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PRESERVATION OF RAMS' SPERM AT +2-+4°C REGION OF MOLDOVA

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Abstract

Quantitative and qualitative indicators of sperm obtained from rams Moldavian Karakul were studied. The average volume of the ejaculate was 0.84 ml, 91% mobility and 2.5 mlrd / ml concentration. The action of the BD-1 preparation synthesized at the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova was experimented as an additional component introduced into the basic environment regarding the quality of the ram seminal material, preserved at 2-4°C. It has been found that the introduction in the composition of the basic medium for 6% dilution of seminal material of BD-1 has a positive influence on the preservation of the semen. After 144 hours of incubation at 2-4°C the sperm mobility was 68% compared to the control group where this index was 45%. Artificial insemination of the sheep with refrigerated sperm after 144 hours allowed 71.9% sheep fecundity. The proposed technology for conserving the semen of ram at 2-4°C which composition proposes the dilution medium for semen, containing: glucose, sodium citrate, egg yolk, BD-1, allows the results of artificial insemination of sheep to be made more efficient.

Key words: *ram, breed, sperm, dilution medium, preservation, fecundity.*

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INTRODUCTION

Theoretical studies in the biology of animal sperm have opened up great prospects for opportunities not only in the accelerated development and widespread introduction of the method of artificial insemination of farm animals, but also in the preservation of genetically most valuable and endangered species and species of animals.

Methods of storing sperm in a non-organism are based on a decrease in the metabolic processes of spermatozoa, which allows them to increase the time of their survival and preserve their fertilizing ability (Milovanov, 1962). Currently, the most widely used in sheep farming is the short-term storage of diluted sperm at a temperature of 2-4°C (Shaydulin, 2007). To store sperm at a temperature of 2-4°C, it is diluted with a special medium (GTsZH) prepared according to the following recipes, per 100 ml of bidistilled water: glucose-0.8 g, sodium citrate-2.8 g, yolk-20 ml. The shelf life of sperm at 2-4°C is very small and as a rule they are used during the day. Even if the spermatozoa have a progressive movement after 3 days or more, their fertilizing ability is sharply reduced. Such a short period of storage of sperm causes difficulties in the work of items of artificial insemination of sheep (Voevodin, 2012). This requires the improvement of this method in order to increase the shelf life of sperm without reducing the fertilizing ability of spermatozoa. In this regard, further research is needed in the field of improving synthetic media for storing sperm, both in freezing conditions and in the frozen state. It is possible to increase the effectiveness of artificial insemination of sheep by using various biological active compounds in environments (Nauk, 1972, 1973).

MATERIALS AND METHODS

The object of research was Moldavian type rams of Karakul breed. In the experiments used clinically healthy rams - producers. Sperm was taken into an artificial vagina, the quality of freshly obtained sperm was determined by standard methods for volume, concentration, and motility was determined using the computer program "CEROS". For the experiments, sperm with a mobility of at least 80% and a concentration of at least 2.5 billion / ml was used.

All the original components intended for the preparation of synthetic media for dilution of sperm had a purity of "HCH" or "analytical grade" and were tested for harmlessness to sperm in accordance with approved quality

control methods. Environments were prepared according to the standard technique; their quality was checked by the method of biocontrol.

As an additional component introduced into the composition of the basic medium (GTsZH), the drug BD-1 was developed, which was developed at the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova in order to increase the shelf life of semen of rams.

The drug BD-1 was tested with its introduction into the composition of the main medium in different concentrations from 1 to 10%.

All comparative experiments in studying the action of the drug BD-1 were studied on separate ejaculates.

The quality of the stored sperm at 2-4°C in each prepared medium after dilution of the sperm was tested for motility every 24 hours, using the CEROS program.

RESULTS AND DISCUSSIONS

Further improvement of the method of storing sperm involves the selection of rams, the sperm of which is suitable for use and does not reduce the loss of spermatozoa during storage, improve the safety of biological usefulness and, accordingly, the effectiveness of artificial insemination. Values of the level of semen products of rams, allows you to send in the right direction their use, which is very important in the effectiveness of their use.

During the experiments, special attention was paid to the study of the quantity and quality of sperm obtained from sheep of the Moldavian type of Karakul breed, as well as improving the synthetic environment for diluting the ram sperm and its protective properties. At first, the assessment of rams producers of the Moldavian type of Karakul breed on the quality of sperm production (Table 1) was reviewed and verified.

