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PRESERVATION OF RAM SEMEN BY REFRIGERATION

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Abstract

The research was performed on ram semen. The biological material was the Moldovan Karakul rams. Ejaculates with a mobility of over 70% and a sperm concentration of over 2 billion / ml were allowed for processing. The basic medium used was SȚJ (sucrose, sodium citrate, egg yolk). An additional component introduced in the basic environment was experienced the biologically active preparation LB / MP obtained from yeast from the brewing by the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova. After diluting and microscopically testing the sperm using the CEROS computer system, the experimental batches were introduced into the cold room at + 2-4 degrees. Mobility assessment was performed at 24 hours intervals. Sperm mobility after 120 hours of storage by refrigeration was maintained at 68.0 ± 5.8 % in the experimental group where the concentration of the preparation in the base medium was 0.8%, mobility allowed for inoculation. Based on the experimental results obtained, it was proposed to dilute and preserve ram semen by refrigerating the SȚJ medium supplemented with 0.8% biologically active preparation LB/MP.

Key words*: ejaculate, mobility, ram, refrigeration, sperm.*

INTRODUCTION

The sensitivity of the sperm cells leads to the obligation that in all the time spent outside the natural conditions, ie between the time of harvesting and sowing, all measures must be taken to protect the sperm from harmful agents on its viability (Ivanova et al., 1999; Tardif et al., 1997).

If the temperature decreases suddenly, the thermal play is installed, which results in an increase in the permeability of the cell membrane and the consequent loss of the proteins of potassium lipids and lipid phosphorus (Ladha et al., 1999).

Thermal shock can be felt even by diluted semen, although diluents provide considerable protection against sudden drops in temperature (Mircu, 2001; Perez-Pe et al., 2010; Tulcan et al., 2004).

In the process of diluting the sperm with dilution media and preserving by refrigeration there are structural changes of the sperm that lead to the deregulation of the transmembrane exchange process that lead to the loss of sperm fertility.

It is not yet clear what is the mechanism of damage to sperm membranes in the process of preserving sperm by refrigeration.

Under these conditions, artificial inseminations as methods of reproduction allow to increase the selection intensity of the production rams and implicitly to increase the selection efficiency.

Of particular importance in this biotechnology is the methods of preserving and diluting ram semen in order to ensure proper fertility and birth. For these reasons, one of the most current perspectives is to develop new environments for preserving ram semen by refrigeration and introducing into their structure as an additional component of various biologically active antioxidants and cryoprotectants.

MATERIALS AND METHODS

The research took place during the breeding season.

The objective of these researches was to determine and statistically research the data on ram semen from the Moldovan Karakul breed. The biological material used was represented by 5 rams aged 3-4 years.
The criteria needed in the producers' choices were age and sexual behavior. The semen was harvested on sheep in heat or in anesthesia with the help of the artificial vagina due to the fact that this method of harvesting is fast and simple and the semen is superior both in terms of quality and quantity. After harvesting, the quantitative and qualitative parameters of the sperm were analyzed.
Macroscopic analysis of the appearance and volume of semen or determined immediately after collection in the graduated container attached to the artificial vagina.
After being followed by the microscopic ones regarding the mobility, concentration, speed of sperm advance (VAP-total speed, VSL-speed of sperm with rectilinear movements, VCL-speed of sperm with curvilinear movements) which were assessed using CEROS computer system.
Ejaculates with a mobility of more than 70% and a sperm concentration of more than 2 billion/ml were allowed for processing.
The ejaculates allowed for processing were diluted with the SȚJ medium. In the composition of which as an additional component was introduced the biologically active preparation LB/MP obtained from yeast yeasts from brewing by the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova.
The LB/MP preparation was introduced into the SȚJ environment component in the concentration range from 0.1% to 1%. After dilution and sperm mobility testing, the experimental batches were introduced into the cold room at +2-+4ºC.
Periodic determination of sperm motility at 24-hours intervals during storage of ram semen at refrigeration temperature in order to ascertain any differences between sperm motility and to establish the optimal storage period by refrigeration until the minimum mobility required for inoculation is reached.
Statistical parameters were calculated using the ISAS computer program.

