THE INFLUENCE OF BIOLOGICALLY ACTIVE PREPARATIONS ON THE PRESERVATION OF BOAR SEMEN

E. Cibotaru[1*], G. Darie[2], I. Djenjera[2], O. Chisaliţa[1], D. Rotari[2], D. Iacob[2]

[1]Practical Scientific Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine, Maximovca village, Anenii-Noi district, Republic of Moldova
[2]Technical University of Moldova, Republic of Moldova

Abstract
The research was conducted on sperm collected from breeding males. Collection was performed using the manual method. For processing, ejaculates with a motility of no less than 70% and a concentration of 0.25 billion per milliliter were accepted. The study utilized the biologically active preparation MP extracted from cyanobacterial yeasts. The biologically active preparation IMB-2 was introduced as an additional component in the GHTS medium at concentrations ranging from 0.2% to 1.2%. The experimental results allowed for the attainment of sperm motility after 120 hours of storage at hypothermic temperatures, measuring 60.3±2.3%, with morphological indices at 63.0±0.7% and a total anomaly rate of 7.8% when the concentration of IMB-2 was added as an additional component at 0.7% in the base medium. In comparison, the control group showed these indices at 50.3±2.9%, 53.5±1.2%, and 10.2%, respectively. The average sperm progression speed after 120 hours was as follows: VAP (velocity average path) - 59.6±5.1%; VSL (velocity straight line) - 28.3±2.4%; and VCL (velocity curve line) - 115.4±1.9%.

Key words: *boar, medium, motility, sperm, concentration*

INTRODUCTION

Animal husbandry is a particularly dynamic branch of science and practice, a characteristic primarily driven by economic factors. Consumer preferences are in constant evolution, which means that the breeder must possess educational capacity, information, and means to anticipate the market and manage production activities accordingly. Breeding strategies used in animal husbandry are continually evolving and include artificial insemination, estrus cycle management, semen and embryo preservation, and sexing, among others [4-6]. Artificial insemination is already a classic reproductive biotechnology that has gradually spread, especially after successful semen preservation at various storage temperatures. [8, 9]. Semen preservation aims to maintain the fertilizing capacity of spermatozoa for a period of time, the duration of which depends on the methods used. [4-6]. This helps to avoid wastage of seminal material and reduces the number of males, as females are inseminated as they come into heat.

The fertilizing capacity of spermatozoa is influenced by a multitude of factors such as motility, viability, ability to achieve capacitation, and acrosomal reaction in the female genital tract [2, 7, 8].

The relationship between sperm motility and fertility remains controversial [1, 3]. The reasons for the approach are determined by the study of the connections and regulations in the field of genetics regarding the

*Corresponding author: lenuta_mar@yahoo.com
The manuscript was received: 18.09.2024
Accepted for publication: 07.10.2024

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

manifestation of morphological traits of growth and development processes in animals, their reproductive processes and qualities, as well as the need to confirm the genetic value of animals through the practice of ecological reproductive biotechnologies, with respect for medical ethics, animal rights, and relevant legislation. Additionally, the goal is to deepen and improve reproductive biotechnologies in animals of zootechnical interest by analyzing their impact on food safety, taking into account the observations and results of other researchers in the field. Thus, the role of reproductive biotechnologies in animals of zootechnical interest in ensuring large quantities of animal-derived products will be highlighted.

MATERIAL AND METHOD

The research was conducted on purebred animals maintained at the SRL, IM "Porco Bello" in Chișinău. The experiments involved breeding males and the sperm collected from them. The sperm was diluted with the GHTS medium, in which the IMB-2 preparation was introduced at concentrations ranging from 0.2% to 1.2%. This biologically active complex was included as an additional component in the commercial medium designed for the storage of boar sperm at temperatures of +16 to +18 °C.

The collected seminal material was analyzed using the computerized semen analysis system (CEROS), which provides information on the motility of each individual sperm cell by projecting electronic images of spermatozoa, constructing the trajectory of each sperm cell, and simultaneously and objectively evaluating minor changes in sperm motility that can be detected. In this study, using the CEROS system, parameters of motility, morphological parameters, and spermatozoa velocity were analyzed.