Table 1. Average data of indicators of freshly received rams semen

	units	*Statistical Parameters*				
		n	M±м	V %	V min	V max
amount	ml	15	0.84±0.06	28.53	0.4	1.2
mobility	%	15	91±1.01	4.38	90	100
concentration	milliard/ml	15	2.78±0.05	24.48	2.06	2.92
The total number of spermatozoa in the ejaculate	milliard	15	2.59±0.05	13.38	2.42	2.86

It has been established that rams of the Moldavian type of Karakul breed is characterized by variability of sperm values. The data presented in the table show that the average volume of ejaculate in ram producers was 0.84 ± 0.06 ml, with fluctuations between rams from 0.4 ml to 1.2 ml.

The mobility of freshly obtained sperm was 91.0 ± 1.01%. The concentration of sperm in 1 ml of sperm was 2.78 billion, and the total number of sperm in the ejaculate averaged 2.59 billion / ml.

The experimental data obtained show that the sperm production of ram-makers of the Moldavian type of Karakul breed is lower compared to the standard indicators of the Karakul breed.

Biocontrol quality of diluents allows to determine the effect of the developed media on the mobility and survival of spermatozoa outside the body.

Another series of experiments was carried out to improve the technology of preserving sperm during cooling. After collecting the semen, the ejaculates were subjected to microscopic and macroscopic analysis using the CEROS program. Ejaculates approved for treatment were diluted with GTG medium, which included BioR with membranotropic and antioxidant properties, synthesized in concentrations from 1 to 10%, developed by the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova.

Biocontrol quality of diluents allows to determine the effect of the developed media on the mobility and survival of spermatozoa outside the body. The mobility and survival of sperm at 2-4^{o}C are presented in Table 2.