RESULTS AND DISCUSSIONS

The study of the conservation of ram's semen by refrigeration, as well as the analysis of their qualitative parameters, the conservation techniques at refrigeration temperatures being the most used in these species have constituted and constitute the research directions in this field.
The success of sperm storage therefore depends on diluting the sperm with an environment containing substances that protect the sperm against the stress associated with lowering the temperature. The addition of different proportions of biologically active substances to a simple diluent, easy to prepare but which has also had a good effect on maintaining sperm motility over time is the main reason for perfecting the protocol for preserving sperm by refrigeration.
To establish the influence of the biologically active preparation on mobility
Experimental research on sperm was performed on ram semen diluted and supplemented with the experimental preparation LB/MP. The freshly sampled semen was diluted with the SȚJ diluent and supplemented with the preparation in various amounts from 0.1 to 1.0%, a total of 10 experimental batches. All batches, both experimental and control were subjected to storage at temperatures of +2-+4°C over a period of 120 hours. During storage, tests were performed every 24 hours to determine sperm motility.
Data on the mobility of sperm processed and stored at +2-+4°C for 120 hours are presented in Table 1.

Table 1. Mobility of ram sperm stored in time at +2-+4°C, %

Parameters		Witness (SŢJ)	LB/MP (%)									
			0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
fresh semen	Mobile, %	87.8 ±0.7	87.8 ±1.0	88.2 ±0.7	88.8 ±1.7	90.2 ±0.7	91.0 ±1.1	91.8 ±0.8*	92.5 ±1.3*	92.0 ±0.9*	91.3 ±0.6*	90.8 ±0.7*
	Progres, %	45.2 ±4.8	50.3 ±3.2	51.2 ±1.4	50.5 ±1.6	42.8 ±2.6	46.5 ±3.6	51.8 ±2.9	51.8 ±2.2	48.0 ±2.2	51.3 ±6.3	46.4 ±3.5
24 h	Mobile, %	83.8 ±2.0	83.8 ±2.0	87.6 ±1.4	86.5 ±1.3	88.8 ±1.7	90.5 ±1.2*	91.0 ±1.1*	91.8 ±1.1*	88.4 ±1.3	88.5 ±1.7	85.6 ±2.2
	progresive, %	36.8 ±2.1	33.0 ±3.9	42.2 ±4.1	38.0 ±6.6	46.2 ±4.4	40.8 ±3.8	47.0 ±3.9	45.8 ±5.3	39.6 ±3.9	37.3 ±5.6	39.6 ±5.6
48 h	mobile, %	81.2 ±1.0	82.0 ±1.4	84.2 ±1.0	85.3 ±1.2	86.6 ±0.7*	86.8 ±0.9*	86.8 ±1.2*	88.5 ±1.3*	87.0 ±1.1*	85.5 ±1.3	83.2 ±1.4
	progresive, %	38.6 ±5.2	39.8 ±5.9	39.8 ±4.4	37.8 ±4.0	34.8 ±1.7	33.0 ±2.2	43.6 ±4.6	42.8 ±3.1	38.6 ±3.8	45.8 ±5.3	40.0 ±5.5
72 h	mobile, %	71.2 ±3.9	78.5 ±1.7	74.0 ±4.5	80.3 ±0.9	77.8 ±4.2	82.5 ±1.0*	77.2 ±5.6	82.5 ±1.0*	78.2 ±3.1	79.0 ±1.6	75.0 ±3.7
	progresive, %	35.2 ±3.9	36.3 ±5.8	32.0 ±5.9	38.0 ±4.4	37.0 ±7.2	29.5 ±3.7	38.8 ±9.4	36.5 ±7.3	33.6 ±6.6	42.8 ±5.8	30.0 ±6.4
96 h	mobile, %	63.0 ±2.5	65.3 ±1.8	69.3 ±3.9	70.5 ±3.9	71.0 ±3.8	72.8 ±3.6	75.3 ±4.9	76.3 ±3.2*	77.0 ±3.5*	74.0 ±3.3	71.0 ±1.8
	progresive, %	26.0 ±4.1	24.8 ±3.3	28.8 ±4.8	24.8 ±3.9	33.0 ±8.4	26.8 ±4.8	33.0 ±5.4	32.8 ±7.5	38.5 ±4.9	30.8 ±5.9	31.8 ±6.7
120 h	mobile, %	58.3 ±2.0	57.3 ±2.7	61.8 ±5.3	62.5 ±4.9	63.0 ±5.1	63.0 ±5.3	66.3 ±5.8	67.5 ±4.6	68.0 ±5.8	65.0 ±5.4	62.3 ±4.8
	progresive, %	12.8 ±1.1	14.3 ±2.9	15.5 ±5.3	15.5 ±5.6	19.0 ±4.8	17.0 ±4.7	18.5 ±6.0	22.3 ±7.6	25.3 ±7.7	17.8 ±3.0	14.8 ±2.6

