STUDIES CONCERNING THE CRYOPRESERVATION OF RAM SPERM

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Abstract

Researches were made on native breeds ram sperm Tigaie, Karakul. It was studied the particularities of sperm cryopreservation. Diluents used in the research were GHŢS, GŢJD, GŢGE. Thus, sperm motility does not change major in the early hours balancing independent of dilution medium and race. It was found that during balancing sperm for 2 hours at a temperature of 4C is optimal. Diluent GŢJG, in the composition of which was introduced glycerol in a ratio of 5%, afforded the best postcongelate mobility, compared with other media taken in research (28.5 ± 0.70).

Key words: ram, semen, volume, mobility, concentration, sperms, ejaculate, race

INTRODUCTION

Indigenous breeds generally manifest a number of problems which should be solved in order to facilitate the expansion of artificial inseminations at sheep. [7,8,9]. Dilution and storage of sperm of rams, and the analysis of qualitative parameters of sperm after resuscitation have a theoretical and practical importance. The diluents, through the property to reduce the number of spermatozoon's in the dose of inoculation of sperm and storage of the capacity of fertilized spermatozoon's, also serve to protect the cryopreserved semen, thereby increasing the duration of storage [1,2,4].

The success of preservation of ram spermatozoon's in vitro depends in a great part on the composition of dilution media which should contain protective substances for spermatozoon's in the process of cryopreservation**.** [3,5,6,10]. This fact is the reason of addition in the composition of mediums of dilution of various protective substances for having positive effects on cryopreservation of ram sperm. Another specific objective of this method of cryopreservation of semen is the improvement of technologies in order to maintain the integrity of spermatozoons. This method allows to create banks of stored semen for a long period of time which will enable the use of this material on time for the amelioration of sheep livestock [2,5,7].

RESEARCH METHOD

For the experience were taken rams of indigenous breeds Karakul and Ţigaie maintained at the sheep farm "Scientific and Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine". In the process of experimentation all animals were maintained and fed in the same conditions. According to morphological and physiological indices of spermatozoon's there was determined the difference of ejaculates depending on breed on the quality of semen. When the rams of interest for the experience have been chosen it was resorted to the collection of the semen. When choosing rams for sperm collection there was taken into account the manifestation of sexual reflexes for obtaining qualitative ejaculates. Semen collection was done with the help of artificial vagina. The volume and the appearance of ejaculates were determined immediately after gathering them in the glass collector. Mobility and spermatozoon's concentration was determined by taking from glass collector a drop of semen which was

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analyzed under a microscope through the program "Ceros".

While obtaining ejaculates their pretability at cryopreservation was studied. The period of equilibration of the mobility of spermatozoon's has been found, the sperm was diluted in different degrees of dilution from 1: 1 to 1: 4. The used diluent was GŢSG. The chosen balancing time was 5, 4 and 2 hours. At the cryopreservation of sperm there was used the diluent proposed by the laboratory staff "Biotechnology of reproduction and embryo transfer" of the Scientific and Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine. The preparation of sperm for freezing supposed the dilution in the ratio of 1: 4 with the mentioned diluent, prewarmed at 37°C. After the dilution the temperature of the samples was gradually decreased till 4°C at the rate of 0.3 degrees / min. The duration of equilibration was at 4 degrees C for 2 hours. Packaging was done in straws of 0.25 ml s, then the straws were transferred to the vat of freezing, initially in nitrogen vapor at 4 cm of the surface level of liquid nitrogen within a period of 10 min., then the straws were hovered in liquid nitrogen (-196 ° C).

RESEARCH RESULTS

The data concerning the mobility of spermatozoon's from ejaculates at Karakul and Ţigaie breeds ire presented in Figure 1.

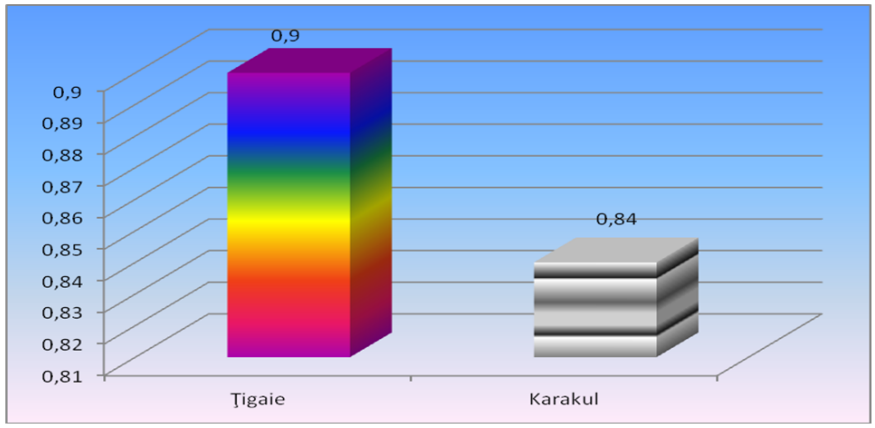

Fig. 1 The mobility of gross sperm obtained from rams of breed Ţigaie and Karakul (%)

Analyzing the data of figure 1, we can find that the mobility of spermatozoons is very high, respectively of 0.9 ± 0.01 ml with reduced limits of variation. The minimum grade for the mobility of 0.8 permitted the use of ejaculates at processing.

