

MAINTENANCE OF REPRODUCTIVE HEALTH WHEN USING MEXIDOLIN THE COURSE OF A SPERM CRYOPRESERVATION

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Abstract

Maintenance and strengthening of reproductive health is defined by need of the solution of problems in the field of veterinary and human medicine. In the conducted researches were used physiological, cryobiological and statistical methods. Their use allowed to establish that mexidol as the substance from a class 3-oxipurin, with concentration of 0,026 mg % in composition of the cryoprotective medium promotes increase of mobility, life expectancy and an absolute index of survival of spermatozoa after thawing of sperm of a bull, a ram and the man. These indicators substantially determines the reproductive health of posterity. The optimal concentration of mexidol has no specific meaning and is common for all of the studied species. Its positive effect is determined by the antioxidant properties, thanks to which it is possible to recover the structure and function of biological membranes after cryopreservation.

Key words: sanogenic sperm, mexidol, reproductive health, sanogenity, freezing, defrosting.

INTRODUCTION

In the case of infertility couples, reproduction is performed through the method of transfer of embryos, *in vitro* fertilization and artificial insemination with the native or frozen sperm (Дунаевская, 2000).

Cryopreservation of sperm causes a decrease of its quality. It is necessary to increase period of storage of frozen sperm and improve their methods of freezing.

It is known, that for increase the fertilization of frozen sperm are used physical factors (ultrasound, laser, permanent magnetic field), biologically active substances (follicular fluid, blood serum) and pharmaceuticals (prostaglandins, pentoxifyllins, progesterone, caffeine, antioxidants) (Борончук, 1999; Евдокимов et al., 2010; Казакова, 1992; Меркуриева, 1964; Наук, 1987; Родионова et al., 2008).

It is necessary to explore new conditions and protectors to improve indices of sperm after freezing and defrosting through the study of antioxidants, which causing inhibition of lipid peroxidation processes and increasing the antioxidant effect and activity of antioxidant

system, providing increased sanogenity of spermatozoa.

Hence the objective of the study was to determine the effect of different concentrations of mexidol on physiological indicators of sperm of human and animals which characterize the sanogenic sperm.

MATERIALS AND METHODS

The material of study was ejaculate from donors with a normal sperm. The sperm of breeding bulls were collected in "vivarium" conditions. The study was conducted in the laboratory of CryosanoCreatology 'V.Nauc'. Mobility of a spermatozoa was determined by the usual method, and the concentration by means of the camera Goreaev (hemocytometer) or photoelectric colorimeter.

The sperm was diluted in proportion of 1:3. The freeze was held in the form of granules on fluoroplastic plate in the volume of 0.1 ml, in pairs of the nitrogen at a temperature of -110 – -120°C with the subsequent diving in liquid nitrogen. Defrosting of the sperm of ram is carried out with the help of a special device to thaw at a temperature of 60°C with the separation of the thawed fractions.

Defrosting of the human sperm and the bull was carried out with the help of a special device at a temperature of 38°C.

The survival of sperm in hours was defined in the conditions of incubation of diluted sperm at a temperature of 38°C, and the absolute survival rate was determined through the method of V. Milovanov.

The obtained data were processed with the help of Student's t-test.

RESULTS AND DISCUSSIONS

It was determined the antioxidant effect of mexidol. This substance belongs to the class of 3-oxypyridine and it is synthesized in the Institute of Physical Chemistry of the Academy of Sciences of Russia.

Taking into account, chemical and biological properties of the mexidol we investigated its effect in the composition of medium — lactose-glycerol-yolk (LGY) in different concentrations for dilution and freezing of the sperm of ram (table 1).

Table 1. The effect of mexidol in the medium lactose-glycerol-yolk on physiological indicators after freezing and defrosting of the sperm of ram, n=8

Variant of the medium	The concentration of mexidol in the medium LGY, mg/%	Physiological indicators which characterize the sanogenic sperm after freezing and defrosting		
		Mobility in points	Survival at 38°C, hours	Absolute survival rate at 38°C, c.u. (conventional units)
	0 (control)	2.6±0.19	6.3±0.27	11.22±1.17
	2.55·10 ⁻⁴	2.7±0.17	6.4±0.20	13.22±1.46
	2.55·10 ⁻³	2.5±0.20	6.6±0.28	14.70±1.67
	2.55·10 ⁻²	2.7±0.10	7.0±0.29	14.75±1.50
	2.55·10 ⁻¹	3.1±0.10*	7.3±0.17*	16.65±0.80**
	2.55	2.8±0.10	7.4±0.2*	14.63±1.30
	25.5	2.8±0.14	6.8±0.27	13.21±1.27
	255.0	2.3±0.20	6.1±0.24	7.25±2.10

The difference is statistically authentic, *P < 0.05; **P < 0.01.