*P≤0.05

Research has shown that the LB / MP preparation produced at the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova introduced as an additional component in the environment of SŢJ in a concentration of 0.1% to 1.0% is not toxic to sperm in the range of the studied concentrations.

The data in Table 1 show that all the lots studied provided satisfactory values for the kinetic parameters, after 120 hours of storage. Sperm mobility was maintained at 68.0 ± 5.8% in the batch where the concentration of the LB / MP preparation introduced as an additional component in the TSJ medium by 0.8%.

Sperm mobility in the batch where the concentration of the LB/MP preparation introduced as an additional component in the TSJ medium in the amount of 0.7 (76.3 ± 3.2) and 0.8% (77.0 ± 3.5), after 96 hours of conservation showed positive values, statistically significant (P≤0.05) compared to the control group.

In batches with diluent supplemented with LB/MP in the amount of 0.5 to 1.0%, the kinetic parameters are maintained at a satisfactory level for a period of up to 6 days at a temperature of +4 degrees.

Further research determined the rate of straight line sperm (VSL), average speed (VAP), and curbolinal speed (VCL).

Data on the results of sperm movement speed in ram semen depending on the concentration of LB/MP introduced as an additional component in the STJ baseline during a shelf life of 120 hours are presented in Table 2.

The analysis of the results presented in Table 2 demonstrates that the speed of sperm advance is not significantly reduced in the experimental groups compared to the control group. Statistically significant differences were found between the diluent variant with the concentration of the preparation LB/MP of 0.8% (78.7 ± 6.2 μm/s,) and the control group after 5 days of keeping the semen diluted at temperatures of +4°C (P 0.05)