RESULTS AND DISCUSSION

The influence of the biologically active complex preparation introduced as an additional component in the composition of the commercial medium intended for boar semen storage at a temperature of +16-+18°C on sperm motility and progressively motile spermatozoa. The experimental data are presented in Figure 1.

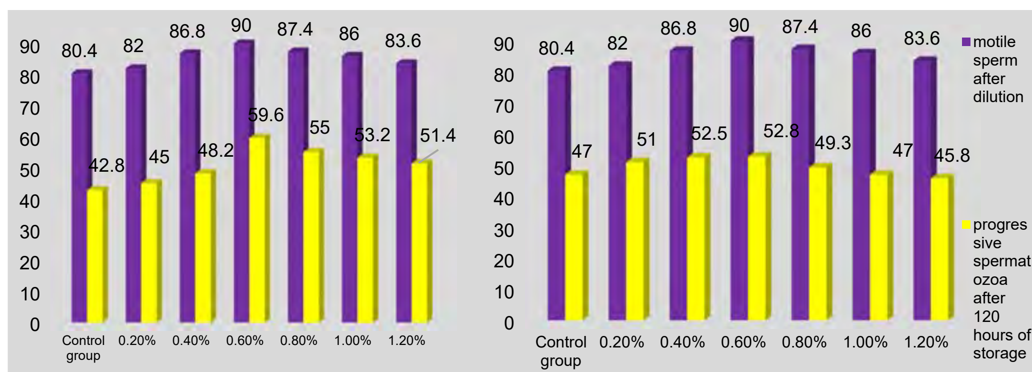


Fig. 1. The influence of the biologically active preparation IMB-2 on the quality of boar semen, %

The experimental data presented in Figure 1 demonstrate that the biologically active preparation IMB-2, within the studied concentration range (0.2-1.2%), introduced as an additional component in the GHȚS medium, is not toxic to spermatozoa. Sperm motility after dilution showed indices ranging from 80.4±0.8% in

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

the control group to 90.0±1.5% in experimental group 3, where the concentration of the IMB-2 preparation was 0.6%. The percentage of spermatozoa with progressive forward movement was highest in experimental group 3 (59.6±0.8%), compared to the control group where this index was 42.8±0.4%.

A more detailed study of how the biologically active preparation affects sperm movement immediately after dilution and after 120 hours of storage at hypothermal temperatures is presented in Table 1.

Table 1. The influence of the biologically active preparation (IMB-2) on the quality of boar semen preserved at hypothermal temperatures (+16-+18°C)

