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OPPORTUNITIES TO IMPROVE THE EFFICIENCY OF REPRODUCTION OF FARM ANIMALS

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Abstract

The method of long-term preservation of mammalian semen in deep-frozen condition provides great opportunities for development and improvement of the system of reproduction of farm animals. Using this method it is possible to check the breeders on the quality of offspring so as to maximum use the improvers. This allows to perform the large-scale genetic selection in animal husbandry, which significantly increases the rate of mass improvement of breeding and productive qualities of animals. However, the existing cryotechnology not provide maximum preservation of the biological integrity of the reproductive cells. Comprehensive research has shown the possibility of increasing the efficiency of cryopreservation by improving of synthetic mediums and the development of optimum process parameters cryopreservation.

Key words*: synthetic mediums, cryopreservation, spermatozoa, efficiency of reproduction, farm animals.*

INTRODUCTION

The most important condition of the dynamic growth of livestock production, along with the providing a full feeding and increasing the productivity of animals, is the intensification of reproduction of the herd, the effective use of the biological capacity of a female livestock and high-value breeders on the basis of wide application of the method of artificial insemination. This method, and especially the possibility of long-term seed storage in deep-frozen state allows to radically accelerate the tempo of evaluation of breeders at quality of offspring, to rational use their genetic potential and in the shortest possible time to increase the potential productivity of the herds, to save and restore the gene pool of rare and endangered species. Further improvement of the method of freezing the semen of animals provides the reduction of losses of spermatozoa during freezing - thawing, increasing of the safety of their functional activity and, consequently, the effectiveness of insemination, as well as elimination of certain technological difficulties. The solution of these problems requires an in-depth study of the mechanisms of cryodamage and cryoprotection of sexual cells, the study of protective properties of the individual components of the synthetic mediums which are used for dilution of the seed and improve of existing cryotechnology. On this concentrated the attention of researchers working in different fields of knowledge (Blesbois et al., 2007; Zamfirescu et al., 2010).

Based on the above, the aim of the research was to explore the possibilities of increasing the efficiency of cryopreservation and the use of seed in artificial insemination of farm animals.

MATERIALS AND METHODS

The object of the study was the semen of the bulls of Black and White breed, the rams of Ţigaie breed, the boars of Large White breed, the roosters of Rhode Island Red breed and seed of carp. The optimal composition and concentration of components of cryoprotective mediums for freezing sperm of animals was determined by the method of consecutive rows (Милованов, 1962).

The semen was frozen in the form of granules with volume of 0,1 - 0,2 ml and in polymer

straws of 0,25 ml. Thawing was performed in water bath at 40 °C or using aerodynamic device.

The amount of cholesterol was determined by the method Ilka (Благоразумова, 1965), at the wavelength of 665 nm with a spectrophotometer SF - 26. The content of loosely bound cholesterol was determined by the formula:

$$X = A*B/C*D$$, where:

X – the amount of cholesterol in μg per one billion of cells,
A – data obtained from the calibration curve,
B – multiplicity of the dilution of seed,
C – the sperm concentration (billion),
D – the volume of the diluted seed, ml.

The activity of aspartate transaminase (AST) were determined by the method of Paskhinna (Пасхина, 1959). Data were obtained on the spectrophotometer SF - 46 at a wavelength of 530 nm. Calculation of AST activity was performed according to the formula:

$$T = A*B/C*D*0,1$$, where:

T – the activity of AST, in IU per 1 billion of spermatozoa,
A - multiplicity of the dilution of seed,
B - the activity of AST was found in the table,
C - the sperm concentration (billion),
D – the volume of the diluted plasma in the sample, ml
0,1 – the volume of plasma required for research.

Study of morphological changes in the acrosome of the spermatozoa was performed using phase-contrast microscopy by the use of interference microscope LPI - 5. Microscopy was performed under immersion at 1500 of times magnification. Smears were fixed with 1% solution of sodium fluoride. The experimental data was processed by the method of variation statistics using the Student's t-test.

RESULTS AND DISCUSSIONS

Raising of the effectiveness of cryopreservation of semen of animals is possible by improving of the cryoprotective agents and the use of effective technological methods. However, they can be developed on the basis of experimental studies, performed at different levels of organization of biological objects using substances distinguished by their mechanism of action.