Table 2. Speed of sperm advance of ram, µm/s

Parameters		Witness (ȘȚJ)	LB/MP (%)									
			0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
fresh semen	VAP	132.9 ±5.9	135.3 ±3.2	135.7 ±6.4	139.5 ±5.5	118.5 ±6.8	125.4 ±6.9	133.1 ±6.7	136.6 ±2.7	130.9 ±2.8	133.3 ±4.1	126.4 ±5.3
	VSL	109.9 ±6.7	113.8 ±4.0	114.0 ±5.2	117.1 ±5.1	94.6 ±5.3	103.8 ±5.9	110.4 ±5.6	112.1 ±3.9	106.6 ±2.5	112.0 ±5.7	106.4 ±7.6
	VCL	192.8 ±9.4	190.7 ±1.9	190.3 ±11.5	203.4 ±5.2	179.2 ±14.9	184.0 ±13.3	191.0 ±10.0	205.4 ±7.2	196.6 ±7.6	193.8 ±6.3	186.1 ±8.7
24 h	VAP	106.9 ±6.5	105.7 ±5.2	101.7 ±4.3	109.3 ±7.2	115.2 ±6.6	111.0 ±1.5	113.0 ±3.3	111.3 ±5.2	102.9 ±6.0	98.6 ±5.1	98.7 ±5.6
	VSL	84.3 ±6.5	78.1 ±2.4	80.5 ±5.3	83.8 ±7.5	92.0 ±6.7	85.0 ±3.0	89.2 ±3.2	89.2 ±5.8	80.4 ±3.9	76.8 ±5.2	80.0 ±5.7
	VCL	172.4 ±9.1	178.8 ±10.4	168.4 ±7.2	180.2 ±12.7	183.5 ±11.4	185.1 ±5.0	183.6 ±9.9	179.2 ±11.0	167.0 ±13.3	162.0 ±11.6	156.7 ±9.0
48 h	VAP	104.6 ±5.7	108.9 ±2.7	102.6 ±3.7	100.4 ±4.9	96.7 ±7.1	100.1 ±5.5	108.3 ±4.5	110.0 ±6.0	99.9 ±5.1	113.3 ±5.2	106.9 ±6.9
	VSL	85.7 ±7.2	86.2 ±4.6	79.7 ±3.9	80.1 ±5.2	71.8 ±1.9	75.9 ±4.6	83.2 ±4.3	82.4 ±4.2	78.5 ±4.2	91.2 ±6.5	86.0 ±6.8
	VCL	166.9 ±7.9	177.1 ±4.4	165.7 ±8.1	167.0 ±7.4	150.3 ±7.6	169.4 ±10.1	156.3 ±8.9	168.8 ±10.1	159.2 ±8.5	172.6 ±6.6	171.4 ±10.8
72 h	VAP	100.5 ±9.7	102.0 ±7.3	88.9 ±6.9	98.4 ±4.5	94.1 ±7.8	92.0 ±3.2	94.3 ±12.0	99.6 ±8.3	85.7 ±4.3	98.6 ±8.4	85.3 ±6.2
	VSL	82.3 ±9.5	83.2 ±7.9	71.0 ±6.8	80.4 ±5.9	77.2 ±7.9	69.7 ±3.7	81.6 ±9.8	78.8 ±9.3	68.3 ±4.6	81.9 ±9.1	67.8 ±6.7
	VCL	160.1 ±9.8	166.4 ±8.3	146.2 ±9.5	158.1 ±3.6	152.9 ±11.1	146.9 ±9.2	156.9 ±15.5	161.3 ±7.2	138.5 ±6.1	159.0 ±7.4	132.5 ±6.6
96 h	VAP	80.7 ±4.6	85.8 ±5.8	85.9 ±2.7	82.7 ±7.0	87.7 ±8.0	86.8 ±4.9	93.9 ±6.8	90.3 ±5.9	97.8 ±5.2	93.3 ±6.5	94.7 ±7.8
	VSL	57.9 ±4.1	67.8 ±5.3	65.5 ±3.1	66.4 ±5.8	73.0 ±8.1	67.0 ±6.0	76.3 ±9.1	71.5 ±8.1	78.7 ±6.2*	74.6 ±9.1	76.4 ±9.7
	VCL	145.1 ±8.1	147.6 ±6.2	150.5 ±2.5	144.6 ±11.8	142.7 ±10.3	155.0 ±8.0	156.8 ±5.1	155.1 ±5.1	158.9 ±4.8	154.1 ±4.8	158.8 ±5.0
120 h	VAP	73.1 ±2.0	76.1 ±1.5	72.7 ±7.9	74.6 ±6.7	79.2 ±6.2	74.1 ±4.8	76.7 ±5.4	76.4 ±6.9	80.3 ±9.4	72.4 ±3.6	71.2 ±2.7
	VSL	54.8 ±0.6	55.4 ±3.9	57.0 ±5.2	52.0 ±3.6	58.5 ±5.2	56.1 ±5.6	56.6 ±6.6	58.2 ±7.5	64.4 ±9.0	52.9 ±3.4	52.8 ±2.7
	VCL	137.6 ±6.1	136.0 ±6.7	125.0 ±14.6	131.2 ±12.1	135.4 ±11.9	132.0 ±6.4	133.0 ±7.6	132.0 ±11.3	136.7 ±12.9	126.3 ±6.9	125.0 ±6.0

*P≤0.05

In conclusion, the diluent supplemented with the LB/MP preparation in the proportions from 0.1 to 1.0%, experienced in our research showed positive results during the longer storage of semen.

