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Use of Thiol Compounds for Preservation of Functional Cryopreserved Sperm of Boar

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Abstract. The purpose of this study is the study of SH-groups of semen of boars, to determine the presence or absence of links between the level of SH-groups and the quality of sperm, to examine the effect of thiol compounds on the functional integrity of cryopreserved sperm of boar usefulness. The aim is to study the effect of thiol compounds on the quality indicators and krioustoychivosti sperm, they can be used for short-term and long-term storage of gametes boars

Keywords. sulfhydryl (SH) groups, biological activity, thiols and proteins, complex biochemical, fertilization process, sperm acrosom, plasma, structural elements, membranes, thiol compounds - unitiol ditioiteitol, effects, temperatures. kriostability of gametes, preservation of their functional usefulness, spermatozoa of boars.

1. Introduction

Among the functional groups of protein molecules there are of distinguished high reactivity and diversity of its sulfur-containing chemical reactions, particularly the sulfhydryl (SH) groups, necessary for the manifestation of biological activity of many proteins and maintenance of their macromolecular structure. A variety of substances containing sulfhydryl (SH-) groups in the germ cells are not less important than in other living systems. W. Green [1]; C. Zittle, R. Odell [2]; R. Berry D. Mayer [3] and others believe that the role of SH-groups of thiols and proteins in sperm is due to their involvement in the complex biochemical processes. They provide the necessary energy reproductive cells, creating conditions for the fertilization process. Despite the contradictory results obtained by researchers, it can be concluded about the important role of sulfhydryl groups in the metabolic processes of living cells, including germ cells and animals.

2. Methods of Research

The experience with 12 boars and 128 sows, manufacturers of large white breed of the pig farms "Dostyk", "Didar" and "Aksunkar" in South-Kazakhstan region. The quality of the ejaculate was evaluated by conventional methods and in accordance with the Regulations. Sperm cryopreservation was performed by the "Instructions of the organizations of technology of artificial insemination and embryo transfer of farm animals."

The content of total sulfhydryl groups in plasma of semen and washed disintegrated spermatozoa was determined by amperometric titration [4], acrosome integrity was assessed by phase-contrast microscopy. To assess the integrity of the sperm acrosome used microscopy stained with semen karboleozinom [5]. In determining the P / O measurement of oxygen uptake and inorganic phosphorus was carried out by LG Frost et al [6]. Total activity of lactate dehydrogenase was determined by fenilgidrazinovym calorimetric method.

3. The Results of the Study

Study of 59 ejaculates obtained from 12 boars of Large White breed, found that in 100 ml of sperm plasma contains in average 67.3 micromoles of sulfhydryl groups (Table 1).

Table 1: Distribution of freshly ejaculates of boars on the content of SH groups in plasma and in spermatozoa

The content of SH-groups in 100 ml of plasma			Content of SH-groups in the 10 billion sperm, mcmol		
mcmol	in the number of ejaculates	in %	mcmol	in the number of ejaculates	in %
6 - 20	1	1,7	5,1-7,0	9	15,2
21- 40	9	15,2	7,1-9,0	15	25,4
4 - 60	19	32,2	9,1-11,0	19	32,2
61 - 80	14	23,8	11,1-13,0	10	17,0
81 - 100	7	11,8	13,1-15,0	4	6,8
101 - 120	4	6,8	15,1-17,0	1	1,7
121 - 140	3	5,1	17,1-19,0	1	1,7
141 - 160	1	1,7			
160 - 200	1	1,7			

Upon transfer to the intensive use of sires in parallel with the deterioration of sperm quality, a sharp decrease in concentration of SH groups in plasma happens. Upon transfer of the boars for everyday use in the first ejaculates there were 52 micromoles, and secondly - 46 mol, in the third - 46 mmol in the fourth - 31 micromol SH groups per 100 ml of plasma. Return to the moderate regime led to the gradual recovery of the initial level of SH-groups. In the spring and summer months, the concentration of SH groups in plasma is 24-29% higher than in autumn and winter. The content of SH-groups in the 10 billion sperm cells in the range from 5.1 to 19.0 mol is in average of 8.7 micromoles.

