

RESEARCH ON CRYOPRESERVATION OF RAM SEMEN

Gregory Darie*, Elena Cibotaru, Nina Bradu, Irina Djenjera

Scientific and Practical Institute of Animal Husbandry and Biotechnologies in Veterinary Medicine

*Corresponding e-mail: darie@mail.ru

Abstract

The research was carried out on semen obtained from Karakul and Tigaie rams. There was revealed a high individual variability of sexual behaviour in Bethaimer and Sulfojaculate efficacy in the cryopreservation process, the dilution medium for cryopreservation of the seminal material was proposed. The evaluation of the results of ram semen freezing supposed the evaluation of the ejaculates obtained from rams of various breeds, the cryopreservation, the storage of the semen and its use for the sowing of the sheep and the stock of semen material in the gene banks.

Keywords: ram, sperm, freezing, mobility, viability, breed, diluent, ejaculate.

Introduction

In countries with a high level of livestock production, began to be designated the limits of increase in productivity. This means that in the future classical technologies alone are no longer able to provide a steady increase in zootechnical production. Hope is based on modern biotechnologies that are called to complement classical technologies and make them more productive and more effective (Anzar et al., 2002; Bonca, 2000; Nauc, 1989; Осташко, 1992; Edyg et al., 1994).

Material and methods

The research was carried out on the ram sperm from Sulfojaculate, Tigaie Karakul and Bethaimer. The sperm was collected by artificial vagina. Ejaculations with mobility of not less than 7-10% and sperm concentration of 2.5 billion per millilitre were taken into processing. For dilution, the compound composed of glucose, sodium cationate, glycerin and egg yolk was used, and as an additional component the substance ND produced at the Institute of Microbiology and Biotechnologies of the ASM with antioxidant and membranotope properties.

Results and discussion

Researches on the optimal concentration of ND substance introduced in the composition of the dilution medium is shown in Table 1.

Table 1. Average values of ram sperm mobility, %

ND	Mobility after					
	Dilution		freezing		reanimation	
	mobile	progressive	mobile	progressive	mobile	progressive
Control	88.7±2.8	46.7±5.5	83.0±3.5	39.3±4.4	30.5±4.5	10.0±2.0
1 %	85.3±5.2	56.7±8.1	81.3±9.3	45.0±6.5	47.0±15.0	21.0±11.0
2 %	88.0±1.7	42.7±7.8	85.3±5.2	36.7±3.0	49.0±5.5	20.0±3.1
3 %	84.0±9.5	45.7±8.4	84.3±6.8	40.3±6.8	36.3±9.1	16.7±7.2
4 %	92.3±1.9	52.7±7.6	87.7±4.5	48.7±2.7	41.0±5.0	14.3±5.0
5 %	83.7±6.4	43.3±1.2	84.3±7.2	42.0±4.0	48.0±7.0	20.5±3.5
6 %	88.7±3.7	46.7±6.1	84.3±7.2	42.0±4.0	62.0±2.9	34.0±5.0
7 %	91.0±1.7	48.0±5.5	89.7±1.8	41.7±1.9	55.0±8.0	26.0±1.0
8 %	85.0±5.6	47.7±8.3	85.7±5.9	39.0±4.0	53.3±4.5	23.0±2.5
9 %	90.7±1.8	50.3±0.9	87.0±4.5	46.3±10.4	57.0±8.0	22.0±2.0
10 %	87.3±8.2	45.3±9.7	83.0±6.0	42.0±3.5	60.0±2.6	30.0±6.0

The analysis of the values in Table 1 demonstrates that sperm mobility is not significantly reduced after dilution in relation to ND concentration. Depending on the concentration of the ND substance in the GTJ dilution medium, sperm motility oscillates between 83.7±6.4 when the concentration of ND in the dilution medium was 5% and 92.3±1.9 when the concentration was 4%. It has been found that the mobility has a first degree of decrease after the equilibration and a second after thawing. After defrosting the sperm motility oscillated between 62.0±2.9 when the ND concentration was 6% and 41.0±5.0% when the ND concentration was 4% and the control group was only 30.5 ± 4.5%.

The study of sperm viability post freezing is presented in Table 2.