Determining the morphological integrity of semen is an important parameter for assessing semen quality. More often, abnormalities are detected in the tail of sperm, the base of the head and neck. A significant number of pathological sperm should certainly be seen as a sign of impotence.

The research conducted aimed to determine how the LB/MP preparation, introduced as an additional component in the ȘȚJ environment. Influences the morphological parameters of ram semen in the storage period of semen at refrigerator temperatures.

For this we identified possible differences in morphological parameters, depending on the percentage of LB/MP added to the diluent ȘȚJ (Table 3).

Table 3. Abnormalities of ram semen

	parameters	Witness (STJ)	STJ + LB/MP, %				
			0.2	0.4	0.6	0.8	1.0
fresh semen	Macrocephaly	0.7±0.3	0.7±0.7	1.0±0.6	0.7±0.3	0.3±0.3	0.3±0.3
	Microcephaly	1.0±0.6	0.7±0.3	0.3±0.3	0.3±0.3	0.7±0.3	0.7±0.3
	Broken neck	6.3±0.9	6.0±0.6	6.0±0.6	5.0±0.6	4.7±0.3	5.0±0.6
	Double head	-	-	-	-	-	-
	Twisted tail	5.3±0.7	5.3±0.3	4.3±0.9	4.0±0.6	4.7±0.9	4.3±0.3
	Headless	4.0±0.6	4.0±0.6	3.3±0.3	3.0±0.6	3.0±0.0	3.7±0.3
	No tail	4.7±0.3	4.0±0.6	4.3±0.7	4.7±1.2	4.3±0.3	4.3±0.7
	Total-Total, %	**22-11**	**20.7-10.4**	**19.2-9.6**	**17.7-8.9**	**17.7-8.9**	**18.3-9.2**
24 h	Macrocephaly	1.0±0.6	1.0±0.6	1.0±0.6	0.7±0.3	0.7±0.3	1.0±0.6
	Microcephaly	1.0±0.6	0.7±0.7	1.0±0.6	1.0±0.6	1.0±0.6	1.0±0.6
	Broken neck	6.7±0.7	6.3±0.3	6.0±0.6	6.0±0.6	6.0±0.6	6.3±0.3
	Double head	-	-	-	-	0.3±0.3	0.3±0.3
	Twisted tail	6.0±0.6	6.0±0.6	5.3±0.9	5.0±0.6	5.0±0.6	5.3±0.3
	Headless	5.7±0.7	5.3±0.3	5.3±0.3	5.0±0.6	5.3±0.9	5.3±0.3
	No tail	5.3±0.9	5.3±0.9	5.3±0.3	5.7±0.7	6.3±0.3	6.3±0.3
	Total-Total, %	**25.7-12.9**	**24.6-12.3**	**23.9-11.9**	**23.4-11.7**	**24.6-12.3**	**25.5-12.8**
48 h	Macrocephaly	1.3±0.3	1.0±0.0	1.0±0.6	0.7±0.3	1.3±0.9	1.3±0.7
	Microcephaly	1.0±0.6	1.3±0.3	0.7±0.3	0.7±0.3	0.7±0.3	1.0±0.6
	Broken neck	7.7±0.9	8.0±0.6	7.7±0.3	7.3±0.9	7.3±0.3	7.7±0.7
	Double head	-	-	-	0.3±0.3	-	-
	Twisted tail	6.7±1.2	6.7±0.9	6.7±1.2	7.0±0.6	6.3±1.2	7.0±0.6
	Headless	7.7±0.9	7.7±0.7	7.3±0.3	7.0±1.5	7.3±1.2	7.7±1.2
	No tail	7.7±2.8	7.7±0.9	8.3±0.3	8.3±1.5	7.7±0.3	8.0±1.0
	Total-Total, %	**32.1-16.05**	**32.4-16.2**	**31.7-15.9**	**31.3-15.7**	**30.6-15.3**	**32.7-16.4**
72 h	Macrocephaly	1.3±0.7	1.0±0.0	1.0±0.0	1.0±0.6	1.0±0.0	1.3±0.3