The parameters		Control group GHŢS	Experimental GHȚS +IMB-2, (%)					
			0.2%	0.4%	0.6%	0.8%	1.0%	1.2%
Fresh sperm	VAP, µm/s	83.2±10.6	78.3±2.0	86.8±6.4	95.3±3.6	88.3±8.7	83.5±3.9	89.2±3.2
	VSL, µm/s	50.1±4.0	50.4±1.9	51.7±4.5	63.0±2.8*	55.5±5.2	53.6±2.4	57.6±1.5
	VCL, µm/s	151.0±21.1	142.4±2.8	158.9±12.6	174.5±8.7	162.3±14.2	150.7±6.9	160.7±6.3
24 ore	VAP, µm/s	80.7±9.3	72.6±5.9	81.8±3.2	80.8±3.7	80.7±3.4	79.7±4.1	76.7±4.5
	VSL, µm/s	46.8±3.2	46.6±3.7	51.4±2.6	51.1±1.4	51.4±3.9	51.8±3.7	48.1±4.2
	VCL, µm/s	147.0±17.0	146.5±14.7	147.0±13.6	148.1±7.8	143.0±11.8	139.9±4.1	141.2±2.9
48 h	VAP, µm/s	66.2±3.9	66.6±2.5	71.7±2.4	71.4±3.2	64.9±3.2	59.5±2.4	59.2±0.8
	VSL, µm/s	40.8±3.3	42.1±3.2	41.8±2.3	46.6±2.2	37.6±1.5	37.4±2.4	37.6±1.0
	VCL, µm/s	115.9±4.7	122.1±4.5	129.3±5.2	134.0±1.8*	122.5±1.8	118.8±3.2	119.6±3.1
72 h	VAP, µm/s	55.9±2.4	57.9±1.8	64.1±5.0	65.4±4.5	58.2±3.7	48.9±0.7	46.4±1.2
	VSL, µm/s	35.1±2.5	37.1±3.0	37.4±4.6	41.4±3.3	37.4±2.1	33.3±1.0	30.1±0.5
	VCL, µm/s	108.3±3.8	115.0±2.8	121.0±4.1*	125.6±4.6*	115.0±4.5	98.3±1.4	95.3±2.3
96 h	VAP, µm/s	48.8±0.6	50.6±1.9	52.9±1.3*	54.0±2.3	46.9±2.2	41.8±2.0	39.0±0.9
	VSL, µm/s	31.5±0.8	32.9±1.0	32.0±1.0	32.2±1.1	28.2±2.0	21.2±0.8***	20.3±0.8***
	VCL, µm/s	97.2±2.3	98.5±2.6	103.8±2.7	106.5±2.9*	92.6±2.8	85.1±2.7	80.9±1.6
120 h	VAP, µm/s	44.5±1.1	44.8±1.4	49.9±1.4*	48.6±1.7	49.5±1.2*	45.7±0.6	45.6±0.8
	VSL, µm/s	26.6±1.1	26.2±0.9	28.3±1.2	29.1±0.9	26.1±1.8	20.5±1.0	18.0±0.8
	VCL, µm/s	87.2±1.4	92.4±2.9	97.0±2.1**	97.8±3.2*	96.1±1.3**	90.4±1.0	83.7±1.6

* P≤0,05;** P≤0,01;*** P≤0,001
IMB-2 – experimental preparation
VAP – velocity average path
VSL – velocity straight line
VCL – velocity curve line
GHŢS – base diluent

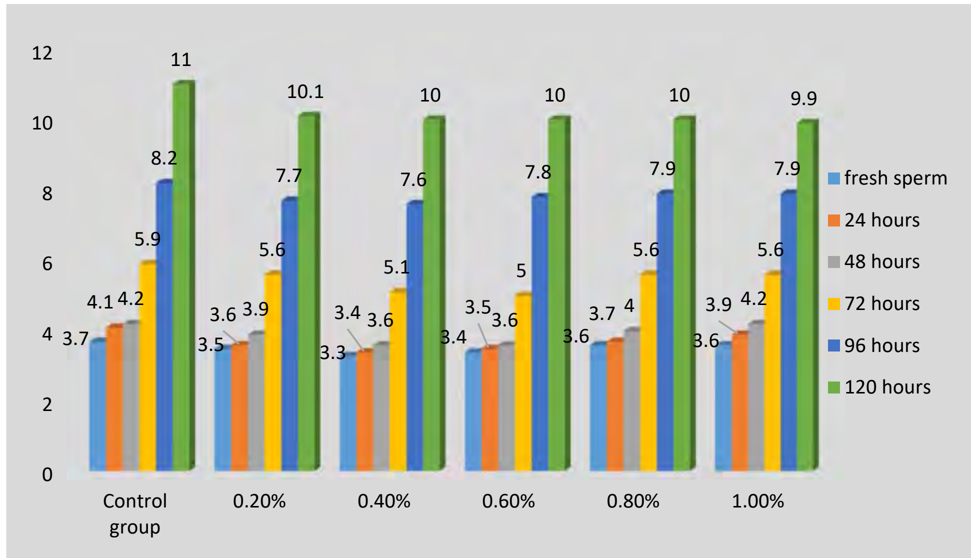
The progression speeds (VAP, VSL, and VCL) were also higher in the experimental groups compared to the control group. After 120 hours of storing the diluted sperm at hypothermic temperatures, the results were superior to those of the control group.

The obtained results are beneficial and advocate for the protective properties of the tested product for spermatozoa. The study of the influence of the biologically active complex preparation introduced as an additional component in the composition of the commercial medium intended for boar semen storage at a temperature of +16-+18°C on the morphological anomalies of spermatozoa fig.2.