Table 2. Mobility and experience of spermatozoa at + 2 - +4°C

Time between researchings, h	indices, %	control GTJ	*BD-1 concentration*									
			1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
After dilution	*motility*	*81.3 ±4.6*	*82.0 ±4.7*	*72.0 ±14.5*	*84.0 ±6.3*	*86.8 ±1.5*	*84.8 ±4.8*	*84.3 ±2.2*	*88.3 ±1.5*	*84.5 ±3.7*	*85.3 ±4.8*	*79.3 ±4.9*
	progressive	*39.3 ±2.3*	*40.8 ±2.7*	*47.0 ±6.9*	*38.3 ±3.3*	*4.3 ±3.0*	*42.3 ±1.6*	*38.5 ±3.2*	*40.8 ±3.4*	*41.0 ±5.0*	*45.5 ±4.3*	*38.5 ±4.3*
24	*motility*	*81.3 ±3.2*	*797 ±6.4*	*80.3 ±5.0*	*82.3 ±3.5*	*88.3 ±3.2*	*83.3 ±3.7*	*81.0 ±3.6*	*82.3 ±3.8*	*80.3 ±2.3*	*83.7 ±0.9*	*82.7 ±4.4*
	progressive	*33.0 ±1.0*	*32.2 ±3.7*	*32.0 ±2.1*	*31.0 ±2.1*	*34.0 ±3.1*	*38.3 ±4.4*	*37.7 ±5.9*	*35.3 ±3.7*	*31.7 ±2.2*	*35.0 ±3.5*	*35.3 ±7.5*
48	*motility*	*81.7 ±3.7*	*71.0 ±5.1*	*82.0 ±6.8*	*73.0 ±2.9*	*76.0 ±2.3*	*78.0 3±5.2*	*77.0 ±5.1*	*84.0 ±5.5*	*84.3 ±6.1*	*81.7 ±3.2*	*80.0 ±3.2*
	progressive	*38.7 ±3.4*	*28.0 ±4.0*	*33.3 ±6.4*	*31.0 ±3.5*	*34.0 ±4.3*	*37.0 ±4.6*	*35.3 ±4.3*	*41.0 ±7.8*	*33.0 ±3.6*	*36.0 ±5.0*	*28.7 ±4.7*
72	*mobility*	*71.3 ±1.8*	*65.7 ±5.9*	*77.7 ±3.4*	*70.3 ±0.9*	*76.3 ±4.5*	*73.3 ±6.2*	*74.7 ±2.7*	*78.0 ±4.0*	*77.7 ±1.8*	*73.0 ±3.0*	*75.3 ±5.5*
	progressive	*28.3 ±1.2*	*18.7 ±1.7*	*25.0 ±2.5*	*31.3 ±3.5*	*29.7 ±4.1*	*31.3 ±5.8*	*30.7 ±2.3*	*32.7 ±5.6*	*27.0 ±1.5*	*32.0 ±3.1*	*27.3 ±4.8*
96	*mobility*	*72.3 ±5.5*	*60.3 ±12.4*	*72.0 ±5.8*	*73.3 ±2.9*	*73.7 ±6.4*	*73.7 ±8.4*	*73.7 ±4.8*	*77.7 ±5.0*	*78.3 ±3.8*	*81.0 ±3.8*	*81.3 ±5.6*
	progressive	*28.3 ±6.1*	*24.0 ±5.5*	*23.0 ±4.7*	*24.3 ±3.3*	*28.7 ±4.3*	*29.0 ±3.5*	*26.7 ±0.9*	*29.7 ±4.8*	*28.3 ±1.8*	*28.3 ±2.4*	*29.7 ±9.6*
120	*mobility*	*52.0 ±6.0*	*52.5 ±12.9*	*53.5 ±5.2*	*64.3 ±5.0*	*65.3 ±3.7*	*68.5 ±6.2*	*67.3 ±8.8*	*74.0 ±4.9*	*69.3 ±9.4*	*68.8 ±4.2*	*65.5 ±4.8*
	progressive	*12.5 ±3.0*	*12.5 ±4.6*	*10.8 ±3.3*	*21.3 ±3.0*	*18.3 ±3.4*	*27.3 ±2.8*	*21.0 ±5.1*	*18.5 ±1.8*	*23.5 ±8.4*	*23.5 ±4.6*	*22.3 ±6.2*
144	*mobility*	*45.7 ±8.2*	*45.7 ±8.2*	*31.7 ±16.6*	*36.3 ±7.4*	*37.0 ±4.7*	*60.3 ±7.6*	*69.0 ±2.5*	*68.0 ±5.0*	*56.3 ±3.3*	*61.0 ±3.6*	*61.7 ±0.3*
	progressive	*8.7 ±5.2*	*3.0 ±2.5*	*4.3 ±1.9*	*8.7 ±2.0*	*6.3 ±2.4*	*13.0 ±6.6*	*16.0 ±6.0*	*14.7 ±0.3*	*14.0 ±1.5*	*15.0 ±3.5*	*13.3 ±4.5*
168	*mobility*	*25.8 ±10.0*	*33.5 ±13.9*	*21.5 ±11.0*	*45.3 ±8.4*	*43.0 ±18.4*	*47.5 ±8.9*	*48.8 ±6.9*	*57.8 ±2.5*	*56.5 ±7.5*	*45.8 ±6.1*	*48.8 ±12.8*
	progressive	*6.0 ±5.4*	*3.8 ±1.9*	*2.5 ±2.2*	*6.8 ±4.9*	*13.3 ±7.5*	*12.0 ±5.6*	*5.5 ±1.7*	*11.8 ±5.7*	*7.5 ±2.0*	*4.0 ±1.3*	*6.3 ±2.8*
192	*mobility*	*15.3 ±8.3*	*12.7 ±7.2*	*21.0 ±3.8*	*31.0 ±6.9*	*37.0 ±14.0*	*44.3 ±5.8*	*50.3 ±6.1*	*53.0 ±3.5*	*45.3 ±11.8*	*29.0 ±5.7*	*44.3 ±9.8*
	progressivee ssive	*1.0 ±0.6*	*1.0 ±0.6*	*1.3 ±0.3*	*1.7 ±0.7*	*5.7 ±3.7*	*6.0 ±2.0*	*7.0 ±4.5*	*8.0 ±3.5*	*8.7 ±5.4*	*2.0 ±0.6*	*3.7 ±1.5*

The experimental data obtained show that the tested drug BD-1 is not toxic to spermatozoa in the tested concentration ranges. After dilution of the sperm with various test media, the motility of the sperm was within 80%, and the number of spermatozoa with straight-line movement was within 40%.

With an increase in the storage time of diluted sperm at 2–4°C, these figures sharply decreased. After 144 hours of sperm storage, sperm motility in the experimental group, where the concentration of the drug BD-1 was in the range of 6-7% was 68-69%, whereas in the control group this indicator decreased and amounted to only 45.7 ± 8.1%.

Similar changes have occurred with the number of sperm with a straight-line movement. In the experimental group, where the concentration of BD-1 was 6-7% after 144 hours of sperm storage, this indicator ranged from 16 to 15%, whereas in the control group, this indicator was 4.7%.

Investigation of the effect of seed dilution on the safety of spermatozoa by cooling was carried out using spermatozoa from designated rams. Ejaculates were diluted 1: 3 and 1: 4. Samples were kept at four degrees Celsius, sperm motility was determined regularly at established intervals (24 hours) until it reached zero. The breeding medium was prepared in the laboratory of the Scientific and Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine.