Thus, the semen of boars contain a significant amount of low molecular SH-groups of thiols and proteins, which apparently are involved in complex biochemical processes occurring in cells, as well as in maintaining the structural elements of cells and membranes of sperm.

Table 2: Effect of cold shock and freezing and thawing on the content of sulfhydryl groups in sperm

Experimental effect on sperm	The number of SH-groups, mcM			
	10 billion spermatozoa		in 100 ml of plasma	
	M+m	%	M+m	%
Freshly	10,3±0,36	100,0	2,4±0,01	100,0
Subjected to the shock of	8,8±0,28	85,4*	2,3±0,01	95,8
Frozen-thawed without kriofilaktov	6,2±0,02	60,2**	2,1±0,01	87,5
Freshly diluted	12,8±0,87	100,0	-	-
Frozen-thawed with kriofilaktom	8,5±0,63	66,4**	-	-

* - P <0,05; ** - P <0,01

In sperm exposed to thermal shock there was a decrease of the quantitative content of SH-groups by an average of 14.9%. The observed reduction in plasma was not significant (Table 2).

For individual boars ejaculate subjected to temperature shocking, reducing the number of sulfhydryl groups in sperm ranged from 14.2 to 15.9%. Freezing semen of boars without the use of cryoprotectants resulted in a decrease in quantitative sulfhydryl groups. Fluctuations in the indicator of sperm ranged from 32.1 to 44.9%. Average for all ejaculate overall reduction of SH-groups was - 39.8%.

In plasma, sperm there was also observed decrease in the SH-groups in average - 13.7%. Compared with baseline (freshly diluted sperm). When freezing and thawing the content of SH-groups in plasma decreased by an average - 32.8%. However, we found no significant difference in reduction of the content of SH-groups in the sperm between boars.

Consequently, the stability of SH-groups of proteins to the sperm damaging effect of low temperatures is a more conservative trait not having individual differences, at least within the same breed.

Among the used thiol compounds cysteamine was toxic to the sperm of boars. When adding cysteamine medium of GHTSZHK sperms lost motility, viability sharply gametes decreased and therefore no further reagent was used.

Cysteine at concentrations of 10-3M caused a significant increase in survival of spermatozoa, but no effect on their survival (Table 3). All other concentrations of cysteine had no significant effect on survival and perezhivaemost sperm.

Mercaptoethanol at concentrations of 3.9 x10-4, and 5 x 10-2M decreased survival time and the experience of spermatozoa after freezing and thawing. Concentration merkatoetanola 6.25 x 10-3 ... 2.5 x 10-2M led to a significant increase at 22 ... 47%-time experience is frozen - thawed sperm without causing significant effect on survival of spermatozoa.

When you add a cysteine (10 • 10-3 M) into medium of GHTSZHK mobility, speed, safety of SH-groups and the absolute survival rate of frozen thawed sperm increased from 3.3% to 16.4%.

The influence of β-mercaptoethanol on the functional usefulness of frozen-thawed semen of boars is slightly higher than of cysteine. It is particularly noticeable in the number of acrosome preservation (25,670,9%) and intracellular sulfhydryl groups (70.9%) of the gametes. β-mercaptoethanol (6,25 • 10-3M) added in medium of GHTSZHK increase the absolute indicator of viability of sperm in 0.9 hour or 36.0% less damage to the acrosome of 16.1%, the enzyme activity of dehydrogenases and cytochrome oxidase remained roughly at the level of control group.

Absolute indicator of viability of spermatozoa after freezing and thawing in a medium with unitiol (at all tested concentrations) are also higher in the control of the experience of time. The greatest increase in absolute survival rate (138%) was observed at a concentration of 0.021% unitiola. Another big difference between the experiment and the control is detected, taking into account the absolute indicator of viability of sperm motility to save 5% of motility of cells.

