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POSSIBILITIES OF INCREASING THE FUNCTIONAL ACTIVITY OF THE ROOSTER DEFROSTED GAMETES

Gheorghe BORONCIUC, Ion BALAN, Vladimir BUZAN, Nicolae ROŞCA, Iulia CAZACOV, Melania BUCARCIUC, Ion MEREUŢA, Nicolai FIODOROV

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

Corresponding author email: vladimirbuzan@yahoo.com

Abstract

As a result of experimental researches it has been established that the optimization of parameters at the development of new mediums helps to reduce the cryogenic risks in the preservation of the genetic resources of the rooster. The best results in the restoring of gametes physiological parameters of after cryopreservation in maltose-petumozid-arginine-glycerin medium and defrozen in isotonic sodium citrate solution are achieved at the dilution of semen 1:3, refrigeration at 2-4°C during 15 minutes and freezing for a duration of 2 minutes. The use of such cryobiological parameters allowed us to obtain similar results when using maltose-arginine-glycerol medium as in the control group. At the same time, the additional use of petumozide in the composition of experimental medium increases the mobility of thawed gametes of cock by 11.4%. The effectiveness of the developed medium was tested and in the production conditions. As a result, it was established that the hatchability of chicks reaches 68.8 ± 6.69 against 41.7 ± 7.12%, which is 27.1% higher in favor of the experimental variant. The results of laboratory and industrial experiments allow to recommend developed medium for cryopreservation of semen of the rooster and use in breeding practice.

Key words*: reproductive cells, rooster, cryopreservation.*

INTRODUCTION

The problems of anabiosis and cryopreservation occupy an important place in modern biology of animal reproduction. Their solution allows to develop various technologies for preservation of gametes, including new cryoprotective mediums and technological methods. It is important to note that the method of long-term storage of semen in a deeply frozen state has found wide application in the field of cattle breeding (Наук, 1991). However, the possibilities of this method are far from exhausted, as about half of gametes does not restore functional activity after thawing.

As for other industries such as sheep, pig, poultry and fish farming, this method has not found wide practical application. This is due to the insufficiently of research to identify the specific features of gametes' cryobiology and technological methods of reproduction of the studied species of animals. At the same time, it should be noted that significant progress has been achieved in solving these problems thanks to the fundamental work of a number of researchers (Нарубина, 1998; Сахацкий, 1990; Kopeika et al., 2000).

Advances in the field of cryobiology made it possible to experiment with other species of domestic and wild animals, and at the beginning of the nineties the list of species on which such experiments were based included more than a hundred species of mammals, birds, reptiles, amphibians, fish and invertebrates-echinoderms and molluscs.

For Cryobiology, the main problem is the preservation of biological objects in a viable state in conditions when active vital activity is turned off, the biological metabolism of substances, energy and information are minimized and at the same time the ability to return after defrozen to polyfunctional activity is provided (Грищенкоет al., 2004).

Research of the mechanisms of cryo-damages, the search of optimal cryoprotection conditions at different levels of the biological organization of gametes, besides theoretical, acquires also practical value.

Thus, at present, there are certain successes in solving a number of fundamental and applied

problems of reproduction of animals using cryopreserved material. However, further trends in the development of research in the field of cryopreservation of semen should be directed to a deeper study of the mechanisms of cryopreservation and cryoprotection of reproductive cells; continuation of research to identify the relationship between the chemical structure, physico-chemical properties and toxicity of cryoprotectants; creation of new more effective cryoprotective mediums. Further increase of the efficiency of the method of storage of semen of animals at liquid nitrogen temperature is currently carried out by creating new cryoprotective mediums, using both penetrating and non-penetrating cryoprotectants in the medium, excluding from the mediums of toxic components (Линникет al., 2010).

Increasing the effectiveness of cryopreservation of genetic resources is possible by carrying out fundamental researches with involvement of the modern equipment, mathematical methods for planning experiments and processing of the obtained digital material (Valcarcel et al., 1994). Thus, the solution of the problems of cryopreservation of the genome has a general biological significance and can be achieved on the basis of an interdisciplinary approach.

It should be noted that in the process of cryopreservation of genetic resources, there are a number of physico-chemical processes such as: temperature shock, changes in osmotic pressure, crystallization and recrystallization, phase transitions of proteins and lipids, morphological changes, condensation and decondensation of chromatin, destruction of nucleoprotein complexes. Therefore, the purpose of the research was to find ways to reduce cryogenic damage during preservation of the rooster sperm.