One of the promising approaches in this direction is the introduction in the composition of the mediums of such components, which provide the possibility of formation of new bio-complexes between components of plasma membranes and synthetic medium. In this regard, we studied the effect of amino acids belonging to different groups, on the functional indices of thawed spermatozoa of the rooster after freezing it in synthetic mediums (Борончук et al., 2008; Фурдуй et al., 2013), which contained amino acids with acidic side chains (aspartic acid), basic side chains (arginine), hydrophobic (valine) and neutral (alanine) (table 1).

Table 1

Effect of exogenous amino acids on the quality of the thawed semen of the rooster

Name of amino acids	Motility of thawed gametes, points
Alanine	3,8 ± 0,14
Arginine	4,4 ± 0,12*
Aspartic acid	3,9 ± 0,21
Valine	4,6 ± 0,11*
Medium without amino acids (control)	3,9 ± 0,11

*The difference is statistically authentic

The data presented in the table 1 show that after freezing semen of the rooster in medium which contain examinee amino acids the motility of spermatozoa varies in the range of 3,8 ± 0,7 to 4,6 ± 0,11 points.

It is known that the surface of the spermatozoa carries a negative electric charge, so the efficiency of amino acid easier to explain from the point of view of their classification, according to the state of residue of amino acids in a protein chain. In this case, amino acids are classified into polar and non-polar. Arginine and aspartic acid, according to this classification refers to polar amino acids. They are able to form hydrogen bonds, acting thus on the structure of water in the cryopreservation process. However, in the physiological range pH is 6 – 8, arginine has a positive charge but aspartic acid - negative. Based on these considerations, the positive effect of arginine may be due to the formation of bio-complexes with components of plasma membranes carrying electronegative charge. Aspartic acid cannot form such biocomplexes which explains its lower efficacy.

Alanine and valine are non-polar amino acids. The different effectiveness of these amino acids can be explained by the fact that valine has a non-polar residue that is inside the protein globule, and at alanine there is no clear distribution of amino acid residues in different parts of the protein molecule (Balan, 2013; Наук, 1991).

Previously, in our laboratory it was shown that steroid glycosides kapsikoside and purpureatoside, possess antioxidant capacity in the cryopreservation of sperm of the bull.

Therefore, we considered it appropriate to investigate a series of steroid glycosides as antioxidants in the cryopreservation of sperm of the different species of animals (table 2).

Table 2

The influence of steroid glycosides on the motility of frozen–thawed gametes of different species of animals

Name of steroid glycosides	Motility of thawed gametes, points
The sperm of the bull	
Petumoside – 2	3,8 ± 0,12*
	3,4 ± 0,10
Rusticoside	3,8 ± 0,12*
	3,4 ± 0,10
Melangoside	3,7 ± 0,18
	3,6 ± 0,07
Lilia – H	4,2 ± 0,28
	3,6 ± 0,33
Trioside – Lilia	4,1 ± 0,11
	3,8 ± 0,22
Asparagoside - H	4,4 ± 0,11*
	3,5 ± 0,11
The sperm of the rooster	
Petumoside – 2	5,8 ± 0,21*
	4,8 ± 0,20
Strophantine	4,7 ± 0,31
	4,2 ± 020
Lilia – H	6,1 ± 0,17*
	5,2 ± 0,12
Trioside – Lilia	6,3 ± 0,23*
	5,4 ± 0,17
Asparagoside - H	6,7 ± 0,18*
	5,9 ± 0,17
The sperm of the boar	
Petumoside – 2	3,7 ± 0,34*
	2,5 ± 0,29
The sperm of the carp	
Balconoside	4,2 ± 0,20
	3,8 ± 0,21
Melangoside	3,3 ± 0,21
	3,8 ± 0,20
Petumoside – 2	4,7 ± 0,74
	3,8 ± 0,14
Rusticoside	4,2 ± 0,20
	3,8 ± 0,21

*The difference is statistically authentic in comparison with the experimental variant. The numerator provides the data of the experimental variants, the denominator - the data of control variants.

