RESEARCH ON THE INFLUENCE OF "BIOR" PREPARATION ON CRIOCONSERVATION OF RAM SPERM

G. Darie[1*], Nina Bradu[1], I. Iurcu[1]

[1]Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine, v. Maximovca, Republic of Moldova

The object of the study was the sperm of sheep-producers of local breeds and the drug BioR (developed by the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova). The drug BioR was added to the commercial environment (GCJ) as an additional component in a concentration of from 1 to 10%. It has been proven that BioR is not toxic to sperm within the studied concentration. The best results in motility and survivability after thawing of sperm were obtained when BioR at a concentration of 6% was introduced into the commercial medium of GC as an additional component. The medium was tested under production conditions for insemination of sheep. It was found that when using a medium in which BioR was introduced as an additional component, the fertility of sheep inseminated by thawed seed was 47.8%. A synthetic sperm cryopreservation medium for rams consists of glucose, sodium citrate, glycerin, egg yolk, BioR and antibiotics (patent 4513).

Key words: cryopreservation, environment, semen, insemination, ram

INTRODUCTION

In conditions of intensification of animal husbandry, an accelerated spread of valuable animal genotypes is observed. This achievement was possible as a result of the development and introduction of artificial insemination of farm animals into production [5,8]. The rational use of the genetic potential of the most valuable animals is possible due to improved breeding work and significant success in developing methods for cryopreservation of ram sperm, in connection with this, the method is widely used in sheep breeding practice. [1,2,3,4,]. In practice, in sheep breeding, the possibilities of this method are not fully realized. The main reason is the low fertility of sheep with frozen semen[5,6]. Finding and solving the problem of a longer shelf life of ram sperm will make it possible to maximize the use of animals throughout the year, store sperm for years and create the necessary reserves of genetic material, transport frozen sperm, which is very important for interbreeding and breeding of new breeds [4]. Based on this problem, there was a need for scientific research to improve the synthetic environment for dilution and storage of ram sperm, stored at a temperature of -196º C and technologies for thawing frozen semen.

MATERIAL AND METHOD

Studies were carried out on sperm of ram-producers in 2016-2017. Sperm for laboratory tests was obtained from clinically healthy animals on an artificial vagina. For research, undiluted seed with a mobility of at least 80% was used. Seed quality was investigated using the CEROS computer program. Research on the development of a synthetic freezing medium was carried out with the inclusion of the biological preparation BioR. The optimal ratio of medium and BioR preparation was determined on the basis of determining the conservation of sperm motility after dilution, cooling, and after freezing and thawing. he degree of dilution was determined arithmetically taking into account the concentration and motility of freshly obtained sperm. The cooling and freezing mode was carried out according to V.

[*]Corresponding author: darie@mail.ru
The manuscript was received: 23.09.2019
Accepted for publication: 04.11.2019

Milovanov. During the random campaign, the selection of ewes in the hunt was carried out using sample rams. The ewes were seeded with sperm frozen in granules. Artificial insemination of the uterus was carried out according to the generally accepted method twice by the intracervical method immediately after sampling and again after 8-10 hours using the necessary tools. A single dose was 0.2 ml. The biological fullness of sperm was determined by the fertilizing ability of the frozen seed, using the individual accounting method for sheep lambing, in scientific and production experiments conducted on the farm of the experimental department of the Moldavian Scientific and Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine. In each case, the results of insemination and lambing were recorded on a commission basis. The conditions for feeding, caring, and keeping the sheep throughout the entire period of the experimental studies were the same and corresponded to zootechnical standards.

RESULTS AND DISCUSSIONS

The rational and long-term use of sheep-producers is the key to improving the breeding and productive qualities of sheep. The main role here is played by sperm freezing, which ensures the accumulation of a large stock of genetic material. Its results depend on the composition of the synthetic medium. To improve the protective environment intended for dilution and freezing of sperm of ram-producers, we studied the new drug BioR developed at the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova. Native sperm was diluted 1: 3 with test variants of synthetic media, to which Bio R was additionally administered at a concentration of 1% to 10%. (Table 1)

Table 1 Average values of ram sperm mobility after dilution,%

To specify		Mobility after dilution	
		Mobility, %	Progressive movements,%
Witness - GŢJ		88.7±2.8	46.7±5.5
BioR, %	1	85.3±5.2	56.7±8.1
	2	88.0±1.7	42.7±7.8
	3	84.0±9.5	45.7±8.4
	4	92.3±1.9	52.7±7.6
	5	83.7±6.4	43.3±1.2
	6	88.7±3.7	46.7±6.1
	7	91.0±1.7	48.0±5.5
	8	85.0±5.6	47.7±8.3
	9	90.7±1.8	50.3±0.9
	10	87.3±8.2	45.3±9.7

Analyzing the data of table 1 we see that the sperm motility after dilution of freshly obtained sperm in all test media was in the range of 83.7-92.3%. Similar changes occurred with sperm motility with rectilinear movement after dilution. Sperm with rectilinear translational motion had 43.3-56.7%.