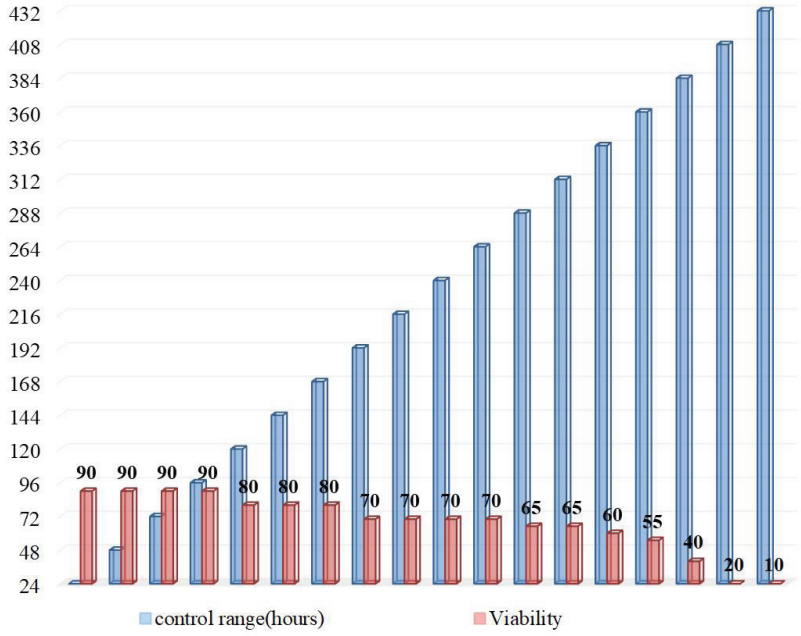

Figure 1. Viability of diluted sperm 1:3

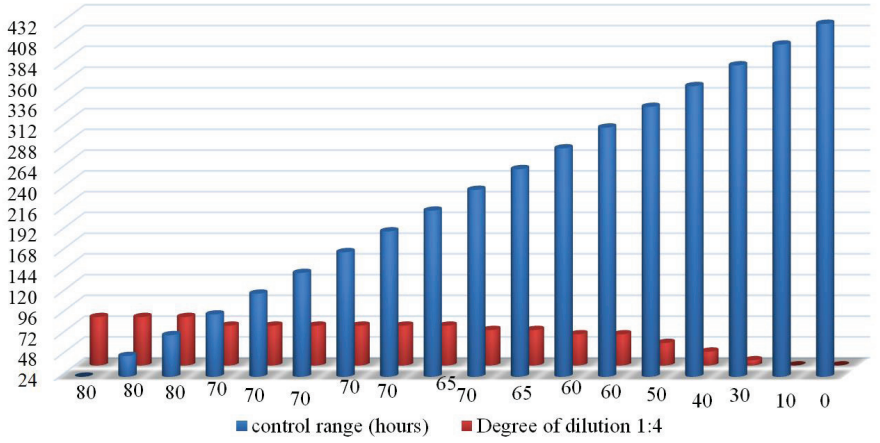

Figure 2. Viability of diluted sperm 1:4

From the data in diagram 1 and 2 it can be seen that, regardless of the degree of dilution, the ability to maintain sperm motility has longer periods. The best results are achieved if the dilution is 1: 3. In this case, after 6 days, the mobility of chilled sperm is still 80%, decreasing to 55% on the 15th day and finally 10% (18th day)

As a result of the research, an improved synthetic medium of the following composition was proposed: for 100 ml of bidistilled water, glucose 0.8 g, sodium citrate - 2.8, chicken egg yolk - 20 ml, BD-1 - 6% and antibiotics.

The proposed improved environment was tested under production conditions by artificial insemination of sheep. After production, the sperm was diluted 1:2 and 1:3 and stored in a refrigerator at 2-4℃. The heat period at sheep's was detected by the special ram-detector. Insemination is twofold. Data on the results of artificial insemination of sheep are presented in Table 3.

Table 3. Results of artificial insemination of sheep

Inseminated sheep, heads	camed back in the period of estrus	
	head	%
74	30	40.5

The data presented show that 30 days after the last insemination 30 heads came to the hunt again, which is 40.5% of the initially inseminated.

CONCLUSIONS

Based on the results of the research it was found:

1. Our improved environment contributes to better preservation and functional usefulness of sperm.
2. Our improved environment contributes to better survival of sperm after thawing (this is confirmed by the results of sperm motility after thawing).

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