent enzymes. The important physiological role of lipid peroxidation processes is confirmed by a number of studies (Бурлакова et al., 1985).

It is shown that lipid peroxidation is a mechanism of membrane disassembly and renewal. The dependence of the rate of lipid peroxidation reactions is demonstrated not only on the degree of saturation of fatty acids, but also on the structural organization of the lipid phase of biological membranes, which is the molecular mobility of lipids, the strength of lipid-lipid and protein-lipid interactions. In this case, covalently bound associations of membrane proteins can form, localization of integral proteins in the hydrophobic interior of membranes can change (Бурлакова et al., 1985).

Thus, lipid peroxidation is not only a universal modifier of the properties of biological membranes, but also an important physiological regulator of their structure and function, a factor establishing and maintaining the stationary functioning of lipid-dependent enzymes, channel formers, receptors, etc. This provision must be considered when developing techniques for modifying membranes in order to create membrane structures resistant to the action of factors of cryopreservation.

Free radical reactions of peroxidation are most effectively developed in lipid (phospholipid) structures and, first of all, in the lipid bilayer of membranes. At the same time, glutathione peroxidase, which adequately destroys lipid peroxides, does not penetrate into the hydrophobic zones, i.e. inside the lipid bilayer. Only the presence in it of tocopherols and carotenoids in it limits the development of lipid peroxidation. Possibly, the concomitant lipid peroxidation activation of phospholipase hydrolysis contributes to the elution of lipoperoxides from the membranes and their subsequent decomposition by glutathione peroxidase (Scherer et al., 1958).

So, in normal conditions of vital activity, in the functioning of living systems in conditions of physiological optimum, there is a pro- and antioxidant balance, which is the most important mechanism of oxidative homeostasis. This equilibrium is mobile in nature, it is an resultant of oppositely directed processes and is characterized by an oscillatory mode of functioning within the limits compatible with the preservation of homeostasis. Extreme conditions and any kind of structural damage of the living system are inevitably accompanied by activation of lipid peroxidation, a shift of pro- and antioxidant equilibrium (Boronciuc et al., 2003; Boronciuc et al., 2005). Moreover, under stress conditions caused by changes in environmental factors, decay processes are enhanced, the amount of oxidized or partially oxidized, including radical products, active forms of oxygen increases, and lipid peroxidation is activated. At the same time, antioxidant systems limit this activation, preventing the continuation and branching of the free radical oxidation chains, keeping the pro- and antioxidants balance within the limits of the optimum functional activity, within the

normal reaction. Only after exhaustion of the power of protective systems, with prolonged loads, when the consumption of antioxidant exceeds its reserve, the number of harmful peroxidation products increases, which leads to the development of damage associated with oxidative destruction of cellular structures, and, above all, biological membranes.

The main factors leading to the initiation of lipid peroxidation processes in the lipid bilayer of membranes during refrigeration, freezing and thawing are hypoxia, the lipotropic action of hypoconcentrated solutions and breach of the structural integrity of membranes. As a result of the action of these factors, in the membranes is observed an accumulation of the anion radical (O_2-), which, under the action of NADH, activates the processes of lipid peroxidation (Tynynyka, 2000).

Based on the above, the purpose of the research, the results of which are presented in this paper was to study the content of lipid peroxidation products and their cryogenic changes in the process of preservation of seed material.

MATERIALS AND METHODS

The object of the study was the sperm of roosters of the Rhode Island breed and the semen material of bulls of the Black-Motley breed which were kept in conditions corresponding to veterinary requirements. In the experiments for dilution and freezing of roosters semen was used maltose-arginine-petumoside-glycerol-yolk medium and for bulls sperm was used lactose-glycerol-yolk medium.

Semen freezing was carried out on the surface of the fluoroplastic plate with a volume of 0.1-0.2 ml, in vapors of liquid nitrogen, at a temperature of minus 110-120°C, followed by the transfer of granules into liquid nitrogen.

So, as in the process of lipid peroxidation there is a transmission of electrons from the donor to the acceptor, we can talk about donor-acceptor interactions, which were judged by the amount of malonic dialdehyde which was determined by the method of (Владимиров et al., 1972) in the modification of the collaborators of our laboratory, which consists in determining the concentration of gametes of the studied samples instead of protein, and concretizing the calculation formula.

Wherein the concentration of malonic dialdehyde was determined in nanomoles in the calculation of 10^9 gametes, taking the extinction coefficient equal to $1{,}56 \times 10^{-5}$ M^{-1} cm^{-1}., diene conjugates were determined spectrophotometrically at a wavelength of 233 nm by the method described by (Стальная, 1977), the content of lipid hydroperoxides was also determined spectrophotometrically at a wavelength of 480 nm by the method described by (Романова, 1977; Стальная, 1977).

Statistical processing of digital material was performed using the Student's t-test.