The same samples were cooled in a refrigerator at T + 4C and analyzed for sperm motility and rectilinear motion after cooling. (table 2).

Table 2 Average values of ram sperm mobility after refrigeration,%

To specify		Mobility after refrigeration	
		Mobility, %	Progressive movements,%
BioR, %	Witness - GŢJ	83.0±3.5	39.3±4.4
	1	81.3±9.3	45.0±6.5
	2	85.3±5.2	36.7±3.0
	3	84.3±6.8	40.3±6.8
	4	87.7±4.5	48.7±2.7
	5	84.3±7.2	42.0±4.0
	6	84.3±7.2	42.0±4.0
	7	89.7±1.8	41.7±1.9
	8	85.7±5.9	39.0±4.0
	9	87.0±4.5	46.3±10.4
	10	83.0±6.0	42.0±3.5

During the equilibration period, sperm motility changed insignificantly compared to freshly diluted sperm and averaged from 81.3% to 89.7% in all experimental media. Of these, rectilinear-translational motion had from 40.3 to 48.7%. To determine sperm survival during freezing, experimental

samples were frozen at T -196 C. Sperm was frozen in the form of granules on a fluoroplastic plate. The results of the evaluation of sperm after thawing are presented in table 3

Table 3 Viability of post-freezing ram sperm at + 37ºC,%

To specify		Iniţial	
		Mobility, %	Progressive movements, %
Witness - GŢJ		31.7±4.4	11.7±2.7
BioR, %	1	42.0±10.0	18.0±7.0
	2	40.7±3.0	18.0±1.2
	3	36.0±5.6	13.3±2.2
	4	40.0±4.0	14.3±5.0
	5	41.7±3.8	19.7±2.2
	6	52.0±4.0	27.3±1.7
	7	50.0±2.5	26.3±0.7
	8	49.3±1.5	23.0±2.5
	9	50.3±2.4	22.7±1.3
	10	50.0±4.0	19.7±3.8

After freezing-thawing, sperm motility decreased markedly and amounted to 36.0-52.0%. The best mobility results after thawing (52.0%) were obtained when 6% of the BioR preparation was injected into the main medium (GC). The obtained experimental data show that the test drug is not an inhibitor for sperm in the tested concentration ranges. Based on laboratory research data, an improved medium was proposed for diluting and freezing sperm of ram-producers of the following composition: glucose - 0.8 g, sodium citrate - 2.8 g, chicken egg yolk 20 ml, glycerin - 7%, Bio R - 6%, antibiotics - 50 UE, distilled water - 100 ml. The proposed medium was tested under production conditions by artificial insemination of sheep. The data of artificial insemination of sheep are presented in table 4.

Table 4 The results of artificial insemination of sheep

Indicators	goals	%
Total inseminated.	67	100
Fertilized	32	47.8
Re-inseminated	35	52.2

The data presented in the table show that out of 67 inseminated sheep 18 days after the last insemination 35 heads returned to the hunt, which is 52.2%. 32 goals (47.8%) remained souign, which is a pretty good indicator.

CONCLUSIONS

1. Sperm motility after dilution of freshly obtained sperm in all test media was in the range of 83.7-92.3%.

2. After cooling, sperm motility changed slightly compared to freshly diluted sperm and was in all experimental media from 81.3 to 89.7%.

3. As a result of freezing-thawing, sperm motility decreased markedly and amounted to 36.0-52.0%.

4. The best mobility results after thawing (52.0%) were obtained when 6% was introduced into the main medium (GC) BioR. Fertilization of sheep after the first insemination according to lambing results was 47.8%.

REFERENCES

[1]Aybazov A.M., Aksenov P.V., AshurbegovK.K., Kovalenko, D.V., 2011: On the question of the preservation of the gene pool and the biological usefulness of cryopreserved sperm. Scientific Works of the All-Russian Research Institute for Sheep and Goat Breeding. 1 (4-1), 24–29. (RU)

[2]Derjazhencev V.I., 2006. Of their insemination. Zhurnal "Veterinarija i kormlenie". 5. 28-29.

[3] Erohin A.S., 2003. Cryoprotective effect on the semen of sheep of various polyethylene glycols. Zhurnal "Ovcy, kozy, sherstnoe delo" .1. 9-11

[4]Gvozdeckij N.A., 2017. Development of technology for the preparation of sperm of rams for extracorporeal fertilization**.** Doctoral dissertation. Krasnodar. (RU)

[5]Kasymov K.T., 1990. Freezing of ram sperm: Theory and practice. Avtoreferat dissertacii doktora sel'skohozjajstvennyh nauk. Alma-Ata. (RU)

[6]Magomedov Z.Z., 2008. Development and improvement of biotechnological methods of sheep reproduction. Doctoral dissertation. Mahachkala. (RU)

[7]Milovanov V.K., 1962. Biology of reproduction and artificial insemination of animals**,** M. 696 p

[8]Nauk V.A., 1991. Structure and function of sperm cells of farm animals in cryopreservation. Kishinev. Shtiintsa. (RU)