	Microcephaly	1.0±0.6	0.7±0.3	1.0±0.6	0.7±0.3	1.0±0.6	1.0±0.6
	Broken neck	8.0±1.5	8.0±2.1	7.7±2.0	7.3±0.9	7.3±0.7	7.7±0.9
	Double head	-	--	-	-	-	-
	Twisted tail	7.0±0.6	7.0±1.2	7.0±0.0	6.7±0.3	6.7±0.9	7.0±0.6
	Headless	8.0±0.6	7.7±0.7	7.0±0.6	6.3±0.9	6.3±0.3	6.3±0.3
	No tail	8.0±0.6	8.0±0.6	8.3±1.5	8.3±1.2	8.0±1.5	8.0±1.0
	Total-Total, %	**33.3-16.7**	**32.4-16.2**	**32-16**	**30.3-15.2**	**30.3-15.2**	**31.3-15.7**
96 h	Macrocephaly	1.7±0.3	1.3±0.3	1.0±0.0	1.0±0.6	1.0±0.6	0.7±0.3
	Microcephaly	1.0±0.6	0.7±0.3	0.7±0.3	0.7±0.3	0.7±0.3	0.7±0.3
	Broken neck	8.3±0.3	8.3±0.9	8.7±0.7	8.0±0.0	7.0±1.0	7.7±0.9
	Double head	-	-	0.3±0.3	-	-	-
	Twisted tail	7.7±0.3	8.0±0.0	7.3±0.3	7.7±0.3	7.3±0.3	7.3±0.9
	Headless	8.0±1.2	8.0±0.6	7.7±0.9	7.3±0.3	6.7±0.9	6.7±0.3
	No tail	8.7±0.9	8.3±1.3	9.0±1.5	8.7±1.5	8.7±0.9	8.3±0.3
	Total-Total, %	**35.4-17.7**	**34.6-17.3**	**34.7-17.4**	**33.4-16.7**	**31.4-15.7**	**31.4-15.7**
120 h	Macrocephaly	2.0±0.6	1.7±0.3	1.7±0.3	1.3±0.7	1.3±0.3	1.7±0.7
	Microcephaly	1.0±0.0	1.0±0.0	1.0±0.0	1.3±0.3	1.0±0.0	1.3±0.3
	Broken neck	8.7±0.3	9.0±0.6	8.7±0.3	8.7±0.9	7.7±0.9	8.3±0.9
	Double head	0.3±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.3	0.0±0.0
	Twisted tail	8.3±0.3	8.0±0.6	7.7±0.9	8.0±0.6	7.7±0.3	8.0±1.2
	Headless	8.3±1.3	8.3±1.2	8.3±0.3	8.3±0.7	7.3±0.3	7.7±0.7
	No tail	9.0±0.0	8.7±0.3	8.7±0.9	9.0±1.5	8.3±0.3	8.7±0.9
	Total-Total, %	**37.6-18.8**	**36.7-18.4**	**36.1-18**	**36.6-18.3**	**33.6-16.8**	**35.7-17.9**

The data in the table show that the highest average value of morphologically abnormal spermatozoa, at the beginning of the experiment, was in the control group in which

the ram semen was diluted with SȚJ medium. When the sperm were diluted with SȚJ medium, in which the LB/MP preparation was introduced as an additional component, at a concentration of 0.8%, the mean value of the abnormal shape indicators was the lowest and was 8.9%. After 120 hours of storage, ram semen diluted and stored at +2-+4°C, the morphological parameters of this experiment indicate that the average value of sperm with abnormality is still high in the control - 18.8%, while in the seminal material with SȚJ medium diluted with the addition of 0.8% of the LB/MP preparation, the morphological parameters corresponded to the standard, the values being the best - 16.8%.