Table 2. Viability of ram sperm post freezing at + 37°C, %

ND	Post freezing	After				
		1h	2h	3h	4h	5h
	Mobile	Mobile	Mobile	Mobile	Mobile	Mobile
Mar-tor	30.5±4.5	42.2±9.2	30.7±10.1	28.5±10.5	19.0±4.0	19.0±2.0
1 %	47.0±15.0	45.5±13.5	54.5±7.5	38.5±6.5	36.0±6.0	18.5±7.5
2 %	49.0±5.5	55.3±5.7	46.3±2.8	44.3±6.4	26.0±3.1	18.0±2.0
3 %	36.3±9.1	49.0±23.0	52.0±17.0	26.3±18.1	43.5±15.5	28.5±3.5
4 %	41.0±5.0	52.3±10.4	38.7±14.2	41.5±9.5	30.0±9.0	27.5±6.5
5 %	48.0±7.0	53.0±12.0	50.5±4.5	36.5±1.5	34.5±3.5	26.0±2.0
6 %	62.0±2.9	58.0±2.1	56.7±5.2	40.0±5.9	41.7±2.7	28.5±8.5
7 %	55.0±8.0	51.5±4.5	59.5±3.5	53.0±11.0	38.0±8.0	23.0±6.0
8 %	53.3±4.5	55.0±6.1	59.0±1.7	46.0±3.6	38.0±4.5	33.0±2.0
9 %	57.0±8.0	56.0±7.0	56.5±8.5	41.0±1.0	27.0±4.0	30.0±10.0
10 %	60.0±2.6	56.0±4.4	56.0±5.5	40.7±1.2	39.7±9.5	26.5±13.5

The analysis of the values in Table 2 demonstrates that the survival of the sperm after 5 hours at 37°C is not significantly reduced in the experimental groups compared to the control group. The best results were obtained in variants 8 and 9- 33.0 ± 2.0 and consecutively $30.0 \pm 10.0\%$. In the results of the research of the qualitative indices of semen after thawing gave us the possibility to develop the dilution medium for the cryoconservation of the ram sperm, which consists of glucose, sodium citrate, egg yolk and as supplementary component ND substance.

The evaluation of the results of freezing of ram semen consisted of evaluation of ejaculates obtained from rams from various breeds, cryopreservation, semen storage and its use for mating the sheep and the semen stock in the gene bank.

Experimental data are presented in Table 3.

Table 3. Storage and use of frozen semen

Breed	Obtained ejaculates	Frozen semen, doses	Stored in the gene bank
Sufolc	26	159	79
Țigaie	64	440	364
Beth.	20	90	38
Karak.	104	278	246
Total	214	967	727

The analysis of the values presented in Table 3 demonstrates that 967 doses of frozen sperm from the ejaculates obtained from processing, of which only 839 doses corresponded to the requirements of the standard against frozen semen.

Conclusions

The following conclusions have been drawn from the researched: The introduction of the dilution medium for ram sperm as an additional component of the ND substance with antioxidant and membranotropic properties demonstrated that after 5 hours of semen storage after thawing at 37 °C the best results of the mobility were obtained when the concentration of the substance ND in the dilution medium was 8-9% (33.0 ± 2.0 and 30.0 ± 10.0 consecutively).

References

- Осташко, Ф.И. (1992): Харьковская технология асептического взятия, криоконсервации, микрохирургии и трансплантации эмбрионов. In: Успехи современной криобиологии: 131-132.
- Anzar, M., Liwei He, M.M. Buhz, T. G. Kroetsch, K. P. Pauls (2002): Sperm apoptosis in fresh and cryopreserved bull semen detected by flow cytometry and its relationship with fertility. In: Biol. Reprod: 354-360.
- Bonca, Gh. (2000): Cercetări privind efectele etapelor de lucru asupra integrității spermatozozilor în timpul conservării spermei prin congelare. In: Proec. of the IV th Int. Simp. ACM-V, Timișoara: 448-459.
- Edyg, E. M., D. O'Brien (1994): The spermatozoon. In: Physiology of Reproduction, Second Edition, E. Knobil and J.D. Neil Eds., Raven Press, FID., New York, 29-77.

Nauc, V. A. (1989): Crioconservația spermî bîcov producătorilor. Crioconservația spermî selischozeistvennîh jivotnîh. L. Agropromizdat: 65-102.