RESEARCH ON RAM SPERM FREEZING

E. Cibotaru[1*], N. Bradu[1], D. Rotari[1], G. Darie[1], I. Djenjera[1]

[1]*Scientific-Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine, Republic of Moldova*

Abstract

The aim of the research was to perfect the ram sperm freezing protocols and develop the dilution medium for cryopreservation. The research was carried out on ram sperm, collected with the help of the artificial vagina. Ejaculates with a mobility of no less than 70% and a concentration of spermatozoa in the ejaculate of 2.5 billion/ml were taken for processing. Dilution medium consisting of sucrose, sodium citrate and egg yolk was used to dilute the semen. As an additional component in the composition of the environment, the preparation MP was introduced in a concentration from 0.1 to 1.0%/v. Post-thaw motility, spermatozoa with rectilinear movements, spermatozoa abnormalities, and spermatozoa with damaged acrosome were assessed. The results of the research demonstrated that the introduction in the dilution medium of the MP preparation in a concentration of 0.5-0.7% as an additional component allowed the mobility to be maintained at the level of 57% and the number of spermatozoa with rectilinear movements of 26-27 % after thawing, or 3.8% higher compared to the control group after thawing, it also allowed to decrease the percentage of spermatozoa with abnormalities by 2.9% and spermatozoa with damaged acrosome by 2%.

Key words: *Ram, Sperm, Diluent, Mobility, Concentration, Preparation, Freezing*

INTRODUCTION

The last decades have brought important data in the development of sperm cryopreservation, a field that constitutes these aspects. The aspects related to the study of ram sperm cryopreservation are far from being exhausted and, in this context, the information related to the protocol for the conservation of genetic resources, is of the greatest scientific and applied interest.

Worldwide, there exists a particular concern for the preservation and increase of sheep flocks in sheep breeding and exploitation. The reduction of sheep breeding and exploitation activity requires the implementation of a program to improve sheep flocks by modifying the productive genetic potential by increasing and improving the quality of meat and milk production (Zamfirescu Stela, 1994; Milovanov V.C., 1962; Miclea V., 2003; Maxwell W.M.C., et.al., 1996). Ensuring genetic progress increases the influence of high-value breeders. In these conditions, artificial inseminations, as methods of reproduction, allow increasing the selection intensity of breeding rams and implicitly increasing the efficiency of the selection flock, the methods of preserving and diluting ram sperm having a particular importance within this biotechnology, in order to ensure a corresponding fecundities and births. (Sonmez M.F., Demirei, 2004; Poulenz H.I. et.al., 2002; Fernandez Abela et.al., 1998; Al-Honak et.al., 1983).

The study of sperm dilution and preservation by freezing, as well as the analysis of qualitative and quantitative parameters, were the main purpose of the research presented in this paper.

MATERIAL AND METHOD

The research took place during the breeding season. The collection of semen was carried out on ewes in heat or on ewes in anestrus, with the help of the artificial vagina, due to the fact that this collection method does

[*]Corresponding author: lenuta_mar@yahoo.com, plescadoina94@gmail.com, darie@mail.ru
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not stress the rams, it is quick and simple, and the semen is superior both in terms of quantity and qualitative. Ejaculates with a mobility of no less than 70% and a concentration of spermatozoa in the ejaculate of 2.5 billion/ml were admitted for processing. Dilution medium composed of sucrose, sodium citrate, egg yolk, glycerin (STJ) was used to dilute the semen. As an additional component in the composition of the STJ dilution medium, the MP preparation obtained from wine yeasts was introduced, in a concentration from 0.1 to 1.0%. The following indices were examined: motility and percentage of spermatozoa with rectilinear movements, using the CEROS computer program, after dilution, refrigeration and resuscitation, spermatozoa viability, spermatozoa abnormalities and spermatozoa with damaged acrosome. The obtained experimental data were statistically processed using a computer method.

Many scientific works on the cryopreservation of ram semen are presented in the specialty literature. The data presented by different authors show different controversial results regarding the quality of semen after thawing and the fecundity of sheep through artificial insemination. Taking into account these results, we proposed to develop a protocol for cryopreservation of ram semen that would allow obtaining positive results regarding the mobility and fecundity of semen obtained by freezing. For this purpose, we used the dilution medium composed of glucose, sodium citrate, egg yolk, glycerin in the composition of which we introduced the biologically active preparation (MP) in different concentrations as an additional component.

The results regarding the influence of the MP preparation on sperm motility after thawing are presented in Table 1.

