

RESEARCHES REGARDING THE CRYOPRESERVATION OF THE SPERM OF THE BOAR

Elena Cibotaru¹, G. Darie^{1*}, Doina Rotari¹

¹Scientific and Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine, Republic of Moldova

Abstract

The research was conducted on the boar sperm. The semen was collected by manual method. After collecting the sperm was subjected to the macroscopic and microscopic examination. Ejaculates admitted for processing were diluted with dilution media in which the CL-1 substance with 7% antioxidant and membranotropic properties was added as an additional component. After dilution, the semen was refrigerated for 4 hours at + 2-4°C. After the term of refrigeration has expired, the semen has frozen in the form of granules. Defrosting was performed in a marine bath at 45°C. Testing of the defrosted material was performed by the CEROS computerized method. The mobility of sperm after thawing was 34-38% with viability at 37°C for 3-5 hours.

Key words: Boar, sperm, ejaculate, substance, dilution, mobility, viability

INTRODUCTION

Reproduction of swine has numerous biological, technological and economic aspects, which are directly influencing the quantitative and qualitative level of animal products, demonstrating the interdependence between production and breeding.

The remarkable progress that has been recorded in swine reproduction has strongly influenced biological and technological thinking on reproduction on the line of creative application of scientific research results. Reproduction of swine, by biotechnology of artificial inseminations leads to an increasing of rhythm and breeding degree of flocks.

At the same time, the new, modern knowledge of the breeding of the swine, acquired under the conditions of the contemporary technical-scientific revolution, must be appropriately appropriated and applied by the specialists working in the zootechnical sector.

A perspective direction in the technologically characterization characterizes the behaviour of the sperm cell in the swine species during cryopreservation.

Decreased semen quality in cryopreservation is only partially accepted in the literature, and the mechanism of the protection system is limited, which is why we have proposed a detailed study of the degree of damage to sperm (1-5).

MATERIAL AND METHODS

The researches were performed on ejaculators obtained from boars raised at the Moldzuinhibrid State Enterprise and accepted for processing, those ejaculates that contained more than 75% of the mobile and normal sperm.

Immediately after determining the volume, mobility and concentration of the semen, it was diluted with different dilution media, being one of the pre-freezing stages.

In order to dilute semen samples, CL-1 was tested by the Institute of Microbiology and Biotechnology ASM as antioxidant and membranotrope introduced as an additional component in the GHTS environment.

After microscope testing the sperm was diluted 1:1 with the dilution medium composed of lactose and distilled water. Sperm balancing was performed at 4 degrees for 4 hours. After the sperm reached the temperature of 4 degrees C, sperm was diluted 1 liter with the medium composed of lactose, glycerol, egg yolk and CL-1

*Corresponding author: darie@mail.ru

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substance. After balancing the sperm, it is subjected to freeze-dried granules with a temperature drop of +4 to -170°C, approximately 30 minutes. Defrosting was performed at 45 degrees C for 30 sec. The qualitative indices of the semen were studied by the CEROS computerized method.

RESULTS AND DISCUSSIONS

Experimental data on the influence of different CL-1 substances introduced into the GHTS dilution medium in a concentration of 1 to 10% are shown in figure 1.

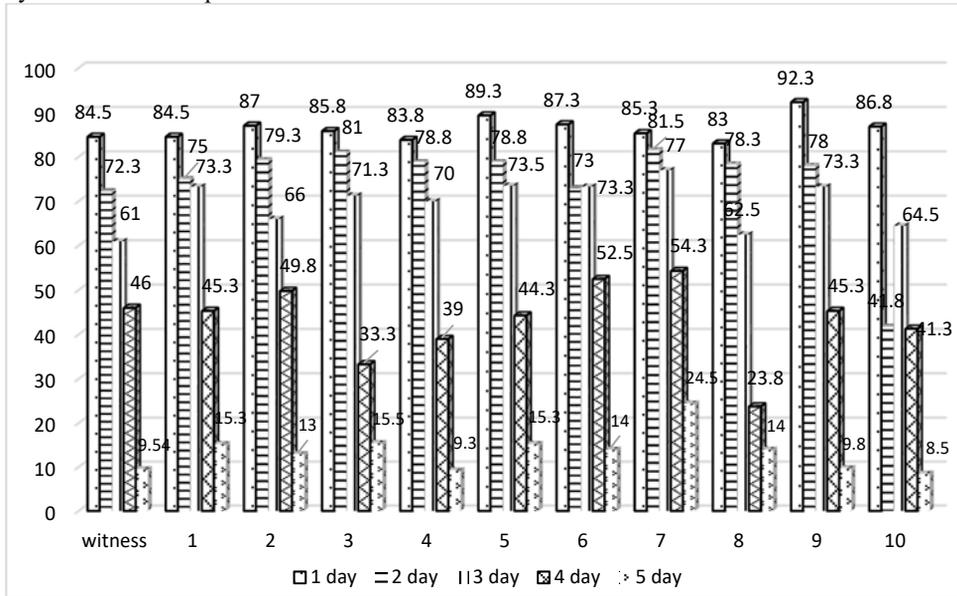


Fig. 1 Sperm motility (%)

The data presented in figure 1 demonstrates that CL-1 introduced as an additional component in the diluted dilution medium at a concentration of 1 to 10% is not toxic to spermatozoa in the concentration slides studied. The best results of mobility were obtained when the CL-1 concentration was 7%, the mobility being 24.5%, after 5 days of keeping the sperm diluted at +4°C.