The experimental data demonstrated that disruptions in spermatogenesis are caused by various internal or external factors, which are highlighted by the appearance of anomalies in the structure of the germ cell. The study focused on

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

anomalies primarily related to head aberrations, termed "primary." Other phenomena, such as sperm aging in the epididymis, the influence of toxic substances, excessive temperatures, etc., mainly lead to tail anomalies, classified as "secondary" and "tertiary." The "total" anomalies, which represent the sum of primary, secondary, and tertiary anomalies, serve as indicators of sperm quality and are illustrated in fig. 2.



* P≤0,05;** P≤0,01

Fig. 2. Morphological abnormalities of boar spermatozoa, %

The experimental data presented in Figure 2 show that, on average, the total percentage of spermatozoa with various morphological anomalies in the control group, after dilution of the seminal material, was 3.7%. In the experimental groups, this index was lower than that of the control group.

After 120 hours of storing the diluted sperm, morphological anomalies in spermatozoa increased in both the control and experimental groups; however, the differences between the groups were not statistically significant.

Another study on the influence of the biologically active complex preparation introduced as an additional component in the composition of the commercial medium intended for boar semen storage at a temperature of +16-+18°C was the analysis of acrosomal damage in boar spermatozoa. It is believed that acrosomal anomalies are of genetic nature. Although degenerative changes in the seminiferous tubules are known to primarily reduce sperm concentration rather than cause abnormalities, when total anomalies exceed 15%, spermatogenesis is considered disrupted. Experimental data on the influence of the biologically active complex preparation on acrosomal damage in boar spermatozoa are presented in Table 2.

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

Table 2. Spermatozoa with damaged acrosomes in boars, (%)

Storage duration hours	Dilution media						
	Control GHȚS	Experimental GHȚS + IMB-2(%)					
		0.2	0.4	0.6	0.8	1.0	1.2
After dilution	22.0±1.1	20.8±1.0	16.6±1.3*	15.6±1.5*	17.0±1.0*	18.2±1.1*	18.6±0.9
24	27.6±1.5	25.2±1.5	20.4±0.8**	20.4±1.2**	23.6±1.4	24.6±1.7	26.6±1.4
48	32.4±0.7	31.2±0.7	30.0±0.9	30.6±0.9	32.4±0.7	33.4±0.7	33.0±1.0
72	42.8±0.9	38.4±1.2*	36.6±1.3**	37.2±0.7**	39.8±0.6*	40.8±0.4	40.6±0.8
96	51.3±1.1	50.3±0.6	46.5±0.6**	46.0±1.1**	48.5±0.9	50.8±0.9	51.5±0.6
120	55.8±0.9	53.8±1.1	52.8±0.8*	52.8±1.1	53.8±0.9	53.0±1.1	53.5±1.2

* P≤0,05;** P≤0,01
IMB-2 – experimental preparation
GHȚS – base diluent

The research conducted demonstrates that semen dilution is generally accompanied by acrosomal changes. The experimental data presented in Table 2 show that for motile spermatozoa diluted with dilution media containing the biologically active preparation IMB-2 in various concentrations, the percentage of spermatozoa with damaged acrosomes was 22.0±1.1% in the control group, while this index decreased in the experimental groups. The lowest percentage of spermatozoa with damaged acrosomes was found in experimental group 3 at 15.6±1.5% ($P \leq 0.05$), in experimental group 2 at 16.6±1.3% ($P \leq 0.05$), and in experimental group 4 at 17.0±1.0% ($P \leq 0.05$), compared to the control group. This can be explained by the buffering of lactic acid from fructolysis by the biologically active preparation IMB-2. Buffering of lactic acid produced during metabolism with biologically active substances halts pathological changes in the acrosome, although the fertilizing capacity of spermatozoa may become uncertain.

Analyzing the study of the influence of the biologically active complex preparation introduced as an additional component in the composition of the commercial medium intended for boar semen storage at a temperature of +16-+18 °C on sperm membrane integrity (HOST test) is presented in Table 3.