RESULTS AND DISCUSSIONS

Table 1 The dynamics of sperm mobility under the influence of the biologically active preparation MP

parameters		Control STJ	MP,%									
			0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
dilution	mobility, %	86.2 ±6.5	92.2 ±0.7	94.2 ±0.7	93.5 ±0.8	94.7 ±0.7	95.0 ±0.7	94.8 ±0.9	93.7 ±0.6	93.5 ±0.4	94.0 ±0.5	93.2 ±0.3
	rectilinear movements,%	49.5 ±2.2	48.8 ±1.6	50.3 ±1.1	47.3 ±1.2	49.7 ±2.1	50.7 ±1.7	49.0 ±2.6	46.8 ±0.6	47.3 ±0.5	44.7 ±1.1	42.3 ±0.7
refrigerati	mobility %	87.5 ±1.1	88.2 ±1.5	89.3 ±1.5	91.0 ±1.2	91.8 ±1.4	92.5 ±0.6	93.0 ±0.5	92.7 ±0.6	91.2 ±0.8	91.0 ±0.9	90.0 ±1.2
	rectilinear movements,%	35.2 ±2.4	39.2 ±2.8	36.3 ±2.1	36.7 ±1.6	42.5 ±1.2	44.2 ±1.7	43.8 ±2.2	42.0 ±0.8	42.7 ±1.1	40.8 ±2.0	39.7 ±2.4
resuscitati	mobility, %	53.4 ±1.5	53.8 ±1.9	53.2 ±2.5	53.8 ±2.0	55.8 ±1.5	57.2 ±1.0	56.8 ±0.9	57.0 ±1.1	54.6 ±1.2	54.4 ±1.7	51.4 ±2.6
	rectilinear movements,%	22.2 ±2.0	23.2 ±2.7	23.8 ±2.9	23.8 ±3.1	25.6 ±4.0	26.0 ±3.5	27.6 ±2.8	25.2 ±2.6	23.2 ±2.9	21.4 ±1.9	21.0 ±2.8

The data presented in Table 1 show that the biologically active preparation MP, introduced as an additional component in the dilution medium composed of sucrose, sodium citrate, glycerin and egg yolk, is not toxic to the spermatozoa in the studied concentration range. Sperm mobility after dilution in the experimental groups practically did not undergo significant changes depending on the medium used for dilution compared to the control group. After refrigeration, sperm mobility oscillated between 88.2±1.5, when the MP preparation was introduced into the dilution medium in a concentration of 0.1% and 93.0+0.5%. When the MP preparation was introduced into the medium in a concentration of 0.6%, in the control group after refrigeration the sperm mobility was 87.5%/v, or 4.8% lower compared to experimental group nr.6. After the resuscitation, the highest mobility index of 57.2+1.0% was in experimental group nr.5,

where the concentration of the preparation in the MP medium was 0.5%. In the control group after resuscitation, sperm mobility was 53.4+1.5%, or 3.8% lower.

The percentage of spermatozoa with rectilinear movement after resuscitation in the experimental groups was higher compared to the control group. The experimental data obtained are not significant compared to the control group.

The experimental data on sperm viability after thawing at 37 degrees C are presented in Table 2.

Table 2 Mobility dynamics of spermatozoa stored at 37 degrees C, under the influence of the biologically active preparation MP