Table 3: Effect of cysteine (10 • 10-3 M) added to the medium GHTSZHK on the functional completeness of frozen-thawed semen of boars

Indicator	options of experience				
	GHTSZHK (control)	GHTSZHK + cysteine	GHTSZHK + mercaptoethanol	GHTSZHK + unitiol	GHTSZHK + ditiotreitol
Mobility, score	3,0±0	3,1	3,2±0,01	3,2	3,1

	,01	±0, 02		±0, 02	±0, 01
Speed, mcM / s	95,4± 6,34	95,8 ±8, 03	95,7±6,93	96,8 ±6, 87	96,2 ±7, 64
Absolute indicator of viability, h	2,5±0 ,01	2,9 ±0, 01	3,4±0,01	3,7 ±0, 03	3,4 ±0, 02
Time reduction of methylene blue, min	16,5± 0,68	15,7 ±1, 97	15,8±1,32	14,9 ±0, 08	14,5 ±2, 03
Preservation of SH groups,%	62,4± 4,83	67,8 ±7, 12	70,9±6,48	72,6 ±6, 52	70,4 ±6, 52
Number of damaged acrosome,%	31,5± 2,89	28,7 ±2, 23	25,6±2,31	21,9 ±1, 23	23,7 ±1, 86
General activity of dehydrogenases, min	54,1± 5,45	52,2 ±3, 76	53,3±4,15	45,8 ±3, 35	48,7 ±4, 63
The activity of cytochrome oxidase, min	6,8±0 ,86	7,9 ±0, 79	7,3±0,79	17,6 ±0, 07	14,4 ±0, 09

At a concentration of 0.021% unitiola the difference is 85.9%. Concentration of 0.014% unitiola though increasing the number of surviving cells after freezing and thawing, does not significant increase of the absolute indicator of viability of gametes (before the loss of mobility). The action is in unitiola concentration 0.035% significantly increases the only absolute survivability indicator of gametes. Analysis of Table 2 shows that, after cryopreservation sperm activity with unitiol is higher by 0.2 points or 6.6%, respectively, the absolute survival rate at 1.1 hour or 42.3%; preservation of SH groups in sperm higher by 10.7 % number of damaged acrosomes is lower by 10.6%. In an environment with the common activity of dehydrogenases 45,8 ± 3,35 min, so in the metabolism of gametes occurs more slow motion.

The most positive cryoprotective effect is obtained by introducing dithiothreitol at a concentration of 1 • 10-5 M. At the indicated concentration frozen-thawed sperm activity is at the level of 5,0-5,1 at 70-71% of the sperm acrosome was intact, but the absolute rate survivability of spermatozoa (at 37 ° C) reached 80,2-81,3 conventional units.

After adding dithiothreitol (at a concentration of 1 • 10-5 M) to medium of GHTSZHK speed of sperm averaged 96.2 mm / s, or roughly at the level of the control group. Dithiothreitol markedly improves the the absolute indicator of of survilability of gametes. Thus, in particular in the prototypes it was an average of 3.4 hour or more at 36.0%. It was also found that the addition of dithiothreitol at sufficiently high level intracellular SH groups remain (70.4%). In general the activity of dehydrogenases dithiothreitol does not change, while at the same time, the activity of cytochrome oxidase increases more than 2 times.

4. Conclusion

Thus, the content of SH-groups in the 10 billion sperm cells in the range from 5.1 to 19.0 mol and an average of 8.7 micromoles, and 100 ml of plasma sperm contains on average 67.3 micromoles. These sulfhydryl groups, semen of boars, belong to low molecular weight thiols (ergotioneinu, cysteine, enzyme cofactors) and proteins that are apparently involved in complex biochemical processes occurring in cells, as well as in maintaining the structural elements of cells and membranes of sperm. The data obtained in the course of studies show that thiol compounds - unitiol ditioiteitol are able to maintain the integrity of the

structural elements of germ cells from the effects of low temperatures and thus increase kriostability of gametes, and thus contribute to the preservation of their functional usefulness. Consequently, they can be used in the media for cryopreservation of spermatozoa of boars.

5. References

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