From the data presented in table 2 follow that the steroid glycosides increases the quality of the thawed semen of the bull, rooster and boar. Thus, the use of the drug Lilia – H allows to increase the motility of thawed spermatozoa of the rooster by 17,3% compared to the control variant, where the antioxidants were not used. However, some glycosides exhibit high protective properties, as shown above, while others, such as Melangoside, at the cryopreservation of sperm of the bull is less effective. It should be noted that the optimal concentration of drugs varies in the range of 0,015 – 0,312 mg. per 100 ml. of the medium, even within the semen of the same species (the semen of the bull). While experimenting with the semen of other species of animals the concentration of substances is also changing. Also noteworthy is the fact that some steroid glycosides (Lilia - H) is effective in the cryopreservation of sperm of some species of animals (rooster), in another case (Asparagoside - H) other animal species (bull). But if there are cases (Petumoside – 2) when the drugs are effective in the cryopreservation of sperm of several species of animals (bull, rooster, boar), still it is different in the cryopreservation of semen of various species of animals (Petumoside – 2 is more effective in the freezing of seed of the boar).

Steroid glycosides were tested by us in the cryopreservation of seed of the carp. It was found that the tested antioxidants do not have a significant protective effect, which may be explained by the fact that their efficiency in the cryopreservation is associated with different hydrophobic–hydrophilic interactions of molecules of steroids. More hydrophobic steroid glycosides have less biological activity (Давыдов, 1986), as well as features of seed of the carp and used cryopreservation techniques.

Since in the process of cryopreservation is disturbed the stability of bond of protein-cholesterol complexes and occur the changes of acrosome of the most labile structures, then one would assume that this is accompanied by loss of enzymes. These enzymes include glutamic-aspartic transaminase, localized in the mitochondria. Leakage of this enzyme into the extracellular space indicates at the serious intracellular damage. These indicators play an important role in maintaining of the functional state of the spermatozoa and can be used to predict of their fertilizing ability. In this regard, we investigated the possibility of stabilizing of these indicators through applying of different cryopreservation techniques (table 3).

The data presented in table 3 indicate a better stabilization of morphological indicators and safety of protein–cholesterol complexes in the case of freezing semen in plastic straws.

Table 3

The indicators of sperm of the ram which were cryopreserved in a different ways

№ of indicators	Method of packing	
	granules	mini straw
I	The content of intact acrosome, %	
	44,0 ± 1,23	48,4 ± 1,09*
II	The content of loosely bound cholesterol, µg/billion	
	328,5 ± 17,94	417,4 27,81*
III	The activity of glutamic-aspartic transaminase IU (billion)	
	144,8 ± 24,87	152,4 ± 27,09

*The difference is statistically authentic between the methods of cryopreservation

What concerns the activity of glutamic–aspartic transaminase, we observed a similar trend. Improving the efficiency of cryopreservation of semen can be explained by the fact that the cylindrical shape of the packaging is more preferable, since it influences the formation of crystals, thus preventing significant morphological and biochemical changes in thawed spermatozoa (Андреев et al., 2014).

In separate production experiments were found that fertility (e.g. cows) is higher by 5,5% in the variant where the sperm of the bulls was frozen in plastic straws in comparison with the packaging in the form of granules. Summarizing the results of the conducted research it can be concluded that the prospect of research in the field of cryopreservation of reproductive cells is purposeful synthesis of components of cryoprotective medium.

CONCLUSIONS

The researches allow making the following conclusions:

1. Increasing the effectiveness of artificial insemination of farm animals is possible by improving of the cryoprotective medium and optimization of the technological methods that would contribute to a more complete manifesttation of the cryoprotective properties of new components of synthetic mediums.
2. Best cryoresistance of semen and reducing of the damaging effects of low temperatures can be achieved by the use of new cryoprotective agents, the regulation of lipid peroxidation, the use of polar compounds and through creating favorable conditions for cryopreservation of seed of animals.
3. Stabilization of resistance of protein-cholesterol complexes, morphological structures, activity of glutamic-aspartic transaminase and motility of spermatozoa of semen of the ram breeders are better achieved with the use of cryopreservation technology in polymer straws.

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