The analysis of the obtained experimental data (tab. 1) shows that mexidol in the concentration of 2.55·10⁻² mg/%, in the medium of lactose-glycerol-yolk for dilution and freezing of the sperm of ram increases physiological indicators which characterize the sanogenic sperm, such

as survivability, mobility and absolute survival rate after freezing and defrosting.

Next, we investigated the effect of mexidol in the medium of lactose-glycerol-yolk (LGY) at the frozen sperm of bull (table 2).

Table 2. The action of mexidol in the composition of medium lactose-glycerol-yolk on physiological indicators after freezing-defrosting of the sperm of bull, n=8

Variant of the medium	The concentration of mexidol in the medium LGY, mg/%	Physiological indicators which characterize the sanogenic sperm after freezing and defrosting		
		Mobility in points	Survival at 38°C, hours	Absolute survival rate at 38°C, c.u. (conventional units)
	0 (control)	3,8±0,14	8,8±0,17	18,70±1,18
	2,55·10 ⁻⁴	3,9±0,26	9,3±0,26	24,21±2,50
	2,55·10 ⁻³	3,8±0,14	8,6±0,49	20,40±2,05
	2,55·10 ⁻²	3,9±0,22	9,9±0,37*	25,43±1,67*
	2,55·10 ⁻¹	3,6±0,13	9,6±0,49	28,60±2,60
	2,55	3,6±0,22	10,0±0,49	25,80±2,76
	25,5	3,4±0,22	9,3±0,17	18,30±1,54
	255,0	2,2±0,28**	6,9±0,98	9,28±1,67**

The difference is statistically authentic, *P < 0,05; **P < 0,01.

Experimental data of table 2 show that mexidol possessing antioxidant and membrane active properties in the composition of medium lactose-glycerol-yolk, depending on the concentration acts differently on the restoration of physiological indicators which provide sanogenity of sperm. Mexidol concentration of $2.55 \cdot 10^{-2}$ mg /% in medium lactose-glycerol-yolk (LGY) provides increase of physiological

indicators which characterize the sanogenic sperm.

The concentration of the mexidol used in the above experiments were extrapolated on the human sperm. It was studied the influence of mexidol in the composition of medium — sucrose-glycerol-yolk, which can be more beneficial for the freezing of human sperm.

Table 3. The action of mexidol in the composition of medium sucrose-glycerol-yolk on the physiological indicators, which characterize the sanogenic sperm after freezing-defrosting of the human sperm, n = 5

Variant of the medium	The concentration of mexidol in the medium SGY, mg/%	Physiological indicators which characterize the sanogenic sperm after freezing and defrosting		
		Mobility in points	Survival at 38°C, hours	Absolute survival rate at 38°C, c.u. (conventional units)
1	0 (control)	2.3±0.16	6.0±0	8.95±0.31
2	$2.55 \cdot 10^{-4}$	2.5±0	6.6±0.27	10.75±0.85
3	$2.55 \cdot 10^{-3}$	2.8±0.14	7.0±0.35	13.05±1.23
4	$2.55 \cdot 10^{-2}$	3.2±0.14	7.6±0.27	14.5±1.38*
5	$2.55 \cdot 10^{-1}$	3.1±0.11	7.8±0.22	14.35±1.35
6	2.55	2.3±0.14	6.6±0.45	10.45±1.27
7	25.55	2.1±0.11	5.8±0.22	7.45±0.59
8	255.0	2.0±0	5.4±0.27	7.0±0.31

The difference is statistically authentic, *P <0.05.

As a result of research it was concluded that the concentration of mexidol of $2.55 \cdot 10^{-2}$ mg/% allows to improve the physiological indicators, which characterize the sanogenic sperm (table 3). A further increase in the concentration of mexidol in the composition of medium leads to decrease of physiological parameters.

The positive impact of mexidol on cryo-preservation of sperm of different species may be explained by the fact that this substance possesses properties which are similar to vitamin B6. This preparation is not toxic and has a wide spectrum of biological action. It is used in medicine for the incubation *in vitro* of the human spermatozoa. Mexidol causes the slowdown of the reactions of peroxidation of lipids, activates the synthesis of proteins, nucleic acids and processes of fermentation of the Krebs cycle, restores the structure and function of membranes (ЕВДОКИМОВ et al., 2010; Казакова, 1992).

Thus the inclusion of mexidol in the composition of medium contributes to the maintenance of the sanogenity indices.

CONCLUSIONS

The researches allow to make the following conclusions:

1. Mexidol has a positive effect on physiological parameters, which characterize the sanogenic sperm.
2. Among all of the studied variants of concentrations of mexidol for the sperm of all species (ram, bull and human) the priority has a concentration of $2,55 \cdot 10^{-2}$ mg/% in the composition of medium for freezing.
3. We suggest for future studying and other representatives from class 3-oxipurin in the composition of medium for freezing of sperm of human and animals.

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