Parameters		Control STJ	MP,%									
			0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
reanimation	mobile,%	53.4 ±1.5	53.8 ±1.9	53.2 ±2.5	53.8 ±2.0	55.8 ±1.5	57.2 ±1.0	56.8 ±0.9	57.0 ±1.1	54.6 ±1.2	54.4 ±1.7	51.4 ±2.6
	progressive, %	22.2 ±2.0	23.2 ±2.7	23.8 ±2.9	23.8 ±3.1	25.6 ±4.0	26.0 ±3.5	27.6 ±2.8	25.2 ±2.6	23.2 ±2.9	21.4 ±1.9	21.0 ±2.8
1 h	mobile,%	46.8 ±1.4	48.2 ±1.1	48.6 ±1.7	50.0 ±1.8	51.2 ±0.4*	52.0 ±0.5	52.8 ±0.9	52.4 ±1.3	51.0 ±0.8	49.4 ±0.7	47.4 ±1.7
	progressive, %	19.8 ±1.2	18.8 ±1.0	19.4 ±1.9	21.4 ±2.1	21.8 ±2.1	22.4 ±2.8	22.4 ±1.7	20.0 ±1.0	19.2 ±0.5	19.4 ±1.7	18.4 ±1.0
2 h	mobile,%	43.2 ±1.9	43.6 ±1.7	46.4 ±2.2	47.8 ±1.8	49.6 ±1.7	51.0 ±1.7	50.6 ±1.1	50.0 ±1.4	49.8 ±1.4	47.8 ±1.2	44.8 ±0.8
	progressive, %	17.4 ±2.0	19.0 ±0.9	18.8 ±1.3	20.8 ±1.4	18.6 ±1.0	21.6 ±2.9	21.2 ±1.1	18.6 ±1.4	18.0 ±0.8	17.4 ±0.7	16.2 ±1.2
3 h	mobile,%	40.5 ±2.0	41.6 ±1.8	44.8 ±1.4	46.4 ±1.4	46.4 ±1.3	48.0 ±1.1	48.4 ±0.9	47.2 ±0.9	46.2 ±0.8	44.6 ±0.7	42.0 ±0.4
	progressive, %	16.2 ±1.1	16.6 ±0.8	17.2 ±1.4	17.2 ±0.6	17.6 ±0.7	17.6 ±1.3	17.8 ±1.0	17.4 ±1.1	16.0 ±1.2	14.6 ±1.4	15.8 ±1.0
4 h	mobile,%	34.3 ±2.7	34.3 ±2.7	35.7 ±2.4	37.8 ±3.1	39.5 ±2.7	41.0 ±2.6	41.3 ±3.0	40.3 ±3.0	39.0 ±2.7	37.5 ±2.8	36.2 ±2.3
	progressive, %	13.0 ±1.8	13.5 ±1.6	15.0 ±1.3	16.2 ±1.5	15.2 ±1.4	15.5 ±1.1	15.0 ±2.1	14.2 ±1.6	12.7 ±1.1	13.0 ±1.2	13.0 ±1.1
5 h	moving,% mobile,%	28.2 ±2.9	28.7 ±2.6	30.8 ±2.8	33.2 ±2.9	34.5 ±3.0	36.2 ±3.6	35.2 ±3.4	34.8 ±3.0	32.2 ±3.1	31.5 ±3.4	30.0 ±2.9
	progressive, %	9.3 ±1.5	10.0 ±1.6	10.0 ±1.8	10.7 ±1.6	12.2 ±1.2	13.0 ±1.8	11.3 ±2.4	11.3 ±1.1	10.0 ±1.3	9.7 ±1.3	8.8 ±1.2

Analyzing the obtained data (Table 2), it was found out that the biologically active preparation had a positive influence on the duration of spermatozoa storage at + 37 degrees C. After 5 hours of storage, the best mobility results were obtained in experimental group nr. 5, with 36.2+3.6% compared to the control group where this index was 28.2+2.9%, or 8.0% lower than in experimental group nr.5. The obtained results correspond to the national standards regarding the quality of frozen semen intended for sheep artificial insemination.

At the same time, the research was carried out on the dynamics of sperm abnormalities in the process of semen cryopreservation under the influence of the biologically active preparation MP. The experimental results are presented in Table 3.

Table 3 The influence of the biologically active preparation MP on the abnormalities of spermatozoa in the process of their cryopreservation

	parameters	Control STJ	MP,%									
			0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
dilution	Macrocephaly	1.3±0.3	1.5±0.3	-	1.0±0.0							1.5±0.5
	Microcephaly	2.5±0.5	-	1.5±0.5	1.0±0.0	1.7±0.7	1.5±0.5	1.5±0.5				1.0±0
	Broken neck	3.0±0.4	3.5±0.3	4.5±0.6	3.3±0.5	3.3±0.5	2.8±0.5	2.8±0.5	5.5±0.6*	4.8±0.5*	6.3±0.5**	6.3±0.6**
	Double head											
	Crooked tail	4.0±0.4	3.8±0.5	3.5±0.6	3.8±0.9	3.3±0.3	3.8±0.6	4.3±0.5	4.8±0.5	6.0±1.1	6.0±0.4*	6.0±0.4*
	No head	3.0±0.4	4.0±0.4	4.0±0.4	4.0±0.4	3.0±0.4	2.5±0.3	4.0±0.4	4.0±0.4	5.8±0.8*	5.0±0.9	4.8±0.9
	No tail	3.5±0.3	4.3±0.6	3.0±0.4	3.3±0.5	3.5±0.5	4.5±0.5	4.3±0.5	4.3±1.3	5.8±1.1	5.0±0.4*	5.3±1.3
	Total %	8.7	8.6	8.3	8.2	7.4	7.6	8.5	9.3	11.2	11.2	12.5
resuscitation	Macrocephaly	1.3±0.3		1.0±0.0	1.5±0.0	1.3±0.3	1.0±0.0	1.0±0.0			1.0±0.0	1.0±0.0
	Microcephaly		1.0±0.0	1.3±0.3	1.0±0.0	1.0±0.0	1.5±0.5			1.0±0.0		
	Broken neck	11.8±0.5	8.3±0.9	8.5±1.0	10.3±0.9	9.3±2.3	7.8±1.1	8.8±0.9	9.3±0.5	9.5±0.6	10.0±0.6	11.0±0.7
	Double head											
	Crooked tail	7.5±0.6	10.8±0.9*	9.5±1.8	9.3±1.7	9.3±0.9	8.5±0.5	9.0±0.9	9.3±1.4	9.5±0.9	10.0±0.9*	10.3±1.4
	No head	10.8±0.5	9.8±0. 9	9.3±0.6	10.0±0.4	8.0±0.4	8.8±0.5	6.8±0.5	10.0±0.7	9.5±0.6	10.0±0.7	9.5±1.2
	No tail	9.8±0. 9	11.3±1.0	11.8±0.5	11.0±0.4	9.5±0.6	10.3±1.2	9.8±0.5	8.5±0.3	11.5±0.6	12.3±0.6*	11.5±1.0
	Total %	20.6	20.6	20.7	21.6	**19.2**	18.9	17.7	18.6	20.5	21.7	21.7