MATERIALS AND METHODS

As an experimental material used the sperm of roosters of the Rhode Island breed contained in the vivarium conditions of the Institute of Physiology and Sanocreatology.

Physiological indicators of gametes were determined according to general accepted methods using the "Ampleval" microscope of the manufacturer Carl Zeiss, at 200-fold magnification. The optimal concentrations of the used medium components were determined by the method of counter series by Милованов (1962). Semen freezing was carried out in liquid nitrogen vapors on the surface of the fluoroplastic plate in the form of open granules with a volume of 0.1-0.2 ml, thawing was carried out in an isotonic solution of sodium citrate at a temperature of 40-42°C.

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

RESULTS AND DISCUSSIONS

Having studied the peculiarities of the spermatozoa metabolism of different species of animals in the process of cryopreservation and having tested in laboratory conditions various substances as possible stabilizers of intermolecular interactions and functional activity of gametes at technological processing, there was arose a question about the development of ways to increase the cryoresistance of animals semen and their reproductive ability.

At developing methods for increasing cryoresistance, not only the specific results of our own research were taken into account, but also proceeded from the concept developed by us, in accordance with which the preservation of structural and functional homeostasis at the initial stages of cryopreservation is predetermined by specific protective reactions, and the occurrence of cryo-damages during freezing and thawing, although in many respects it has a specific character, is caused mainly by non-specific changes in the structural-biochemical homeostasis.

To improve the efficiency of cryogenic risks reduction we have developed a new cryoprotective medium for conservation of rooster semen.

Taking into account the influence of carbohydrates and antioxidants on the quality of the stored seed, which suggested the relative regulation of the non-electrolyte composition, the inhibition of lipid peroxidation processes, the intensification of the water vitrification process and maintenance of the charge of plasma membranes in the process of gamete cryoconservation, we performed research on the development of a new medium and the determination of optimal parameters cryopreservation of rooster sperm.

The components of the medium and their content are presented in Table 1.

Table 1.Composition of the medium for cryopreservation of the rooster sperm

Component name	Quantity
Maltose, g	11.5
Petumozide, mg	0.05
Glycerol, ml	7.0
Water, ml	100
Arginine hydrochloride, to pH 6.8-6.9	

Using this medium in subsequent experiments, was determined the effect of the degree of dilution on the quality of the defrozen rooster semen (Table 2) and investigated the optimal parameters of its cooling (Tables 3 and 4).

Table 2. Restoration of gamete mobility in dependence on the degree of dilution of the rooster sperm

Experimental variants	Degree of dilution	Mobility of gametes, point
		M±m
1	1:1	4.3 ± 0.14
2	1:2	4.1 ± 0.21
3	1:3	4.6 ± 0.11
4	1:4	4.4 ± 0.11
5	1:5	4.3 ± 0.14
6	1:6	4.1 ± 0.11*
7	1:7	4.0 ± 0.18*
8	1:8	3.2 ± 0.14*
9	1:9	3.0 ± 0.18*
10	1:10	3.0 ± 0.18*

Note: * Differences are statistically authentic in comparison with the third variant of the experiment

From Table 2 it follows that the best results on restoring the mobility of gametes after their thawing were obtained in the third variant, in case of dilution of the semen in a ratio of 1:3 with the elaborated medium.

The increase of the degree of dilution successively reduces the mobility of the gametes, which can be explained by a change in the buffer capacity in the system of the medium-semen of the cock (Balan, 2012).

The presented data in Table 3 show that the best results in restoring the mobility of gametes after their thawing were obtained in the third variant of the experiment, when the studied indicator reached 5.1±0.27 points.

Reducing or increasing of the duration of cooling does not improve the life longevity of gametes.

The reasons for this may be insufficient cooling time at which the entire volume did not reach 2-4°C or too long a time during which the products of metabolic processes can accumulate or a temperature shock may occur (Ostashko et. al., 2004).