The concentration of spermatozoons in the ejaculate is presented in figure 2.

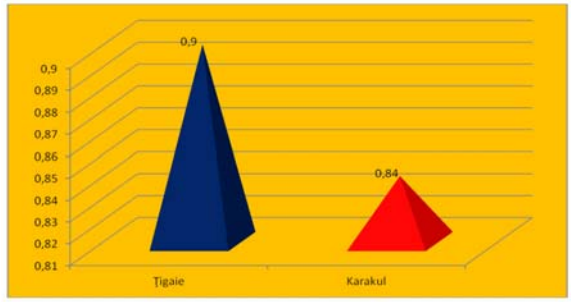

Fig. 2 The concentration of spermatozoons at Ţigaie and Karakul breed, bln / ml

The experimental results demonstrate that the concentration of spermatozoon's ranges between 0.687 bln / ml and 2.13 bln / ml with an average of 1.47 ± 0.10 bln / ml at Ţigaie breed and 1.98 ± 0.12 bln / ml at Karakul breed.

The determination of the equilibration period on the mobility of spermatozoon's is presented in Table 1

Table 1 The mobility of spermatozoon's in the period of equilibration with duration of 5 hours

Rams breed	Degree dilution	Mobility at:					
		Initial	1h	2h	3h	4h	5h
Breed Ţigaie	1:1	0.8	0.8	0.8	0.8	0.7	0.7
	1:2	0.7	0.7	0.7	0.7	0.7	0.7
	1:3	0.9	0.9	0.9	0.8	0.8	0.8
	1:4	0.7	0.7	0.7	0.7	0.7	0.6
Breed Karakul	1:1	0.8	0.8	0.8	0.8	0.7	0.7
	1:2	0.9	0.9	0.9	0.8	0.8	0.8
	1:3	0.8	0.8	0.8	0.7	0.7	0.7
	1:4	0.9	0.9	0.8	0.8	0.7	0.7

The analysis of values in table 1 demonstrates that spermatozoon's mobility does not reduce very much in the first hours of equilibration regardless of the degree of dilution and of the breed to which the rams belong.

The dynamics of mobility of spermatozoon's during the period of equilibration for 4 hours is presented in table 2.

Table 2 The mobility of spermatozoon's in the period of equilibration with duration of 4 hours

Rams breed	Degree dilution	Mobility at:				
		Initial	1h	2h	3h	4h
Breed Ţigaie	1:1	0.8	0.8	0.8	0.7	0.7
	1:2	0.8	0.8	0.7	0.7	0.7
	1:3	0.9	0.9	0.9	0.9	0.8
	1:4	0.9	0.9	0.9	0.8	0.8
Breed Karakul	1:1	0.9	0.8	0.8	0.8	0.7
	1:2	0.9	0.9	0.9	0.8	0.8
	1:3	0.9	0.9	0.9	0.8	0.8
	1:4	0.9	0.9	0.9	0.9	0.9

Compared to the previous carried out analysis when the dynamics of the decrease of temperature during the period of equilibration was slow (5 hours), at the equilibration with duration of 4 hours, in general, the same aspects have been established. Increasing the speed of temperature reduction, up to + 4 ° C does not affect very much the spermatozoon's mobility for the first two hours. It remains at high values respectively of 91.42 - 97.29% from the initial mobility.

The degree of dilution on the mobility in this case demonstrates that after 3-4 hours of equilibration the diluted sperm in ratio of 1: 4 has the highest values.

The dynamics of mobility of spermatozoon's during the period of equilibration with duration of 2 hours is presented in table 3.

Table 3 The mobility of spermatozoon's in the period of equilibration with duration of 2 hours

Rams breed	Degree dilution	Mobility at:		
		Initial	1h	2h
Breed Ţigaie	1:1	0.8	0.8	0.7
	1:2	0.8	0.8	0.8
	1:3	0.8	0.8	0.8
	1:4	0.8	0.8	0.7
Breed Karakul	1:1	0.8	0.8	0.7
	1:2	1.0	1.0	0.9
	1:3	0.9	0.9	0.9
	1:4	0.9	0.9	0.8

From the table data it comes out that all spermatozoons after an hour of equilibration retain their mobility regardless of the degree of dilution. It reduces a little after 2 hours, but remains at high levels and close to initial mobility.

Table 4 Evaluation of the semen post freezing is presented in Table 4

Dilution media	n	Mobility M±m (%)	Viability (hours)	Spermatozoon's morphology	
				Mobile, %	Progressive, %
GŢJG	22	28.5±0.70	4.5	27.56	10.44
GŢJD	18	5.11±0.26	4	4.29	0.71
GŢJE	12	1.0±0.01	0	0.90	0.21

The data analysis from table 4 demonstrates that spermatozoon's mobility post freezing reduces very much. Depending on the dilution media the mobility of sperms post freezing represents 28.5 ± 0.70 and 1.0 ± 0.001%. It has been established that diluent GŢJG, in the composition of which was introduced glycerin with a ratio of 5%, spermatozoon's had an improved mobility after defrosting, compared with other media involved in the research.

CONCLUSIONS

To ensure the morpho-functional integrity of spermatozoon's during freezing, there must be respected strictly both the proportion of cryoprotector material in diluent as well as and the actual dilution technology, freezing, cryopreservation and thawing.

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