The results presented in the table show that the biologically active preparation MP introduced as an additional component in a concentration of 0.4-0.5% had a positive influence on sperm abnormalities. The lowest percentage of sperm abnormalities was obtained in experimental group nr. 4 – 7.4% compared to the control group where this index was 8.7% or 1.3% lower.

After resuscitation, the lowest percentage of abnormal spermatozoa was obtained in experimental group nr. 4 - where the concentration of the MP preparation was 0.4 with 37.9% compared to the control group where this index was 41%. In conclusion, the biologically active preparation MP had a positive influence on sperm abnormalities after thawing. The obtained results correspond to the standards in force regarding frozen ram sperm accepted for sheep artificial insemination.

In our research, the influence of the biologically active preparation MP on the integrity of the acrosome after resuscitation was studied.

The experimental data are presented in Figure 1.

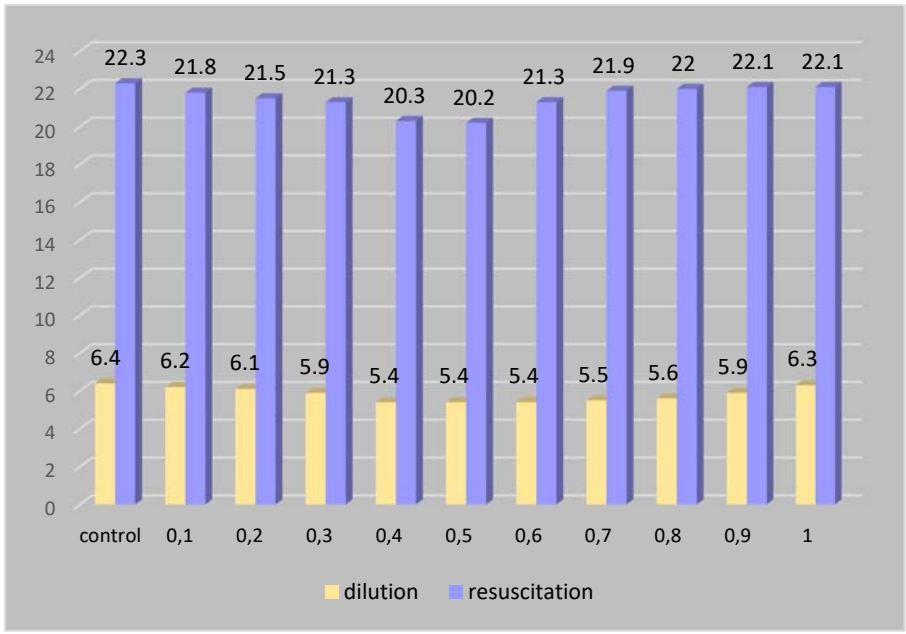

Fig. 1 Acrosome integrity, %

Analyzing the data presented in Figure 1, we can conclude that the lowest percentage of acrosome damage was obtained in experimental group nr. 4, where the concentration of the preparation introduced as a supplementary component in the dilution medium was 0.4%. The percentage of spermatozoa with damaged acrosome in experimental group nr. 4 was 20.3+0.7%, compared to the control group where this index was 22.3+0.7%.

Based on the experimental data, the dilution medium for cryopreservation of ram semen was obtained in the following component: sucrose – 6.4%; sodium citrate - 0.6%; egg yolk -10%; mannoproteic preparation (MP) -0.6-0.8%; double-distilled water up to 100 ml.

CONCLUSIONS

1. Semen freezing can be done with good results by using the STJ commercial diluent.

2. The biologically active substances, used as a supplement for the STJ commercial diluent, improve sperm parameters and mobility, upon thawing, by more than 50%, the number of spermatozoa with rectilinear movements by 26-28%, the percentage of spermatozoa with abnormalities by 2.8% and with the acrosome damaged by 2%.

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