Table 3.Restoration of gamete mobility in dependence on the duration of cooling of the rooster sperm

Experimental variants	Cooling of sperm, min	Mobility of thawed gametes, point
		M±m
1	5	4.1 ± 0.21
2	10	4.8 ± 0.29
3	15	5.1 ± 0.27
4	20	4.9 ± 0.33
5	25	4.5 ± 0.18
6	30	4.1 ± 0.21
7	35	3.8 ± 0.33
8	40	3.4 ± 0.21
9	45	3.2 ± 0.14*
10	60	2.7 ± 0.32*

Note: * Differences are statistically authentic in comparison with the third variant of the experiment

Table 4.Restoration of gamete mobility depending on the duration of freezing of the cock sperm

Experimental variants	Freezing of sperm, min	Mobility of thawed gametes, point
		M±m
1	1.0	2.4 ± 0.21*
2	1.5	3.4 ± 0.11*
3	2.0	5.3 ± 0.28
4	2.5	4.8 ± 0.22
5	3.0	4.4 ± 0.27*
6	4.0	4.0 ± 0.18*

Note: * Differences are statistically authentic in comparison with the third variant of the experiment

The presented digital material allows us to answer that the optimal duration of freezing is 2 minutes. Deviation from this parameter reduces the physiological parameters of thawed gametes, which may be due by crystallization or recrystallization processes (Crister et. al., 2004).

From the data of Tables 2, 3, 4 it follows that the best results on the recovery of thawed sperm motility are achieved by diluting it with ratio of 1:3, cooling at 2°C for 15 minutes and freezing for 2 minutes.

In order to determine the effectiveness of the new medium and the optimal technological parameters, a comparative experiment was conducted, as a control - the Watanabe medium (Table 5).

Table 5. Physiological indicator of rooster gametes cryopreserved in various mediums

Cryoprotectant medium	Mobility of thawed gametes, point
	M±m
1. Watanabe medium (control)	6.1 ± 0.11
2. Maltose-arginine-glycerin	6.3 ± 0.13
3. Maltose-arginine-glycerin-petumozide	6.8 ± 0.14*

Note: * Differences are statistically authentic

The data presented in the table show that the mobility of thawed gametes after cryopreservation using the maltose-arginine-glycerin medium is not significantly different from that in the control variant.

At the same time, the use of petumozide in the experimental medium increases the mobility of the thawed gametes of the cock.

Thus, for example, the mobility of gametes cryopreserved in the control medium reached 6.1 ± 0.11, and in a medium with petumozide – 6.8 ± 0.14 points.

Based on the above results obtained in the laboratory conditions, the experience was performed in production conditions.

The research was conducted using the semen of roosters belonging to the "Chetrosu" breeding center of the Criuleni district of the Republic of Moldova. The results are presented in Table 6.

Table 6. Results of incubation of eggs obtained from hens inseminated with cryopreserved rooster sperm in the form of granules

Indicators of experience	Name of mediums			
	Maltose-arginine-glycerin	Maltose-arginine-glycerin with petumozide	Watanabe medium (control)	Watanabe medium (control) with petumozide
Incubated eggs, pieces	48	48	48	48
Fertilized eggs, pieces	19	34	23	31
Fertilization of eggs, %	39.6 ± 7.06	70.8 ± 6.56*	47.9 ± 7.21	64.6 ± 6.90
Chickens were hatched, pieces	19	33	20	31
Hatchability of chickens, %	39.6 ± 7.06	68.8 ± 6.69*	41.7 ± 7.12	64.6 ± 6.90

Note: * Differences are statistically authentic

From the data of Table 6, it can be seen that the use of maltose-arginine-glycerin medium with petumozide makes it possible to achieve egg fecundity equal to 70.8±6.5%.

In the control variant, the investigated indicator is 23% lower. It should be noted that the introduction of petumozide in the composition of control medium Watanabe also increased the fertilization of eggs. However, the studied indicator was still higher in the case of application of developed by us maltose-arginine-glycerine-petumozide medium. A similar conclusion can be made on the hatchability of chickens.

Thus, the results of laboratory and production experiments allow us to recommend the medium which we developed for cryopreservation of the rooster sperm and use it in breeding practice.

CONCLUSIONS

The researches allow making the following conclusions:

1. Optimization of technological parameters at the development of new mediums allows reduce cryogenic damage in the process of preserving the genetic resources of the rooster.

2. The composition of synthetic mediums determines the degree of preservation of the structural and functional state, and the technological parameters contribute to strengthening of their cryoprotective properties at the conservation of rooster sperm.
3. The best results in restoring of the physiological indicators of gametes after their cryopreservation in maltose-arginine-glycerin-petumozide medium and deconservation in isotonic solution of sodium citrate are achieved at the dilution of semen 1:3, cooling at 2-4°C for 15 minutes and freezing for a duration of 2 minutes.

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