Table 3. Integrity of boar spermatozoa membranes (HOST test)

Integrity of membranes after, hour (h)	Dilution media						
	Experimental GHȚS +IMB-2 (%)						
	Control GHȚS	0.2	0.4	0.6	0.8	1.0	1.2
Sp/pr diluted	77.6±1.1	78.4±0.9	83.6±0.9**	86.0±1.2**	83.6±1.3*	81.4±0.9*	79.2±1.1
24 h	73.8±1.3	75.0±1.8	78.4±1.2*	78.4±0.8*	78.0±2.2	77.0±1.9	74.6±0.9
48 h	70.0±0.7	73.0±1.0*	75.4±0.5***	75.0±0.7**	73.6±0.5**	71.2±0.7	69.4±0.5
72 h	64.0±0.3	67.4±0.5**	69.8±0.6***	69.6±0.8***	67.4±1.4	65.0±1.0	64.2±0.7
96 h	51.0±0.7	52.0±0.8	54.5±0.6*	56.3±0.6**	53.8±0.6*	50.8±0.5	48.8±0.5
120 h	45.0±1.4	50.3±1.8	51.8±1.0**	50.0±0.7*	48.5±0.6	45.5±1.2	43.5±0.6

* P≤0,05;** P≤0,01;*** P≤0,001
IMB-2 – experimental preparation
GHȚS – base diluent

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

The hypo-osmotic swelling test (HOST) for evaluating the fertilizing capacity of spermatozoa and the functional integrity of the plasma membrane is a simple and economical test. For its execution, the seminal material was incubated with hypo-osmotic medium at a ratio of 1 to 10. After incubation for 30-60 minutes at +37 °C, evaluation was performed using phase-contrast microscopy, observing spermatozoa with a twisted tail end, and the results were expressed as a percentage. Experimental data regarding the influence of the biologically active preparation on sperm membrane integrity in boars are presented in Table 3.

From the data presented in Table 3, indicate that the IMB-2 preparation had a positive effect on maintaining membrane integrity compared to the control group. In the control group, after diluting the seminal material with the GHTS medium, the percentage of spermatozoa with intact membranes was 77.6±1.1% ($P \leq 0.01$), compared to experimental group 2 (83.6±0.9%) and experimental group 3 (86.0±1.2%) after 120 hours of storing the diluted sperm at temperatures of +16 to +18°C.

CONCLUSIONS

The obtained data confirm the importance of correctly selecting the composition of dilution media to enhance the efficiency of boar sperm conservation. They indicate the potential for using the biologically active preparation IMB-2 as an additional component introduced into the base diluent GHTS for the preservation of boar sperm at hypothermic temperatures.

REFERENCES

1. Alvarez J.G., B.T. Storey, Role of glutathione peroxidase protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation. 1989, Gamete Res, 23:77–90.
2. Mann T., C. Lutwak-Mann, Male reproductive function and semen. 1981, New York: Springer.
3. Harrison R.A.P., H.M. Dott, G.C. Foster, Bovine serum albumin, sperm motility, and the ‘‘dilution effect’’. 1982, J Exp Zool, 222:81–88
4. Miclea V., Zahan M., Miclea I., Reproducția animalelor de fermă. 2010, *Editura. Accent*, Cluj-Napoca, 334 p.
5. Miclea V. Însămânţarea artificială la animale de fermă. Ed.Agronaut. 2003, Cluj-Napoca.
6. Zahan M.,. Conservarea resurselor genetice în zootehnic. Accent, 2017. Cluj-Napoca p. 34-35, 225.
7. Milovanov V. Biologija vosproizvedennija i iskusstvennoe osemenenie životnyh. M.Sel′shozgiz, 1962, c.696.
8. Milovanov V.K.., Sokolovskaja I.I. Teorija i praktika vosproizvedenija životnyh, M. 1984, 186 c.
9. Ostaško, F.I. Glubokoe zamoraživanie i dlitel′noe hranenie spermy proizvoditelej. Kiev: Urožaj. 1978, 254 c.

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)