The effect of the LB/MP preparation introduced as an additional component in the SȚJ environment on the condition of the acrosome of ram sperm during storage of sperm at refrigeration temperatures was determined (Table 4).

Table 4. Spermatozoa with damaged acrosome in ram semen stored at temperature +2-+4°C

Specification		Sp/pr	24 h	48 h	72 h	96 h	120 h
Witness (SȚJ)		4.0±0.8	9.0±0.7	24.5±3.5	31.0±3.5	45.3±3.7	60.8±5.3
LB/MP, %	0.1	4.0±1.0	8.2±0.9	14.2±3.7	27.0±4.0	41.7±4.9	55.2±3.5
	0.2	4.0±0.7	8.1±0.5	14.5±2.9	28.3±3.5	42.4±3.7	56.5±4.9
	0.3	3.3±0.9	7.5±0.3	13.0±3.3	24.7±4.2	41.2±4.6	52.3±4.6
	0.4	3.5±0.9	7.5±0.6	11.9±2.0	25.8±3.5	40.9±3.6	56.1±4.4
	0.5	3.0±0.9	7.3±0.3	11.0±2.8	22.8±4.4	38.3±3.6	52.7±4.4
	0.6	3.1±0.5	6.8±0.3	10.5±2.0*	24.9±3.7	39.8±3.2	55.1±4.6
	0.7	2.7±1.0	6.5±0.3*	10.3±3.3	21.8±4.1	37.0±3.8	51.7±4.4
	0.8	2.6±0.7	6.6±0.2*	10.3±2.4*	24.5±3.9	36.4±2.2	52.4±3.4
	0.9	2.8±0.7	6.5±0.6	10.5±3.8	23.2±3.4	30.3±3.9	52.2±4.4
	1.0	3.1±0.6	7.6±0.6	11.9±2.3	25.8±3.5	40.1±3.4	56.4±4.8

*P≤0.05

The data presented in the table show that at the initial dilution, the lowest percentage of sperm with damaged acrosome was in the sperm with SȚJ medium, with the addition of 0.7 to 0.9% of the LB/MP preparation, which constituted 2.6-2.8% compared to the control group, where the percentage of damaged acrosomes was 4.0%.

The most efficient variant was experienced in sowing sheep. For this purpose, the ejaculates allowed for processing were diluted with the SȚJ medium in the composition of which as an additional component the LB/MP preparation was introduced in a concentration of 8%. After dilution the sperm was exposed to temperatures of 2-4 degrees for 120 hours, after which the refrigerated semen was used for artificial insemination of sheep. Experimental data on sheep sowing are presented in Table 5.

Table 5. The results of artificial insemination of sheep

Breed	They were Sown (heads)	They didn't repeat		The heat gave birth	
		heads	%	heads	%
Karakul	74	41	53.4	36	48.6

The detection of sheep in heat was carried out with the help of test rams. The first time the sheep were sown after being detected, and the second sowing over 10-12 hours after the first sowing. The results of artificial insemination showed that out of 74 sheep artificially seeded with refrigerated semen and stored for 120 hours, they gave birth to 36 heads or 48.6%.

CONCLUSIONS

The LB/MP preparation introduced as an additional component in commercial dilution media is not toxic for ram sperm in the range of concentrations studied (0.1-1.0%).

After 6 days of storage of the temperature +2-+4°C the best results were obtained when the concentration of the LB/MP preparation introduced in the dilution media was 0.6-0.8%: - mobility 67.5-68.0%, permissible mobility for artificial insemination; VSL - 58.2-64.4 μm/s, VCL - 132.0-136.0 μm/s;

The percentage of abnormal sperm was 33.6-36.6% in the experimental groups compared to the control group in which the number of sperm was 37.6%.

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