Data on the number of sperm with progressive forward movements diluted with dilution medium BD-1 at +4°C temperature depending on the concentration of the

substance introduced as an additional component in the GHJ basal medium are shown in Figure 2.

Figure 2 shows that CL-1, introduced as an additional component in the dilution medium GHTS in a concentration of 1 to 10%, allowed to achieve the best results of progressive movements of the sperm when the CL-1 concentration was 7%, being 8.5% compared to the control group where this index was 3.5% after 5 days of storage of the diluted sperm at +4°C.

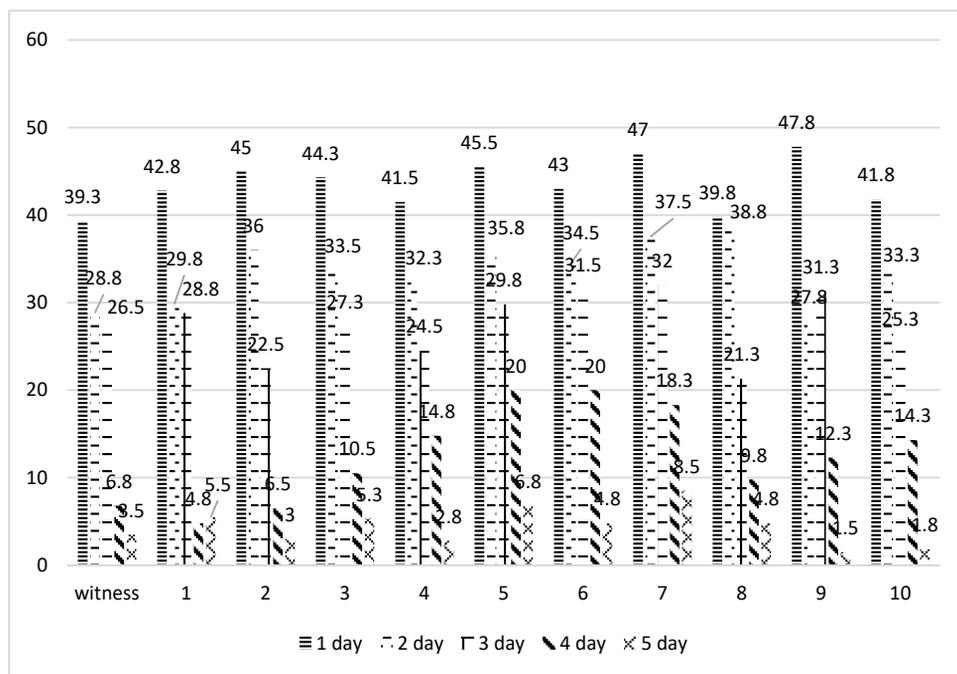


Fig. 2 Boar spermatozoa with progressive movement, (%)

Criocervice or freezing is a method by which space-time barriers are exceeded, biological material being available at any time and part of the world. Cryopreservation techniques are based on lower animal temperature from 37C to -196C (liquid nitrogen temperature). A number of methods have been developed for this purpose.

In order to dilute semen samples for cryopreservation, the CL-1 substance was tested by the Institute of Microbiology and Biotechnologies ASM as an antioxidant and membranotrope introduced as an additional component in the basic environment.

The mobility of the semen after freezing is shown in Table 1.

Table 1 Sperm motility at boar

Specification	After dilution		After refrigeration		Post freezing	
	Motile, %	Progressive, %	Motile, %	Progressive, %	Motile, %	Progressive, %
Witness	84.5±4.8	39.3±6.8	61.0±8.8	26.5±7.0	18.0±5.0	3.0±2.0
Experimental	85.3±3.2	47.0±4.6	73.3±8.9	31.3±6.2	38.0±6.5	17.0±4.0

The values of the experimental data presented in Table 1 show that after dilution the sperm motility was 85.3% compared to 84.5% in the control group. After refragmentation the sperm motility decreased to 73.3% in the experimental group compared to the group witness where these indexes were 61.0%. Cryopreservation (-196°C) produced major sperm dysplasia. After resuscitation the sperm motility in the experimental group was

38.0% compared to the control group where the sperm motility was 18.0%.

CONCLUSIONS

The CL-1 preparation introduced as a supplemental component in the dilution medium for conserving the boar sperm by refrigeration is not toxic to spermatozoa in the concentration range studied. The best results were obtained when CL-1 sunlight

concentration was 7% introduced as an additional component in the studied experimental environment.

The cryopreservation of the boar sperm resulted in major damage to the sperm after resuscitation, with the mobility being 38.0% compared to the control group of only 18.0%.

REFERENCES

- [1] Bonca Gh. Cercetările privind efectele etapelor de lucru asupra integrității spermatozozilor în timpul conservării spermei prin congelare. Proect. of the IV th Int. Simp. ACM-V. Timișoara. 200. p.448-459.
- [2] Осташко Ф.И. – Харьковская технология асептического взятия. криоконсервации. микрохирургии и трансплантации эмбрионов. Успехи современной криобиологии. 1992. с. 131-132
- [3] Милованов В. К. Биология воспроизведения и искусственное осеменение сельскохозяйственных животных. Москва: Изд-во с-х литературы. 1962. с. 499-503.
- [4] Zăhan Marius (2017). Conservarea resurselor genetice în zootehnie. Ed. Accent. Cluj-Napoca.
- [5] Anzar M., He. Liwei, Buhz M.M., Kroetsch T.G., Pauls K.P., – Sperm apoptosis in fresh and cryopreserved bull semen detected by flow cytometry and its relationship with fertility. Biol. Reprod. 2002.66. p.354-360.