About donor-acceptor interactions were judged by the intensity of lipid peroxidation, by studying the cryogenic changes of content in the gametes of the final product of this process - malonic dialdehyde.

RESULTS AND DISCUSSIONS

Lipid metabolism, the functioning of lipid-dependent enzymes, proliferation rate and membrane permeability are determined by the level of lipid peroxidation. However, the increase in its speed relative to the norm may be the cause or a contributing factor of the damage of the gametes.

In the conducted experiments, about the intensity of lipid peroxidation in the bull and rooster semen was judged by the content of its primary, intermediate and final products. The results are presented in Table 1.

Table 1. The content of products of lipid peroxidation in the bull and rooster semen during the stages of cryopreservation

Technological stage	Lipid oxidation products		
	Diene conjugates, conventional units	Hydroperoxide, extinction units	Malonic dialdehyde, nm/billion.
	M ± m	M ± m	M ± m
Bull semen			
Dilution	1.6 ± 0.44	0.03 ± 0.004	20.4 ± 1.34
Refrigeration	1.3 ± 0.32	0.20 ± 0.02*	30.8 ± 3.02
Thawing	1.4 ± 0.11	0.87 ± 0.09*	50.5 ± 2.01*
Rooster semen			
Dilution	3.8 ± 0.39	0.26 ± 0.20	38.6 ± 7.82
Refrigeration	2.1 ± 0.06	0.3 ± 0.02	40.2 ± 1.08
Thawing	4.0 ± 0.47	0.72 ± 0.02	67.0 ± 2.48*
Note: * Cryogenic changes are statistically authentic			

From the table it follows that lipid peroxidation is of a species-specific nature. At the same time, the number of diene conjugates in the rooster semen is more than twice its amount in the bull semen. The content of this product is not subject to cryogenic changes. The amount of hydroperoxides in the rooster sperm is much higher than in the bull sperm. It should be noted that the cryogenic changes of this indicator are observed only in the bull semen. While the content of malonic dialdehyde increases in the semen of both species in the process of its cryopreservation.

In the process of technological treatment of semen of various species of animals, the increase in the content of lipid peroxidation products is due mainly to malonic dialdehyde, rather than diene conjugates and hydroperoxides and is also species-specific character; the effect of certain antioxidants on the functional state of gametes depends on the degree of stabilization of intermediate and final products of lipid peroxidation (Boronchuk and Balan, 2005).

Due to the fact that the content of malonic dialdehyde is more susceptible to cryogenic changes, in the following experiment was conducted a comparative study of the intensity of lipid peroxidation in bull and boar sperm (Table 2).

Table 2. The content of malonic dialdehyde in the gametes during cryopreservation of bull and boar sperm, nmol/billion gametes

Species of animals	Sperm processing stage		
	Dilution	Refrigeration	Thawing
	M ± m	M ± m	M ± m
Bull	13.7 ± 0.75	18.3 ± 2.13	22.3 ± 2.10*
Boar	22.5 ± 3.10*	26.4 ± 1.70	37.8 ± 3.80*
Note: * Cryogenic changes are statistically authentic			

The data of the table show that the greatest amount of malonic dialdehyde is found after thawing of boar sperm, in the gametes of the bull its amount is almost two times less. Consequently, the gametes of the first are more sensitive to peroxidation during the process of sperm cryopreservation.

In the process of refrigerating and freezing-thawing of the semen of the studied animal species, as in the previous experiment, the results of which are presented in Table 1, lipid peroxidation is enhanced in comparison with freshly diluted material by a statistically authentic value in all variants of the experiment.

Extreme environmental factors causing a disturbance of the homeostatic balance between pro- and antioxidant factors in cells contribute to the activation of lipid peroxidation, autocatalytic accumulation of peroxides, epoxides, oxidative radicals, etc. Obtained data convincingly show the need for regulation of lipid peroxidation of animal's semen.

Thus, lipid peroxidation is an important element in the cryo-damage of cells due to changes in the state of the membranes. Intensive development of lipid peroxidation deeply affects the basic functional systems of cells, causing a decrease in viability or complete destruction. However, lipid peroxidation can be regulated both endogenously due to the whole system of protective mechanisms and exogenously by the use of antioxidants and the creation of favorable conditions for the manifestation of the cell's own protective functions realized through donor-acceptor interactions.

CONCLUSIONS

At the regulation of the process of lipid peroxidation, the most likely are methods, as well as substances, that eliminate the initiation of chain branching and in particular its rupture.

Lipid peroxidation occurs at all technological stages and reaches the highest activity after freezing-thawing of the semen.

The change in the content of lipid peroxidation products in the process of cryopreservation is mainly due to malonic dialdehyde.

The more intense accumulation of lipid peroxidation products in the boar semen is presumably due to the higher content of unsaturated fatty acids in their lipids, whose double bonds are easily broken under the influence of various environmental factors.

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